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THE UNITED STATES PHARMACOPEIAL CONVENTION  
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## SIX-MONTH IMPLEMENTATION GUIDELINE

The *United States Pharmacopeia–National Formulary* and its supplements become official **six months** after being released to the public. The *USP–NF*, which is released on November 1 of each year, becomes official on May 1 of the following year. This six-month implementation timing gives users more time to bring their methods and procedures into compliance with new and revised *USP–NF* requirements.

The table below describes the official dates of the *USP–NF* and its supplements. The 2011 *USP 35–NF 30*, and its supplements, *Interim Revision Announcements (IRAs)* and *Revision Bulletins* to that edition, will be official until May 1, 2013, at which time the *USP 36–NF 31* becomes official.

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# GENERAL NOTICES AND REQUIREMENTS

The *General Notices and Requirements* section (the *General Notices*) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia* (USP) and the *National Formulary* (NF).

Requirements stated in these *General Notices* apply to all articles recognized in the USP and NF (the “compendia”) and to all general chapters unless specifically stated otherwise. Where the requirements of an individual monograph differ from the *General Notices* or a general chapter, the monograph requirements apply and supersede the requirements of the *General Notices* or the general chapter, whether or not the monograph explicitly states the difference.

## 1. TITLE AND REVISION

The full title of this publication (consisting of three volumes and including its *Supplements*), is *The Pharmacopeia of the United States of America*, Thirty-Sixth Revision and the *National Formulary*, Thirty-First Edition. These titles may be abbreviated to USP 36, to NF 31, and to USP 36–NF 31. The *United States Pharmacopeia*, Thirty-Sixth Revision, and the *National Formulary*, Thirty-First Edition, supersede all earlier revisions. Where the terms “USP,” “NF,” or “USP–NF” are used without further qualification during the period in which these compendia are official, they refer only to USP 36, NF 31, and any *Supplement(s)* thereto. The same titles, with no further distinction, apply equally to print or electronic presentation of these contents. Although USP and NF are published under one cover and share these *General Notices*, they are separate compendia.

This revision is official beginning May 1, 2013, unless otherwise indicated in specific text.

*Supplements* to USP and NF are published periodically. *Interim Revision Announcements* are revisions to USP and NF that are published on the USP website. *Interim Revision Announcements* contain official revisions and their effective dates. Announcements of the availability of new USP Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required USP Reference Standards are also available on the “New Official Text” tab of USP’s website.

*Revision Bulletins* are revisions to official text or postponements that require expedited publication. They are published on the USP website and generally are official immediately unless otherwise specified in the *Revision Bulletin*.

*Errata* are corrections to items erroneously published that have not received the approval of the Council of Experts and that do not reflect the official requirements. *Errata* are effective upon publication.

## 2. OFFICIAL STATUS AND LEGAL RECOGNITION

### 2.10. Official Text

*Official text* is text contained in USP and NF, including monographs, general chapters, and these *General Notices*. Revisions to official text are provided in *Supplements*, *Interim Revision Announcements*, and *Revision Bulletins*. General chapters numbered from 1000 to 1999 are considered interpretive and are intended to provide information on, give definition to, or describe a particular subject. They contain no mandatory requirements applicable to any official article unless specifically referenced in *General Notices*, a monograph, or a general chapter numbered below 1000. General chapters numbered above 2000 apply only to articles that are

intended for use as dietary ingredients and dietary supplements.

### 2.20. Official Articles

An *official article* is an article that is recognized in USP or NF. An article is deemed to be recognized and included in a compendium when a monograph for the article is published in the compendium and an official date is generally or specifically assigned to the monograph.

The title specified in a monograph is the *official title* for such article. Other names considered to be synonyms of the official titles may not be used as substitutes for official titles.

*Official articles* include both *official substances* and *official products*. An *official substance* is a drug substance, excipient, dietary ingredient, other ingredient, or component of a finished device for which the monograph title includes no indication of the nature of the finished form.

An *official product* is a drug product, dietary supplement, compounded preparation, or finished device for which a monograph is provided.

### 2.30. Legal Recognition

The USP and NF are recognized in the laws and regulations of many countries throughout the world. Regulatory authorities may enforce the standards presented in the USP and NF, but because recognition of the USP and NF may vary by country, users should understand applicable laws and regulations. In the United States under the Federal Food, Drug, and Cosmetic Act (FDCA), both USP and NF are recognized as official compendia. A drug with a name recognized in USP–NF must comply with compendial identity standards or be deemed adulterated, misbranded, or both. See, e.g., FDCA § 501(b) and 502(e)(3)(b); also FDA regulations, 21 CFR § 299.5(a&b). To avoid being deemed adulterated, such drugs must also comply with compendial standards for strength, quality, and purity, unless labeled to show all respects in which the drug differs. See, e.g., FDCA § 501(b) and 21 CFR § 299.5(c). In addition, to avoid being deemed misbranded, drugs recognized in USP–NF must also be packaged and labeled in compliance with compendial standards. See FDCA § 502(g).

A dietary supplement represented as conforming to specifications in USP will be deemed a misbranded food if it fails to so conform. See FDCA § 403(s)(2)(D).

Enforcement of USP standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere. USP has no role in enforcement.

## 3. CONFORMANCE TO STANDARDS

### 3.10. Applicability of Standards

Standards for an article recognized in a USP compendium are expressed in the article’s monograph, applicable general chapters, and *General Notices*. Unless specifically exempted elsewhere in a compendium, the identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, whether incorporated in the monograph itself, in the *General Notices*, or in the applicable general chapters. Early adoption of revised standards is allowed. Where revised standards for an existing article have been published as final approved “official text” (as approved in section 2.10) but are not yet official (six months after publication, unless otherwise specified; see “official date,” section 2.20) compliance with the revised standard shall not preclude a finding or indication of conformance with USP official standards, unless USP specifies

otherwise by prohibiting early adoption in a particular standard.

The standards in the relevant monograph, general chapter(s), and *General Notices* apply at all times in the life of the article from production to expiration. The manufacturer's specifications, and good manufacturing practices generally (including, e.g., Quality by Design initiatives), are developed and followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.

At times, compendial standards take on the character of statistical procedures, with multiple units involved and perhaps a sequential procedural design to allow the user to determine that the tested article meets or does not meet the standard. The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all cases, statements about whether the compendial standard is met apply only to the units tested. Repeats, replicates, statistical rejection of outliers, or extrapolations of results to larger populations, as well as the necessity and appropriate frequency of batch testing, are neither specified nor proscribed by the compendia. Frequency of testing and sampling are left to the preferences or direction of those performing compliance testing, and other users of *USP-NF*, including manufacturers, buyers, or regulatory authorities.

Official products are prepared according to recognized principles of good manufacturing practice and from ingredients that meet *USP* or *NF* standards, where standards for such ingredients exist (for dietary supplements, see section 3.10.20).

Official substances are prepared according to recognized principles of good manufacturing practice and from ingredients complying with specifications designed to ensure that the resultant substances meet the requirements of the compendial monographs.

### **3.10.10. Applicability of Standards to Drug Products, Drug Substances, and Excipients**

The applicable *USP* or *NF* standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient in a drug. The applicable standard applies to such articles whether or not the added designation "*USP*" or "*NF*" is used. The standards apply equally to articles bearing the official titles or names derived by transposition of the definitive words of official titles or transposition in the order of the names of two or more active ingredients in official titles, or where there is use of synonyms with the intent or effect of suggesting a significant degree of identity with the official title or name.

### **3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients**

An article recognized in *USP* or *NF* shall comply with the compendial standards if the article is a medical device, component intended for a medical device, dietary supplement, dietary ingredient, or other ingredient that is intended for incorporation into a dietary supplement, and is labeled as conforming to the *USP* or *NF*.

Generally, dietary supplements are prepared from ingredients that meet *USP*, *NF*, or *Food Chemicals Codex* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be of acceptable food grade quality using other suitable procedures.

### **3.20. Indicating Conformance**

A drug product, drug substance, or excipient may use the designation "*USP*" or "*NF*" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article

complies with the identity prescribed in the specified compendium.

When a drug product, drug substance, or excipient differs from the relevant *USP* or *NF* standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label.

When a drug product, drug substance, or excipient fails to comply with the identity prescribed in *USP* or *NF* or contains an added substance that interferes with the prescribed tests and procedures, the article shall be designated by a name that is clearly distinguishing and differentiating from any name recognized in *USP* or *NF*.

A medical device, dietary supplement, or ingredient or component of a medical device or dietary supplement may use the designation "*USP*" or "*NF*" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the monograph standards and other applicable standards in the compendium.

The designation "*USP*" or "*NF*" on the label may not and does not constitute an endorsement by *USP* and does not represent assurance by *USP* that the article is known to comply with the relevant standards. *USP* may seek legal redress if an article purports to be or is represented as an official article in one of *USP*'s compendia and such claim is determined by *USP* not to be made in good faith.

The designation "*USP-NF*" may be used on the label of an article provided that the label also bears a statement such as "*Meets NF standards as published by USP,*" indicating the particular compendium to which the article purports to apply.

When the letters "*USP*," "*NF*," or "*USP-NF*" are used on the label of an article to indicate compliance with compendial standards, the letters shall appear in conjunction with the official title of the article. The letters are not to be enclosed in any symbol such as a circle, square, etc., and shall appear in capital letters.

If a dietary supplement does not comply with all applicable compendial requirements but contains one or more dietary ingredients or other ingredients that are recognized in *USP* or *NF*, the individual ingredient(s) may be designated as complying with *USP* or *NF* standards or being of *USP* or *NF* quality provided that the designation is limited to the individual ingredient(s) and does not suggest that the dietary supplement complies with *USP* standards.

## **4. MONOGRAPHS AND GENERAL CHAPTERS**

### **4.10. Monographs**

Monographs set forth the article's name, definition, specification, and other requirements related to packaging, storage, and labeling. The specification consists of tests, procedures, and acceptance criteria that help ensure the identity, strength, quality, and purity of the article. For general requirements relating to specific monograph sections, see section 5, *Monograph Components*.

Because monographs may not provide standards for all relevant characteristics, some official substances may conform to the *USP* or *NF* standard but differ with regard to nonstandardized properties that are relevant to their use in specific preparations. To assure interchangeability in such instances, users may wish to ascertain functional equivalence or determine such characteristics before use.

### **4.10.10. Applicability of Test Procedures**

A single monograph may include several different tests, procedures, and/or acceptance criteria that reflect attributes of different manufacturers' articles. Such alternatives may be presented for different polymorphic forms, impurities, hydrates, and dissolution cases. Monographs indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling.

A test in a monograph may contain and require multiple procedures. However, multiple procedures may be included in particular monographs specifically for the purpose of assuring the availability of an appropriate procedure for a par-



ticular product. In such cases, a labeling statement to indicate the appropriate application of the procedure(s) will be included in the monograph. A labeling statement is not required if Test 1 is used.

#### 4.10.20. Acceptance Criteria

The acceptance criteria allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. The existence of compendial acceptance criteria does not constitute a basis for a claim that an official substance that more nearly approaches 100 percent purity "exceeds" compendial quality. Similarly, the fact that an article has been prepared to tighter criteria than those specified in the monograph does not constitute a basis for a claim that the article "exceeds" the compendial requirements.

An official product shall be formulated with the intent to provide 100 percent of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, the upper acceptance criterion contained in the monograph may be increased by a corresponding amount.

The acceptance criteria specified in individual monographs and in the general chapters for compounded preparations are based on such attributes of quality as might be expected to characterize an article compounded from suitable bulk drug substances and ingredients, using the procedures provided or recognized principles of good compounding practice, as described in these compendia.

#### 4.20. General Chapters

Each general chapter is assigned a number that appears in angle brackets adjacent to the chapter name (e.g., *Chromatography* (621)). General chapters may contain the following:

- Descriptions of tests and procedures for application through individual monographs,
- Descriptions and specifications of conditions and practices for pharmaceutical compounding,
- General information for the interpretation of the compendial requirements,
- Descriptions of general pharmaceutical storage, dispensing, and packaging practices, or
- General guidance to manufacturers of official substances or official products.

When a general chapter is referenced in a monograph, acceptance criteria may be presented after a colon.

Some chapters may serve as introductory overviews of a test or of analytical techniques. They may reference other general chapters that contain techniques, details of the procedures, and, at times, acceptance criteria.

### 5. MONOGRAPH COMPONENTS

#### 5.10. Molecular Formula

The use of the molecular formula for the active ingredient(s) named in defining the required strength of a compendial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 percent) purity.

#### 5.20. Added Substances

Added substances are presumed to be unsuitable for inclusion in an official article and therefore prohibited, if: (1) they exceed the minimum quantity required for providing their intended effect; (2) their presence impairs the bioavailability, therapeutic efficacy, or safety of the official article; or (3) they interfere with the assays and tests prescribed for determining compliance with the compendial standards.

The air in a container of an official article may, where appropriate, be evacuated or be replaced by carbon dioxide, helium, argon, or nitrogen, or by a mixture of these gases. The use of such gas need not be declared in the labeling.

#### 5.20.10. Added Substances, Excipients, and Ingredients in Official Substances

Official substances may contain only the specific added substances that are permitted by the individual monograph. Where such addition is permitted, the label shall indicate the name(s) and amount(s) of any added substance(s).

#### 5.20.20. Added Substances, Excipients, and Ingredients in Official Products

Suitable substances and excipients such as antimicrobial agents, pharmaceutical bases, carriers, coatings, flavors, preservatives, stabilizers, and vehicles may be added to an official product to enhance its stability, usefulness, or elegance, or to facilitate its preparation, unless otherwise specified in the individual monograph.

Added substances and excipients employed solely to impart color may be incorporated into official products other than those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colors issued by the U.S. Food and Drug Administration (FDA), provided such added substances or excipients are otherwise appropriate in all respects. (See also *Added Substances under Injections* (1).)

The proportions of the substances constituting the base in ointment and suppository products and preparations may be varied to maintain a suitable consistency under different climatic conditions, provided that the concentrations of active ingredients are not varied and provided that the bioavailability, therapeutic efficacy, and safety of the preparation are not impaired.

#### 5.20.20.1. In Compounded Preparations

Compounded preparations for which a complete composition is given shall contain only the ingredients named in the formulas unless specifically exempted herein or in the individual monograph. Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the relevant standards and to preparations produced by following the specified process.

Where a monograph for a compounded preparation calls for an ingredient in an amount expressed on the dried basis, the ingredient need not be dried before use if due allowance is made for the water or other volatile substances present in the quantity taken.

Specially denatured alcohol formulas are available for use in accordance with federal statutes and regulations of the Internal Revenue Service. A suitable formula of specially denatured alcohol may be substituted for Alcohol in the manufacture of official preparations intended for internal or topical use, provided that the denaturant is volatile and does not remain in the finished product. A preparation that is intended for topical application to the skin may contain specially denatured alcohol, provided that the denaturant is either a usual ingredient in the preparation or a permissible added substance; in either case the denaturant shall be identified on the label of the topical preparation. Where a process is given in the individual monograph, any preparation compounded using denatured alcohol shall be identical to that prepared by the monograph process.

#### 5.20.20.2. In Dietary Supplements

Additional ingredients may be added to dietary supplement products provided that the additional ingredients: (1) comply with applicable regulatory requirements; and (2) do not interfere with the assays and tests prescribed for determining compliance with compendial standards.

#### 5.30. Description and Solubility

Only where a quantitative solubility test is given in a monograph and is designated as such is it a test for purity.

A monograph may include information regarding the article's description. Information about an article's "description and solubility" also is provided in the reference table *Description and Relative Solubility of USP and NF Articles*. The reference table merely denotes the properties of articles that comply with monograph standards. The reference table is

intended primarily for those who use, prepare, and dispense drugs and/or related articles. Although the information provided in monographs and the information in the reference table may indirectly assist in the preliminary evaluation of an article, it is not intended to serve as a standard or test for purity.

The approximate solubility of a compendial substance is indicated by one of the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble, or Insoluble	Greater than or equal to 10,000

#### 5.40. Identity

A compendial test titled *Identity* or *Identification* is provided as an aid in verifying the identity of articles as they are purported to be, e.g., those taken from labeled containers, and to establish whether it is the article named in *USP–NF*. The *Identity* or *Identification* test for a particular article may consist of one or more procedures. When a compendial test for *Identity* or *Identification* is undertaken, all requirements of all specified procedures in the test must be met to satisfy the requirements of the test. Failure of an article to meet all the requirements of a prescribed *Identity* or *Identification* test (i.e., failure to meet the requirements of all of the specified procedures that are components of that test) indicates that the article is mislabeled and/or adulterated.

#### 5.50. Assay

Assay tests for compounded preparations are not intended for evaluating a compounded preparation before dispensing, but instead are intended to serve as the official test in the event of a question or dispute regarding the preparation's conformance to official standards.

##### 5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical and physical means, it may be necessary to express quantities of activity in biological units of potency, each defined by an authoritative, designated reference standard.

Units of biological potency defined by the World Health Organization (WHO) for International Biological Standards and International Biological Reference Preparations are termed International Units (IU). Monographs refer to the units defined by USP Reference Standards as "USP Units." For biological products, units of potency are defined by the corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* <1041>).

#### 5.60. Impurities and Foreign Substances

Tests for the presence of impurities and foreign substances are provided to limit such substances to amounts that are unobjectionable under conditions in which the article is customarily employed (see also *Impurities in Official Articles* <1086>).

Nonmonograph tests and acceptance criteria suitable for detecting and controlling impurities that may result from a change in the processing methods or that may be introduced from external sources should be employed in addition to the tests provided in the individual monograph, where the presence of the impurity is inconsistent with applicable good manufacturing practices or good pharmaceutical practice.

#### 5.60.10. Other Impurities in USP and NF Articles

If a *USP* or *NF* monograph includes an assay or organic impurity test based on chromatography, other than a test for residual solvents, and that monograph procedure does not detect an impurity present in the substance, the amount and identity of the impurity, where both are known, shall be stated in the labeling (certificate of analysis) of the official substance, under the heading *Other Impurity(ies)*.

The presence of any unlabeled other impurity in an official substance is a variance from the standard if the content is 0.1% or greater. The sum of all *Other Impurities* combined with the monograph-detected impurities may not exceed 2.0% (see *Ordinary Impurities* <466>), unless otherwise stated in the monograph.

The following categories of drug substances are excluded from *Other Impurities* requirements:

- fermentation products and semi-synthetics derived therefrom,
- radiopharmaceuticals,
- biologics,
- biotechnology-derived products,
- peptides,
- herbals, and
- crude products of animal or plant origin.

Any substance known to be toxic shall not be listed under *Other Impurities*.

#### 5.60.20. Residual Solvents in USP and NF Articles

All *USP* and *NF* articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in *Residual Solvents* <467>, using the general methods presented therein or other suitable methods.

#### 5.70. Performance Tests

Where content uniformity determinations have been made using the same analytical methodology specified in the Assay, with appropriate allowances made for differences in sample preparation, the average of all of the individual content uniformity determinations may be used as the Assay value.

#### 5.80. USP Reference Standards

USP Reference Standards are authentic specimens that have been approved as suitable for use as comparison standards in *USP* or *NF* tests and assays. (See *USP Reference Standards* <11>.) Where a procedure calls for the use of a compendial article rather than for a USP Reference Standard as a material standard of reference, a substance meeting all of the compendial monograph requirements for that article shall be used. If any new *USP* or *NF* standard requires the use of a new USP Reference Standard that is not yet available, that portion of the standard containing the requirement shall not be official until the specified USP reference material is available.

Unless a reference standard label bears a specific potency or content, assume the reference standard is 100.0% pure in the official application. Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP Reference Standards are to be used in accordance with the instructions on the label of the Reference Standard.

### 6. TESTING PRACTICES AND PROCEDURES

#### 6.10. Safe Laboratory Practices

In performing compendial procedures, safe laboratory practices shall be followed, including precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any procedure described in the compendia, the analyst should be aware of the hazards associated with the chemicals and the techniques and means of protecting against them. These compendia are not designed to describe such hazards or protective measures.

## 6.20. Automated Procedures

Automated and manual procedures employing the same basic chemistry are considered equivalent.

## 6.30. Alternative and Harmonized Methods and Procedures

Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in the general chapter *Validation of Compendial Procedures* (1225) and must be shown to give equivalent or better results. Only those results obtained by the methods and procedures given in the compendium are conclusive.

Alternative procedures should be submitted to USP for evaluation as a potential replacement or addition to the standard (see section 4.10, *Monographs*).

Certain general chapters contain a statement that the text in question is harmonized with the corresponding text of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* and that these texts are interchangeable. Therefore, if a substance or preparation is found to comply with a requirement using an interchangeable method or procedure from one of these pharmacopoeias, it should comply with the requirements of the *USP*. When a difference appears, or in the event of dispute, only the result obtained by the method and/or procedure given in the *USP* is conclusive.

## 6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis

All calculations in the compendia assume an "as-is" basis unless otherwise specified.

Test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for *Loss on Drying*, or *Water*, or *Loss on Ignition*, respectively, is given in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

The term "solvent-free" signifies that the calculation shall be corrected for the presence of known solvents as determined using the methods described in *Residual Solvents* (467) unless a test for limit of organic solvents is provided in the monograph.

The term "previously dried" without qualification signifies that the substance shall be dried as directed under *Loss on Drying* (731) or *Water Determination* (921) (gravimetric determination).

Where drying in vacuum over a desiccant is directed, a vacuum desiccator, a vacuum drying pistol, or other suitable vacuum drying apparatus shall be used.

### 6.40.10. Ignite To Constant Weight

"Ignite to constant weight" means that ignition shall be continued at  $800 \pm 25^\circ$ , unless otherwise indicated, until two consecutive weighings, the second of which is taken after an additional period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

### 6.40.20. Dried To Constant Weight

"Dried to constant weight" means that drying shall be continued until two consecutive weighings, the second of which is taken after an additional drying period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

## 6.50. Preparation of Solutions

### 6.50.10. Filtration

Where a procedure gives direction to "filter" without further qualification, the liquid shall be passed through suitable filter paper or equivalent device until the filtrate is clear. Due to the possibility of filter effects, the initial volumes of a filtrate may be discarded.

### 6.50.20. Solutions

Unless otherwise specified, all solutions shall be prepared with Purified Water. Solutions for quantitative measures shall

be prepared using accurately weighed or accurately measured analytes (see section 8.20, *About*).

An expression such as "(1 in 10)" means that 1 part *by volume* of a liquid shall be diluted with, or 1 part *by weight* of a solid shall be dissolved in, a sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts *by volume*. An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids shall be mixed, unless otherwise indicated.

### 6.50.20.1. Adjustments to Solutions

When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement.

Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the special case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument.

When adjustment of pH is indicated with either an acid or base and the concentration is not indicated, appropriate concentrations of that acid or base may be used.

### 6.50.20.2. Test Solutions

Information on Test Solutions (TS) is provided in the *Test Solutions* portion of the *Reagents, Indicators, and Solutions* section of the *USP-NF*. Use of an alternative Test Solution or a change in the Test Solution used may require validation.

### 6.50.20.3. Indicator Solutions

Where a procedure specifies the use of an indicator TS, approximately 0.2 mL, or 3 drops, of the solution shall be added unless otherwise directed.

## 6.60. Units Necessary to Complete a Test

Unless otherwise specified, a sufficient number of units to ensure a suitable analytical result shall be taken.

### 6.60.10. Tablets

Where the procedure of a Tablet monograph directs to weigh and finely powder not fewer than a given number of Tablets, a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered Tablets taken shall be representative of the whole Tablets and shall, in turn, be weighed accurately.

### 6.60.20. Capsules

Where the procedure of a Capsule monograph gives direction to remove, as completely as possible, the contents of not fewer than a given number of the Capsules, a counted number of Capsules shall be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken shall be representative of the contents of the Capsules and shall, in turn, be weighed accurately.

## 6.70. Reagents

The proper conduct of the compendial procedures and the reliability of the results depend, in part, upon the quality of the reagents used in the performance of the procedures. Unless otherwise specified, reagents conforming to the specifications set forth in the current edition of *Reagent Chemicals* published by the American Chemical Society (ACS) shall be used. Where such ACS reagent specifications are not available or where the required purity differs, compendial specifications for reagents of acceptable quality are provided (see the *Reagents, Indicators, and Solutions* section of the *USP-NF*). Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Listing of these reagents, including the indicators and solutions employed as reagents, in no way implies that they have therapeutic utility; furthermore, any reference to *USP* or *NF* in their labeling shall include also the term "reagent"

or “reagent grade.” USP may supply reagents if they otherwise may not be generally commercially available.

#### 6.80. Equipment

Unless otherwise specified, a specification for a definite size or type of container or apparatus in a procedure is given solely as a recommendation. Other dimensions or types may be used if they are suitable for the intended use.

##### 6.80.10. Apparatus for Measurement

Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed.

##### 6.80.10.1. Pipet

Where a pipet is specified, a suitable buret may be substituted. Where a “to contain” pipet is specified, a suitable volumetric flask may be substituted.

##### 6.80.10.2. Light Protection

Where low-actinic or light-resistant containers are specified, either containers specially treated to protect contents from light or clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

##### 6.80.20. Instrumental Apparatus

An instrument may be substituted for the specified instrument if the substitute uses the same fundamental principles of operation and is of equivalent or greater sensitivity and accuracy. These characteristics shall be qualified as appropriate. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

##### 6.80.20.1. Chromatographic Tubes and Columns

The term “diameter” refers to internal diameter (ID).

##### 6.80.20.2. Tubing

The term “diameter” refers to outside diameter (OD).

##### 6.80.20.3. Steam Bath

Where use of a steam bath is directed, use actively flowing steam or another regulated heat source controlled at an equivalent temperature.

##### 6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

### 7. TEST RESULTS

#### 7.10. Interpretation of Requirements

Analytical results observed in the laboratory (or calculated from experimental measurements) are compared with stated acceptance criteria to determine whether the article conforms to compendial requirements.

The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable value is the end result of a completed measurement procedure, as documented.

Where acceptance criteria are expressed numerically herein through specification of an upper and/or lower limit, permitted values include the specified values themselves, but no values outside the limit(s). Acceptance criteria are considered significant to the last digit shown.

#### 7.10.5. Nominal Concentrations in Equations

Where a “nominal concentration” is specified, calculate the concentration based on the label claim. In assay procedures, water correction is typically stated in the Definition and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

#### 7.10.10. Equivalence Statements in Titrimetric Procedures

The directions for titrimetric procedures conclude with a statement of the weight of the analyte that is equivalent to each mL of the standardized titrant. In such an equivalence statement, the number of significant figures in the concentration of the titrant should be understood to correspond to the number of significant figures in the weight of the analyte. Corrections to calculations based on the blank determination are to be made for all titrimetric assays where appropriate (see *Titrimetry* (541)).

#### 7.20. Rounding Rules

The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

### 8. TERMS AND DEFINITIONS

#### 8.10. Abbreviations

- RS refers to a USP Reference Standard.
- CS refers to a Colorimetric Solution.
- TS refers to a Test Solution.
- VS refers to a Volumetric Solution that is standardized in accordance with directions given in the individual monograph or in the *Reagents, Indicators, and Solutions* section of USP–NF.

#### 8.20. About

“About” indicates a quantity within 10%.

If the measurement is stated to be “accurately measured” or “accurately weighed,” follow the statements in the gen-

Illustration of Rounding Numerical Values for Comparison with Requirements			
Compendial Requirement	Unrounded Value	Rounded Result	Conforms
Assay limit $\geq 98.0\%$	97.96%	98.0%	Yes
	97.92%	97.9%	No
	97.95%	98.0%	Yes
Assay limit $\leq 101.5\%$	101.55%	101.6%	No
	101.46%	101.5%	Yes
	101.45%	101.5%	Yes
Limit test $\leq 0.02\%$	0.025%	0.03%	No
	0.015%	0.02%	Yes
	0.027%	0.03%	No
Limit test $\leq 3$ ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes

eral chapters *Volumetric Apparatus* (31) and *Weights and Balances* (41), respectively.

### 8.30. Alcohol Content

Percentages of alcohol, such as those under the heading *Alcohol Content*, refer to percentage by volume of  $C_2H_5OH$  at 15.56°. Where a formula, test, or assay calls for alcohol, ethyl alcohol, or ethanol, the *USP* monograph article Alcohol shall be used. Where reference is made to " $C_2H_5OH$ ," absolute (100 percent) ethanol is intended. Where a procedure calls for dehydrated alcohol, alcohol absolute, or anhydrous alcohol, the *USP* monograph article Dehydrated Alcohol shall be used.

### 8.40. Atomic Weights

Atomic weights used in computing molecular weights and the factors in the assays and elsewhere are those established by the IUPAC Commission on Atomic Weights and Isotopic Abundances.

### 8.50. Blank Determinations

Where it is directed that "any necessary correction" be made by a blank determination, the determination shall be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

### 8.60. Concomitantly

"Concomitantly" denotes that the determinations or measurements are to be performed in immediate succession.

### 8.70. Desiccator

The instruction "in a desiccator" indicates use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of a suitable desiccant such as anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, or silica gel. See also section 8.220, *Vacuum Desiccator*.

### 8.80. Logarithms

Logarithms are to the base 10.

### 8.90. Microbial Strain

A microbial strain cited and identified by its ATCC catalog number shall be used directly or, if subcultured, shall be used not more than five passages removed from the original strain.

### 8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

### 8.110. NLT/NMT

"NLT" means "not less than." "NMT" means "not more than."

### 8.120. Odor

"Odorless," "practically odorless," "a faint characteristic odor," and variations thereof indicate evaluation of a suitable quantity of freshly opened material after exposure to the air for 15 minutes. An odor designation is descriptive only and should not be regarded as a standard of purity for a particular lot of an article.

### 8.130. Percent

"Percent" used without qualification means:

- For mixtures of solids and semisolids, percent weight in weight;
- For solutions or suspensions of solids in liquids, percent weight in volume;
- For solutions of liquids in liquids, percent volume in volume;
- For solutions of gases in liquids, percent weight in volume.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

### 8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

- *Percent Weight in Weight (w/w)* is defined as the number of g of a solute in 100 g of solution.

- *Percent Weight in Volume (w/v)* is defined as number of g of a solute in 100 mL of solution.
- *Percent Volume in Volume (v/v)* is defined as the number of mL of a solute in 100 mL of solution.

### 8.150. Pressure

Pressure is determined by use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

### 8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

### 8.170. Specific Gravity

Specific gravity is the weight of a substance in air at 25° divided by the weight of an equal volume of water at the same temperature.

### 8.180. Temperatures

Temperatures are expressed in centigrade (Celsius) degrees, and all measurements are made at 25° unless otherwise indicated. Where moderate heat is specified, any temperature not higher than 45° (113° F) is indicated.

### 8.190. Time

Unless otherwise specified, rounding rules, as described in section 7.20, *Rounding Rules*, apply to any time specified.

### 8.200. Transfer

"Transfer" indicates a quantitative manipulation.

### 8.210. Vacuum

"Vacuum" denotes exposure to a pressure of less than 20 mm of mercury (2.67 kPa), unless otherwise indicated.

### 8.220. Vacuum Desiccator

"Vacuum desiccator" indicates a desiccator that maintains a low-moisture atmosphere at a reduced pressure of not more than 20 mm of mercury (2.67 kPa) or at the pressure designated in the individual monograph.

### 8.230. Water

#### 8.230.10. Water as an Ingredient in an Official Product

As an ingredient in an official product, water meets the requirements of the appropriate water monograph in *USP* or *NF*.

#### 8.230.20. Water in the Manufacture of Official Substances

When used in the manufacture of official substances, water may meet the requirements for drinking water as set forth in the regulations of the U.S. Environmental Protection Agency (potable water).

#### 8.230.30. Water in a Compendial Procedure

When water is called for in a compendial procedure, the *USP* article Purified Water shall be used unless otherwise specified. Definitions for *High-Purity Water* and *Carbon Dioxide-Free Water* are provided in *Containers—Glass* (660). Definitions of other types of water are provided in *Water for Pharmaceutical Purposes* (1231).

### 8.240. Weights and Measures

In general, weights and measures are expressed in the International System of Units (SI) as established and revised by the *Conférence générale des poids et mesures*. For compendial purposes, the term "weight" is considered to be synonymous with "mass."

Molality is designated by the symbol *m* preceded by a number that represents the number of moles of the designated solute contained in 1 kilogram of the designated solvent.

Molarity is designated by the symbol *M* preceded by a number that represents the number of moles of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

Normality is designated by the symbol *N* preceded by a number that represents the number of equivalents of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

Symbols commonly employed for SI metric units and other units are as follows:

Bq = becquerel	dL = deciliter
kBq = kilobecquerel	L = liter
MBq = megabecquerel	mL = milliliter <sup>c</sup>
GBq = gigabecquerel	μL = microliter
Ci = curie	Eq = gram-equivalent weight
mCi = millicurie	mEq = milliequivalent
μCi = microcurie	mol = gram-molecular weight (mole)
nCi = nanocurie	Da = dalton (relative molecular mass)
Gy = gray	mmol = millimole
mGy = milligray	Osmol = osmole
m = meter	mOsmol = milliosmole
dm = decimeter	Hz = hertz
cm = centimeter	kHz = kilohertz
mm = millimeter	MHz = megahertz
μm = micrometer (0.001 mm)	V = volts
nm = nanometer <sup>a</sup>	MeV = million electron volts
kg = kilogram	keV = kilo-electron volt
g = gram	mV = millivolt
mg = milligram	psi = pounds per square inch
μg; mcg = microgram <sup>b</sup>	Pa = pascal
ng = nanogram	kPa = kilopascal
pg = picogram	g = gravity (in centrifugation)
fg = femtogram	

<sup>a</sup> Previously the symbol mμ (for millimicron) was used.

<sup>b</sup> The symbol μg is used in the *USP* and *NF* to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ, frequently is used to represent micrograms in biochemical literature.

<sup>c</sup> One milliliter (mL) is used herein as the equivalent of one cubic centimeter (cc).

## 9. PRESCRIBING AND DISPENSING

### 9.10 Use of Metric Units

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph (see also *Units of Potency*, section 5.50.10 above). If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the prescribed amount shall be dispensed. Apothecary unit designations on labels and labeling shall not be used.

### 9.20 Changes in Volume

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

## 10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

### 10.10. Storage Under Nonspecific Conditions

If no specific directions or limitations are provided in the *Packaging and Storage* section of an individual *USP* monograph or in the labeling of an article recognized in *USP*, the conditions of storage shall include storage at controlled room temperature, protection from moisture, and, where necessary, protection from light. Such articles shall be protected from moisture, freezing, and excessive heat, and, where necessary, from light during shipping and distribution. Drug substances are exempt from the requirements in this paragraph.

Regardless of quantity, where no specific storage directions or limitations are provided in an individual *NF* monograph or stated in the labeling of an article recognized in *NF*, the conditions of storage and distribution shall include protection from moisture, freezing, excessive heat, and, where necessary, from light.

### 10.20. Containers

The container is that which holds the article and is or may be in direct contact with the article. The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container.

Before being filled, the container should be clean. Special precautions and cleaning procedures may be necessary to ensure that each container is clean and that extraneous matter is not introduced into or onto the article.

The container does not interact physically or chemically with the article placed in it so as to alter the strength, quality, or purity of the article beyond the official requirements.

The compendial requirements for the use of specified containers apply also to articles as packaged by the pharmacist or other dispenser, unless otherwise indicated in the individual monograph.

#### 10.20.10. Tamper-Evident Packaging

The container or individual carton of a sterile article intended for ophthalmic or otic use, except where extemporaneously compounded for immediate dispensing on prescription, shall be so sealed that the contents cannot be used without obvious destruction of the seal.

Articles intended for sale without prescription are also required to comply with the tamper-evident packaging and labeling requirements of the FDA where applicable.

Preferably, the immediate container and/or the outer container or protective packaging used by a manufacturer or distributor for all dosage forms that are not specifically exempt is designed so as to show evidence of any tampering with the contents.

#### 10.20.20. Light-Resistant Container

A light-resistant container (see *Light Transmission Test* under *Containers—Performance Testing* (671)) protects the contents from the effects of light by virtue of the specific properties of the material of which it is composed, including any coating applied to it. Alternatively, a clear and colorless or a translucent container may be made light-resistant by means of an opaque covering, in which case the label of the container bears a statement that the opaque covering is needed until the contents are to be used or administered. Where it is directed to "protect from light" in an individual monograph, preservation in a light-resistant container is intended.

Where an article is required to be packaged in a light-resistant container, and if the container is made light-resistant by means of an opaque covering, a single-use, unit-dose container or mnemonic pack for dispensing may not be removed from the outer opaque covering before dispensing.

#### 10.20.30. Well-Closed Container

A well-closed container protects the contents from extraneous solids and from loss of the article under the ordinary or customary conditions of handling, shipment, storage, and distribution.

#### 10.20.40. Tight Container

A tight container protects the contents from contamination by extraneous liquids, solids, or vapors; from loss of the article; and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and distribution; and is capable of tight reclosure. Where a tight container is specified, it may be replaced by a hermetic container for a single dose of an article.

A gas cylinder is a metallic container designed to hold a gas under pressure. As a safety measure, for carbon dioxide, cyclopropane, helium, nitrous oxide, and oxygen, the Pin-Index Safety System of matched fittings is recommended for cylinders of Size E or smaller.

[NOTE—Where packaging and storage in a *tight container* or a *well-closed container* is specified in the individual monograph, the container used for an article when dispensed on prescription meets the requirements under *Containers—Performance Testing* (671).]

**10.20.50. Hermetic Container**

A hermetic container is impervious to air or any other gas under the ordinary or customary conditions of handling, shipment, storage, and distribution.

**10.20.60. Single-Unit Container**

A single-unit container is one that is designed to hold a quantity of drug product intended for administration as a single dose or a single finished device intended for use promptly after the container is opened. Preferably, the immediate container and/or the outer container or protective packaging shall be so designed as to show evidence of any tampering with the contents. Each single-unit container shall be labeled to indicate the identity, quantity and/or strength, name of the manufacturer, lot number, and expiration date of the article.

**10.20.70. Single-Dose Container**

A single-dose container is a single-unit container for articles intended for parenteral administration only. A single-dose container is labeled as such. Examples of single-dose containers include prefilled syringes, cartridges, fusion-sealed containers, and closure-sealed containers when so labeled. (See also *Containers for Injections* under *Injections* (1).)

**10.20.80. Unit-Dose Container**

A unit-dose container is a single-unit container for articles intended for administration by other than the parenteral route as a single dose, direct from the container.

**10.20.90. Unit-of-Use Container**

A unit-of-use container is one that contains a specific quantity of a drug product and that is intended to be dispensed as such without further modification except for the addition of appropriate labeling. A unit-of-use container is labeled as such.

**10.20.100. Multiple-Unit Container**

A multiple-unit container is a container that permits withdrawal of successive portions of the contents without changing the strength, quality, or purity of the remaining portion.

**10.20.110. Multiple-Dose Container**

A multiple-dose container is a multiple-unit container for articles intended for parenteral administration only. (See also *Containers for Injections* under *Injections* (1).)

**10.20.120. Requirements under the Poison Prevention Packaging Act (PPPA)**

This act (see the website, [www.cpsc.gov/businfo/pppa.html](http://www.cpsc.gov/businfo/pppa.html)) requires special packaging of most human oral prescription drugs, oral controlled drugs, certain non-oral prescription drugs, certain dietary supplements, and many over-the-counter (OTC) drug preparations in order to protect the public from personal injury or illness from misuse of these preparations (16 CFR § 1700.14).

The immediate packaging of substances regulated under the PPPA shall comply with the special packaging standards (16 CFR § 1700.15 and 16 CFR § 1700.20). The PPPA regulations for special packaging apply to all packaging types including reclosable, nonclosable, and unit-dose types.

Special packaging is not required for drugs dispensed within a hospital setting for inpatient administration. Manufacturers and packagers of bulk-packaged prescription drugs do not have to use special packaging if the drug will be repackaged by the pharmacist. PPPA-regulated prescription drugs may be dispensed in non-child-resistant packaging upon the request of the purchaser or when directed in a legitimate prescription (15 U.S.C. § 1473).

Manufacturers or packagers of PPPA-regulated OTC preparations are allowed to package one size in non-child-resistant packaging as long as popular-size, special packages are also supplied. The non-child-resistant package requires special labeling (16 CFR § 1700.5).

Various types of child-resistant packages are covered in ASTM International Standard D-3475, *Standard Classification of Child-Resistant Packaging*. Examples are included as an aid in the understanding and comprehension of each type of classification.

**10.30. Storage Temperature and Humidity**

Specific directions are stated in some monographs with respect to the temperatures and humidity at which official articles shall be stored and distributed (including the shipment of articles to the consumer) when stability data indicate that storage and distribution at a lower or a higher temperature and a higher humidity produce undesirable results. Such directions apply except where the label on an article states a different storage temperature on the basis of stability studies of that particular formulation. Where no specific storage directions or limitations are provided in the individual monograph, but the label of an article states a storage temperature that is based on stability studies of that particular formulation, such labeled storage directions apply. The conditions are defined by the following terms.

**10.30.10. Freezer**

"Freezer" indicates a place in which the temperature is maintained thermostatically between  $-25^{\circ}$  and  $-10^{\circ}$  ( $-13^{\circ}$  and  $14^{\circ}$ F).

**10.30.20. Cold**

Any temperature not exceeding  $8^{\circ}$  ( $46^{\circ}$ F) is "cold." A "refrigerator" is a cold place in which the temperature is maintained thermostatically between  $2^{\circ}$  and  $8^{\circ}$  ( $36^{\circ}$  and  $46^{\circ}$ F).

**10.30.30. Cool**

Any temperature between  $8^{\circ}$  and  $15^{\circ}$  ( $46^{\circ}$  and  $59^{\circ}$ F) is "cool." An article for which storage in a *cool place* is directed may, alternatively, be stored and distributed in a *refrigerator*, unless otherwise specified by the individual monograph.

**10.30.40. Controlled Cold Temperature**

"Controlled cold temperature" is defined as temperature maintained thermostatically between  $2^{\circ}$  and  $8^{\circ}$  ( $36^{\circ}$  and  $46^{\circ}$  F), that allows for excursions in temperature between  $0^{\circ}$  and  $15^{\circ}$  ( $32^{\circ}$  and  $59^{\circ}$  F) that may be experienced during storage, shipping, and distribution such that the allowable calculated mean kinetic temperature is not more than  $8^{\circ}$  ( $46^{\circ}$  F). Transient spikes up to  $25^{\circ}$  ( $77^{\circ}$  F) may be permitted if the manufacturer so instructs and provided that such spikes do not exceed 24 hours unless supported by stability data or the manufacturer instructs otherwise.

**10.30.50. Room Temperature**

"Room temperature" indicates the temperature prevailing in a working area.

**10.30.60. Controlled Room Temperature**

"Controlled room temperature" indicates a temperature maintained thermostatically that encompasses the usual and customary working environment of  $20^{\circ}$  to  $25^{\circ}$  ( $68^{\circ}$  to  $77^{\circ}$ F); that results in a mean kinetic temperature calculated to be not more than  $25^{\circ}$ ; and that allows for excursions between  $15^{\circ}$  and  $30^{\circ}$  ( $59^{\circ}$  and  $86^{\circ}$ F) that are experienced in pharmacies, hospitals, and warehouses. Provided the mean kinetic temperature remains in the allowed range, transient spikes up to  $40^{\circ}$  are permitted as long as they do not exceed 24 hours. Spikes above  $40^{\circ}$  may be permitted if the manufacturer so instructs. Articles may be labeled for storage at "controlled room temperature" or at "up to  $25^{\circ}$ ", or other wording based on the same mean kinetic temperature. The mean kinetic temperature is a calculated value that may be used as an isothermal storage temperature that simulates the nonisothermal effects of storage temperature variations.

An article for which storage at *controlled room temperature* is directed may, alternatively, be stored and distributed in a *cool place*, unless otherwise specified in the individual monograph or on the label.

**10.30.70. Warm**

Any temperature between  $30^{\circ}$  and  $40^{\circ}$  ( $86^{\circ}$  and  $104^{\circ}$ F) is "warm."

**10.30.80. Excessive Heat**

"Excessive heat" means any temperature above  $40^{\circ}$  ( $104^{\circ}$ F).

**10.30.90. Protection From Freezing**

Where, in addition to the risk of breakage of the container, freezing subjects an article to loss of strength or potency, or to destructive alteration of its characteristics, the container label bears an appropriate instruction to protect the article from freezing.

**10.30.100. Dry Place**

The term "dry place" denotes a place that does not exceed 40% average relative humidity at *Controlled Room Temperature* or the equivalent water vapor pressure at other temperatures. The determination may be made by direct measurement at the place or may be based on reported climatic conditions. Determination is based on not less than 12 equally spaced measurements that encompass either a season, a year, or, where recorded data demonstrate, the storage period of the article. There may be values of up to 45% relative humidity provided that the average value is 40% relative humidity.

Storage in a container validated to protect the article from moisture vapor, including storage in bulk, is considered storage in a dry place.

**10.40. Labeling**

The term "labeling" designates all labels and other written, printed, or graphic matter upon an immediate container of an article or upon, or in, any package or wrapper in which it is enclosed, except any outer shipping container. The term "label" designates that part of the labeling upon the immediate container.

A shipping container containing a single article, unless such container is also essentially the immediate container or the outside of the consumer package, is labeled with a minimum of product identification (except for controlled articles), lot number, expiration date, and conditions for storage and distribution.

Articles in these compendia are subject to compliance with such labeling requirements as may be promulgated by governmental bodies in addition to the compendial requirements set forth for the articles.

**10.40.10. Amount of Ingredient Per Dosage Unit**

The strength of a drug product is expressed on the container label in terms of micrograms or milligrams or grams or percentage of the therapeutically active moiety or drug substance, whichever form is used in the title, unless otherwise indicated in an individual monograph. Both the active moiety and drug substance names and their equivalent amounts are then provided in the labeling.

Official articles in capsule, tablet, or other unit dosage form shall be labeled to express the quantity of each active ingredient or recognized nutrient contained in each such unit; except that, in the case of unit-dose oral solutions or suspensions, whether supplied as liquid preparations or as liquid preparations that are constituted from solids upon addition of a designated volume of a specific diluent, the label shall express the quantity of each active ingredient or recognized nutrient delivered under the conditions prescribed in *Deliverable Volume* (698). Official drug products not in unit dosage form shall be labeled to express the quantity of each active ingredient in each milliliter or in each gram, or to express the percentage of each such ingredient (see 8.140., *Percentage Concentrations*), except that oral liquids or solids intended to be constituted to yield oral liquids may, alternatively, be labeled in terms of each 5-mL portion of the liquid or resulting liquid. Unless otherwise indicated in a monograph or chapter, such declarations of strength or quantity shall be stated only in metric units. See also 5.50.10., *Units of Potency (Biological)*.

**10.40.20. Use of Leading and Terminal Zeros**

To help minimize the possibility of errors in the dispensing and administration of drugs, the quantity of active ingredient when expressed in whole numbers shall be shown without a decimal point that is followed by a terminal zero (e.g., express as 4 mg [not 4.0 mg]). The quantity of active ingredient when expressed as a decimal number smaller than 1

shall be shown with a zero preceding the decimal point (e.g., express as 0.2 mg [not .2 mg]).

**10.40.30. Labeling of Salts of Drugs**

It is an established principle that official articles shall have only one official title. For purposes of saving space on labels, and because chemical symbols for the most common inorganic salts of drugs are well known to practitioners as synonymous with the written forms, the following alternatives are permitted in labeling official articles that are salts: HCl for hydrochloride; HBr for hydrobromide; Na for sodium; and K for potassium. The symbols Na and K are intended for use in abbreviating names of the salts of organic acids, but these symbols are not used where the word Sodium or Potassium appears at the beginning of an official title (e.g., Phenobarbital Na is acceptable, but Na Salicylate is not to be written).

**10.40.40. Labeling Vitamin-Containing Products**

The vitamin content of an official drug product shall be stated on the label in metric units per dosage unit. The amounts of vitamins A, D, and E may be stated also in USP Units. Quantities of vitamin A declared in metric units refer to the equivalent amounts of retinol (vitamin A alcohol). The label of a nutritional supplement shall bear an identifying lot number, control number, or batch number.

**10.40.50. Labeling Botanical-Containing Products**

The label of an herb or other botanical intended for use as a dietary supplement bears the statement, "If you are pregnant or nursing a baby, seek the advice of a health professional before using this product."

**10.40.60. Labeling Parenteral And Topical Preparations**

The label of a preparation intended for parenteral or topical use states the names of all added substances (see 5.20., *Added Substances, Excipients, and Ingredients* and see *Labeling* under *Injections* (1)), and, in the case of parenteral preparations, also their amounts or proportions, except that for substances added for adjustment of pH or to achieve isotonicity, the label may indicate only their presence and the reason for their addition.

**10.40.70. Labeling Electrolytes**

The concentration and dosage of electrolytes for replacement therapy (e.g., sodium chloride or potassium chloride) shall be stated on the label in milliequivalents (mEq). The label of the product shall indicate also the quantity of ingredient(s) in terms of weight or percentage concentration.

**10.40.80. Labeling Alcohol**

The content of alcohol in a liquid preparation shall be stated on the label as a percentage (v/v) of C<sub>2</sub>H<sub>5</sub>OH.

**10.40.90. Special Capsules and Tablets**

The label of any form of Capsule or Tablet intended for administration other than by swallowing intact bears a prominent indication of the manner in which it shall be used.

**10.40.100. Expiration Date and Beyond-Use Date**

The label of an official drug product or nutritional or dietary supplement product shall bear an expiration date. All articles shall display the expiration date so that it can be read by an ordinary individual under customary conditions of purchase and use. The expiration date shall be prominently displayed in high contrast to the background or sharply embossed, and easily understood (e.g., "EXP 6/08," "Exp. June 08," or "Expires 6/08"). [NOTE—For additional information and guidance, refer to the Consumer Healthcare Products Association's *Voluntary Codes and Guidelines of the Self-Medication Industry*.]

The monographs for some preparations state how the expiration date that shall appear on the label shall be determined. In the absence of a specific requirement in the individual monograph for a drug product or nutritional supplement, the label shall bear an expiration date assigned for the particular formulation and package of the article, with the following exception: the label need not show an expiration date in the case of a drug product or nutritional supplement packaged in a container that is intended for sale



without prescription and the labeling of which states no dosage limitations, and which is stable for not less than 3 years when stored under the prescribed conditions.

Where an official article is required to bear an expiration date, such article shall be dispensed solely in, or from, a container labeled with an expiration date, and the date on which the article is dispensed shall be within the labeled expiry period. The expiration date identifies the time during which the article may be expected to meet the requirements of the compendial monograph, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the article may be dispensed or used. Where an expiration date is stated only in terms of the month and the year, it is a representation that the intended expiration date is the last day of the stated month. The beyond-use date is the date after which an article shall not be used. The dispenser shall place on the label of the prescription container a suitable beyond-use date to limit the patient's use of the article based on any information supplied by the manufacturer and the *General Notices*. The beyond-use date placed on the label shall not be later than the expiration date on the manufacturer's container.

For articles requiring constitution before use, a suitable beyond-use date for the constituted product shall be identified in the labeling.

For all other dosage forms, in determining an appropriate period of time during which a prescription drug may be retained by a patient after its dispensing, the dispenser shall take into account, in addition to any other relevant factors, the nature of the drug; the container in which it was packaged by the manufacturer and the expiration date thereon; the characteristics of the patient's container, if the article is repackaged for dispensing; the expected storage conditions to which the article may be exposed; any unusual storage conditions to which the article may be exposed; and the expected length of time of the course of therapy. The dispenser shall, on taking into account the foregoing, place on the label of a multiple-unit container a suitable beyond-use date to limit the patient's use of the article. Unless otherwise specified in the individual monograph, or in the absence of stability data to the contrary, such beyond-use date shall be not later than (a) the expiration date on the manufacturer's container, or (b) 1 year from the date the drug is dispensed, whichever is earlier. For nonsterile solid and liquid dosage forms that are packaged in single-unit and unit-dose containers, the beyond-use date shall be 1 year from the date the drug is packaged into the single-unit or unit-dose container or the expiration date on the manufacturer's container, whichever is earlier, unless stability data or the manufacturer's labeling indicates otherwise.

The dispenser shall maintain the facility where the dosage forms are packaged and stored, at a temperature such that the mean kinetic temperature is not greater than 25°. The

plastic material used in packaging the dosage forms shall afford better protection than polyvinyl chloride, which does not provide adequate protection against moisture permeation. Records shall be kept of the temperature of the facility where the dosage forms are stored, and of the plastic materials used in packaging.

#### 10.40.100.1. Compounded Preparations

The label on the container or package of an official compounded preparation shall bear a beyond-use date. The beyond-use date is the date after which a compounded preparation is not to be used. Because compounded preparations are intended for administration immediately or following short-term storage, their beyond-use dates may be assigned based on criteria different from those applied to assigning expiration dates to manufactured drug products.

The monograph for an official compounded preparation typically includes a beyond-use requirement that states the time period following the date of compounding during which the preparation, properly stored, may be used. In the absence of stability information that is applicable to a specific drug and preparation, recommendations for maximum beyond-use dates have been devised for nonsterile compounded drug preparations that are packaged in tight, light-resistant containers and stored at controlled room temperature unless otherwise indicated (see *Stability Criteria and Beyond-Use Dating under Stability of Compounded Preparations* in the general test chapter *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

#### 10.50. Guidelines for Packaging and Storage Statements in USP–NF Monographs

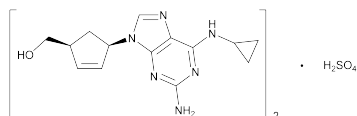
In order to provide users of the *USP* and *NF* with proper guidance on how to package and store official articles, every monograph in the *USP* and *NF* shall have a packaging and storage specification.

For the packaging portion of the statement, the choice of containers is given in this section 10, *Preservation, Packaging, Storage, and Labeling*, and includes *Light-Resistant Container*, *Well-Closed Container*, *Tight Container*, *Hermetic Container*, *Single-Unit Container*, *Single-Dose Container*, *Unit-Dose Container*, and *Unit-of-Use Container*. For most preparations, the choice is determined by the container in which it shall be dispensed (e.g., tight, well-closed, hermetic, unit-of-use, etc.). For drug substances, the choice would appear to be tight, well-closed, or, where needed, a light-resistant container. For excipients, given their typical nature as large-volume commodity items, with containers ranging from drums to tank cars, a well-closed container is an appropriate default. Therefore, in the absence of data indicating a need for a more protective class of container, the phrase "Preserve in well-closed containers" should be used as a default for excipients.



# Official Monographs for USP 36

## Abacavir Sulfate



$(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$  670.74  
2-Cyclopentene-1-methanol, 4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-, (1S-cis)-, sulfate (salt) (2:1);  
(1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1)  
[188062-50-2].

### DEFINITION

Abacavir Sulfate contains NLT 97.0% and NMT 102.0% of  $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ , calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, obtained as directed in the test for *Organic Impurities*, Procedure 2.
- C. IDENTIFICATION TESTS—GENERAL**, Sulfate <191>  
Sample solution: 5 mg/mL

### ASSAY

- PROCEDURE**  
Mobile phase: Acetonitrile, phosphoric acid, and water (20:1:180)  
Standard solution: 0.04 mg/mL of USP Abacavir Sulfate RS in water  
Sample solution: 0.04 mg/mL of Abacavir Sulfate in water  
Chromatographic system  
(See *Chromatography* <621>, *System Suitability*.)  
Mode: LC  
Detector: UV 254 nm  
Column: 4.6-mm × 5-cm; 5-μm packing L1  
Column temperature: 30°  
Flow rate: 1 mL/min  
Injection size: 20 μL  
System suitability  
Sample: *Standard solution*  
Suitability requirements  
Relative standard deviation: NMT 1.5%  
Analysis  
Samples: *Standard solution* and *Sample solution*  
Calculate the percentage of  $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$  in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of abacavir from the *Sample solution*  
 $r_S$  = peak area of abacavir from the *Standard solution*  
 $C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Abacavir Sulfate in the *Sample solution* (mg/mL)  
Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** <281>: NMT 0.2%

#### Organic Impurities

- PROCEDURE 1: RELATED COMPOUNDS**

Solution A: Trifluoroacetic acid and water (0.05:99.95)

Solution B: Methanol and water (17:3)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
35.1	95	5
50	95	5

System suitability solution: 0.25 mg/mL of USP Abacavir Related Compounds Mixture RS in water

Sample solution: 0.25 mg/mL of Abacavir Sulfate in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 μL

#### System suitability

Sample: *System suitability solution*

#### Suitability requirements

Resolution: NLT 1.5 between abacavir and *trans*-abacavir

#### Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_T$  = sum of the areas of all the peaks from the *Sample solution*

**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 0.8%**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Descyclopropyl abacavir <sup>a</sup>	0.65	0.2
Abacavir	1.00	—
<i>trans</i> -Abacavir <sup>b</sup>	1.04	0.2
O-Pyrimidine derivative abacavir <sup>c</sup>	1.33	0.2
<i>t</i> -Butyl derivative abacavir <sup>d</sup>	1.67	0.2
Any unspecified impurity	—	0.1

<sup>a</sup> [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.<sup>b</sup> {(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-cyclopent-2-enyl}methanol.<sup>c</sup> *N*<sup>6</sup>-Cyclopropyl-9-[(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yl-oxy)methyl]cyclopent-2-enyl]-9*H*-purine-2,6-diamine.<sup>d</sup> 9-[(1*R*,4*S*)-4-(*tert*-Butoxymethyl)cyclopent-2-enyl]-*N*<sup>6</sup>-cyclopropyl-9*H*-purine-2,6-diamine.**PROCEDURE 2: ENANTIOMERIC PURITY****Solution A:** Heptane, 2-propanol, and diethylamine (850:150:1).**Solution B:** Heptane and 2-propanol (1:1)**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	1.0
25	100	0	1.0
27	0	100	0.8
37	0	100	0.8
39	100	0	1.0
55	100	0	1.0

**Diluent:** Methanol and trifluoroacetic acid (200:1)**System suitability solution:** Transfer a quantity of USP Abacavir Stereoisomers Mixture RS to a suitable volumetric flask, add a volume of *Diluent* equivalent to 30% of the final volume, and sonicate until the solid is fully dissolved. Add a volume of 2-propanol equivalent to about 30% of the final volume, mix, and dilute with heptane to volume to obtain 0.4 mg/mL of USP Abacavir Stereoisomers Mixture RS.**Sample solution:** Transfer 4 mg of Abacavir Sulfate to a 10-mL volumetric flask. Add 3 mL of *Diluent*, and sonicate until the solid is fully dissolved. Add 3 mL of 2-propanol, mix, and dilute with heptane to volume.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 286 nm**Column:** 4.6-mm × 25-cm; 10-μm packing L51**Column temperature:** 30°**Injection size:** 20 μL**System suitability**[NOTE—The relative retention times for *trans*-abacavir, abacavir enantiomer, and abacavir are 0.8, 0.9, and 1.0, respectively.]**Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 1.0 between *trans*-abacavir and abacavir enantiomer; NLT 1.5 between abacavir enantiomer and abacavir**Analysis****Sample:** *Sample solution*

Calculate the percentage of abacavir enantiomer in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak area of abacavir enantiomer from the *Sample solution* $r_T$  = total peak areas of abacavir and abacavir enantiomer from the *Sample solution***Acceptance criteria****Individual impurities:** NMT 0.3% of abacavir enantiomer**SPECIFIC TESTS**

- WATER DETERMINATION, Method 1c <921>:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

**USP REFERENCE STANDARDS <11>**

USP Abacavir Sulfate RS

USP Abacavir Stereoisomers Mixture RS

A mixture of abacavir sulfate, abacavir enantiomer, and *trans*-abacavir.

USP Abacavir Related Compounds Mixture RS

A mixture of abacavir glutarate, O-pyrimidine derivative abacavir, descyclopropyl abacavir, *trans*-abacavir, and *t*-butyl derivative abacavir.**Abacavir Oral Solution****DEFINITION**Abacavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O).**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE****Solution A:** Trifluoroacetic acid and water (0.05:99.95)**Solution B:** Methanol and water (17:3)**Diluent:** 1 mL of phosphoric acid diluted with water to 1000 mL**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	0	100
50	0	100
51	95	5
55	95	5

**System suitability solution:** 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent***Standard solution:** 0.46 mg/mL of USP Abacavir Sulfate RS in *Diluent***Sample solution:** Equivalent to 0.4 mg/mL of abacavir in *Diluent*, from Oral Solution. [NOTE—Sonicate, if necessary.]

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 15-cm; 5-μm packing L1**Column temperature:** 30°**Flow rate:** 0.8 mL/min**Injection size:** 10 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak area of abacavir from the *Sample solution* $r_S$  = peak area of abacavir from the *Standard solution* $C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66 $M_{r2}$  = molecular weight of abacavir sulfate, 670.74**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **DELIVERABLE VOLUME** <698>: Meets the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE**

Solution A, Solution B, Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Sensitivity solution:** 0.2 μg/mL of USP Abacavir Sulfate RS in *Diluent*, from the *Standard solution*. [NOTE—The concentration of this solution is 0.05% of the nominal concentration of the *Sample solution*.]**Analysis****Samples:** *Diluent*, *Standard solution*, *Sample solution*, and *Sensitivity solution*. [NOTE— In the *Sample solution* disregard any peaks corresponding to peaks identified in the *Diluent* and any peak with a peak area less than the abacavir peak area in the *Sensitivity solution*.]

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak area of abacavir from the *Sample solution* $r_S$  = peak area of abacavir from the *Standard solution* $C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL) $F$  = relative response factor for each impurity from *Impurity Table 1* $M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66 $M_{r2}$  = molecular weight of abacavir sulfate, 670.74**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 2.0%**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropylidaminopurine abacavir <sup>a</sup>	0.57	1.4	0.3
Descyclopropyl abacavir <sup>b</sup>	0.68	1.0	0.8
Abacavir	1.00	—	—
<i>trans</i> -Abacavir <sup>c</sup>	1.04	1.0	—
Any individual unspecified impurity	—	1.0	0.2

<sup>a</sup> N<sup>6</sup>-Cyclopropyl-9H-purine-2,6-diamine.<sup>b</sup> [(1S,4R)-4-(2,6-Diamino-9H-purin-9-yl)cyclopent-2-enyl]methanol.<sup>c</sup> [(1R,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-cyclopent-2-enyl]methanol. It is a process impurity and monitored in the drug substance.**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeast count does not exceed 10 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.
- **PH** <791>: 3.8–4.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>
  - USP Abacavir Sulfate RS
  - USP Abacavir System Suitability Mixture RS
  - A mixture containing abacavir sulfate and *trans*-abacavir

**Abacavir Tablets****DEFINITION**Abacavir Tablets contain Abacavir Sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O).**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE****Diluent:** 1.0 mL of phosphoric acid in 1 L of water**Solution A:** Trifluoroacetic acid and water (0.05: 99.95)**Solution B:** Methanol and water (85:15)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	95	5
50	95	5

**System suitability solution:** 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent*

**Standard solution:** 0.21 mg/mL of abacavir sulfate in *Diluent* (equivalent to 0.18 mg/mL of abacavir), from USP Abacavir Sulfate RS

**Sample stock solution:** Transfer the equivalent to 1500 mg of abacavir, from a portion of Tablets, into a 250-mL volumetric flask. Add 150 mL of *Diluent*. Shake mechanically for 45 min. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45- $\mu$ m or finer pore size. Discard the first 3 mL of the filtrate.

**Sample solution:** 0.18 mg/mL of abacavir in *Diluent* using the filtrate obtained in the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  15-cm; packing L1

**Flow rate:** 0.8 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of abacavir from the *Sample solution*

$r_S$  = peak response of abacavir from the *Standard solution*

$C_S$  = concentration of abacavir sulfate in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 15 min

**Standard solution:** 0.39 mg/mL of USP Abacavir Sulfate RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** 254 nm

**Blank:** *Medium*

Calculate the percentage of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Change to read:

##### • ORGANIC IMPURITIES

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

#### Analysis

[NOTE—Record the chromatograms for 2.5 times the retention time of abacavir.]

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of abacavir from the *Standard solution*

$C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity (see *Table 2*)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** See *Table 2*.

▲ **Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropyldiaminopurine abacavir <sup>a</sup>	0.57	1.4	0.2
Descyclopropyl abacavir <sup>b</sup>	0.68	1.0	0.2
Abacavir	1.0	—	—
<i>trans</i> -Abacavir <sup>c,d</sup>	1.04	—	—
O-Pyrimidine derivative abacavir <sup>d,e</sup>	1.24	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

<sup>a</sup> N<sup>6</sup>-Cyclopropyl-9H-purine-2,6-diamine.

<sup>b</sup> [(1*S*,4*R*)-4-(2,6-Diamino-9H-purin-9-yl)-cyclopent-2-enyl]methanol.

<sup>c</sup> {(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-cyclopent-2-enyl}methanol.

<sup>d</sup> Process impurity monitored in the drug substance and not included in the total impurities.

<sup>e</sup> N<sup>6</sup>-Cyclopropyl-9-[(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yl)-methyl]cyclopent-2-enyl]-9H-purine-2,6-diamine.

▲ USP 36

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

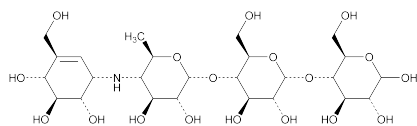
##### • USP REFERENCE STANDARDS <11>

USP Abacavir Sulfate RS

USP Abacavir System Suitability Mixture RS

A mixture of abacavir sulfate and *trans*-abacavir.

## Acarbose



$C_{25}H_{43}NO_{18}$  645.60

D-Glucose, O-4,6-dideoxy-4-[[[1S-(1 $\alpha$ ,4 $\alpha$ ,5 $\beta$ ,6 $\alpha$ )]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucose [56180-94-0].

» Acarbose is produced by certain strains of *Actinoplanes utahensis*. It contains not less than 95.0 percent and not more than 102.0 percent of  $C_{25}H_{43}NO_{18}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acarbose RS

USP Acarbose System Suitability Mixture RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the acarbose peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between +168° and +183°.

*Test solution:* 10 mg per mL, in water.

**pH** (791): between 5.5 and 7.5, in a solution containing 50 mg per mL.

**Water**, *Method 1c* (921): not more than 4.0%.

**Residue on ignition** (281): not more than 0.2% determined on 1.0 g.

**Heavy metals**, *Method II* (231): 0.002%.

**Chromatographic purity**—

*Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Diluted test solution*—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Test solution* and the *Diluted test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Acarbose taken by the formula:

$$(1/F)(r_i / r_A)$$

in which *F* is the relative response factor for each impurity, as listed in *Table 1*; *r<sub>i</sub>* is the individual peak response for each impurity; and *r<sub>A</sub>* is the response of the main acarbose peak in the chromatogram obtained from the *Diluted test solution*. In addition to not exceeding the limits for each impurity in *Table 1*, not more than 3.0% of total impurities is found.

**Assay**—

*Phosphate buffer*—Dissolve 0.6 g of monobasic potassium phosphate and 0.35 g of dibasic sodium phosphate in 900 mL of water, dilute with water to 1 L, and mix.

*Mobile phase*—Prepare a mixture of acetonitrile and *Phosphate buffer* (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Reconstitute a vial of USP Acarbose RS in 5.0 mL of water.

*System suitability solution*—Reconstitute a vial of USP Acarbose System Suitability Mixture RS in 1 mL of water.

**Table 1**

Name	Approximate Relative Retention Time	Relative Response Factor ( <i>F</i> )	Limit (%)
Impurity A <sup>1</sup>	0.9	1	0.6
Impurity B <sup>2</sup>	0.8	1.6	0.5
Impurity C <sup>3</sup>	1.2	1	1.5
Impurity D <sup>4</sup>	0.5	1.33	1.0
Impurity E <sup>5</sup>	1.7	0.8	0.2
Impurity F <sup>6</sup>	1.9	0.8	0.3
Impurity G <sup>7</sup>	2.2	0.8	0.3
Impurity H <sup>8</sup>	0.6	1	0.2
Any individual unknown impurity			0.2

<sup>1</sup>O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-arabino-hex-2-ulopyranose.

<sup>2</sup>(1R,4R,5S,6R)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-O-[4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside.

<sup>3</sup> $\alpha$ -D-Glucopyranosyl 4-O-[4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside.

<sup>4</sup>4-O-[4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]-D-glucopyranose.

<sup>5</sup>O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-arabino-hex-2-ulopyranose (4-O- $\alpha$ -acarboseyl-D-fructopyranose).

<sup>6</sup>O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose (4-O- $\alpha$ -acarboseyl-D-glucopyranose).

<sup>7</sup> $\alpha$ -D-Glucopyranosyl O-4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside ( $\alpha$ -D-glucopyranosyl  $\alpha$ -acarbose).

<sup>8</sup>O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-6-deoxy- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose.

**Assay preparation**—Transfer about 200 mg of Acarbose, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

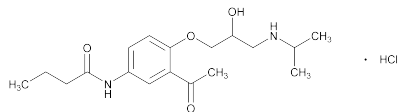
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm × 25-cm column that contains packing L8. The flow rate is about 2 mL per minute. The column temperature is maintained at 35°. Chromatograph the *System suitability solution*, and identify the acarbose peak and the peaks due to the impurities listed in *Table 1*. Record the peak responses as directed for *Procedure*: the ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and the acarbose peak is not less than 1.2. The chromatogram obtained is similar to the chromatogram supplied with USP Acarbose System Suitability Mixture RS.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of C<sub>25</sub>H<sub>43</sub>NO<sub>18</sub> in the portion of Acarbose taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acarbose RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acarbose peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acebutolol Hydrochloride



C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> · HCl 372.89

Butanamide, N-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-, monohydrochloride, (±)-. (±)-3'-Acetyl-4'-[2-hydroxy-3-(isopropylamino)propoxy]-butyranilide monohydrochloride [34381-68-5].

» Acebutolol Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> · HCl, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** <11>—

USP Acebutolol Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** Prepare a mixture of the *Standard preparation* and the *Assay preparation* (1:1), and chromatograph the mixture as directed in the *Assay*: the chromatogram thus obtained exhibits a single major peak due to acebutolol.

**C:** It responds to the tests for *Chloride* <191>, when tested as directed for alkaloidal hydrochlorides.

**pH** <791>: between 4.5 and 7.0, in a solution (1 in 100).

**Melting range** <741>: between 140° and 144°.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method II** <231>: 0.002%.

**Chromatographic purity**—

**Standard solution**—Prepare a solution of USP Acebutolol Hydrochloride RS in methanol containing 1.0 mg per mL.

**Test solution 1**—Prepare a solution of Acebutolol Hydrochloride in methanol containing 10 mg per mL.

**Test solution 2**—Mix 1 mL of *Test solution 1* and 9 mL of methanol.

**Reference solution 1**—Transfer 3.0 mL of the *Standard solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

**Reference solution 2**—Mix 5.0 mL of *Reference solution 1* and 10.0 mL of methanol.

**Procedure**—Apply separate 20-µL portions of the *Standard solution*, *Test solution 1*, *Test Solution 2*, *Reference solution 1*, and *Reference solution 2* to a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography* under *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of the upper layer of a mixture of water, butyl alcohol, and glacial acetic acid (50:40:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Examine the plate under short-wavelength UV light: the chromatograms from *Test solution 2* and the *Standard solution* show principal spots at about the same  $R_f$  value. No secondary spot in the chromatogram from *Test solution 1*, excluding the area at the point of application, is more intense than the principal spot obtained from *Reference solution 1* (0.3%), and not more than two secondary spots in the chromatogram from *Test solution 1* are more intense than the principal spot obtained from *Reference solution 2* (0.1%), and the total of all impurities detected in the chromatogram of *Test solution 1* is not more than 0.5%.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, a 0.3% aqueous solution of sodium dodecyl sulfate, and glacial acetic acid (675:325:20). Make adjustments if necessary to achieve a retention time for acebutolol of between 4 minutes and 7 minutes (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Acebutolol Hydrochloride RS in water to obtain a solution having a known concentration of about 0.14 mg per mL.

**Assay preparation**—Transfer about 35 mg of Acebutolol Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> · HCl in the portion of Acebutolol Hydrochloride taken by the formula:

$$250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acebutolol Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acebutolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Acebutolol Hydrochloride Capsules

» Acebutolol Hydrochloride Capsules contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acebutolol ( $C_{18}H_{28}N_2O_4$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acebutolol Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{18}H_{28}N_2O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 232 nm on filtered portions of the solution under test in comparison with a Standard solution having a known concentration of USP Acebutolol Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of acebutolol ( $C_{18}H_{28}N_2O_4$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Chromatographic purity—

#### TEST 1—

**Buffer solution**—Prepare as directed in the *Assay*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (56:44). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of *Buffer solution* and methanol (50:50).

**Standard solution**—Transfer about 30 mg of USP Acebutolol Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask. Add about 12 mL of methanol, swirl to dissolve, dilute with *Diluent* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.4 µg of USP Acebutolol Hydrochloride RS per mL.

**Test solution**—Transfer an accurately weighed portion of the contents of 20 opened Capsules, equivalent to about 250 mg of acebutolol, to a 100-mL volumetric flask, add about 25 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of this solution, and transfer 10.0 mL of the clear supernatant to a 100-mL volumetric flask. Dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 6.0%.

**Procedure**—Separately inject equal volumes (about 35 µL) of the *Standard solution*, *Test solution*, and *Diluent* into the chromatograph, record the chromatograms for about two times the retention time of acebutolol, and measure the responses for all the peaks, disregarding any peaks corresponding to those obtained from the *Diluent*. Calculate the

percentage of each impurity eluting prior to the acebutolol peak in the portion of Capsules taken by the formula:

$$(336.44/372.89)(0.4C)(r_i / r_s)$$

in which 336.44 and 372.89 are the molecular weights of acebutolol and acebutolol hydrochloride, respectively; C is the concentration, in µg per mL, of USP Acebutolol Hydrochloride RS in the *Standard solution*;  $r_i$  is the peak response of any individual impurity obtained from the *Test solution*; and  $r_s$  is the peak response of acebutolol obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found.

#### TEST 2—

**Buffer solution**—Prepare as directed in the *Assay*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 30 mg of USP Acebutolol Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask. Add about 12 mL of methanol, swirl to dissolve, dilute with *Mobile phase* to volume, and mix. Dilute an accurately measured volume of this stock solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.4 µg of USP Acebutolol Hydrochloride RS per mL.

**Test solution**—Transfer an accurately weighed portion of the contents of 20 opened Capsules, equivalent to about 250 mg of acebutolol, to a 100-mL volumetric flask, add about 25 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with *Mobile phase* to volume, and mix. Centrifuge a portion of this solution, and transfer 10.0 mL of the clear supernatant to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 6.0%.

**Procedure**—Separately inject equal volumes (about 70 µL) of the *Standard solution*, *Test solution*, and *Mobile phase* into the chromatograph, record the chromatograms for about three times the retention time of acebutolol, and measure the responses for all the peaks, disregarding any peaks corresponding to those obtained from the *Mobile phase*. Calculate the percentage of each impurity eluting after the acebutolol peak in the portion of Capsules taken by the formula:

$$(336.44/372.89)(0.4C)(r_i / r_s)$$

in which 336.44 and 372.89 are the molecular weights of acebutolol and acebutolol hydrochloride, respectively; C is the concentration, in µg per mL, of USP Acebutolol Hydrochloride RS in the *Standard solution*;  $r_i$  is the peak response of any individual impurity obtained from the *Test solution*; and  $r_s$  is the peak response of acebutolol obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found. The sum of all individual impurities found in *Test 1* and *Test 2* is not more than 1.0%.

### Assay—

**Buffer solution**—Dissolve about 2.4 g of sodium 1-decanesulfonate in 1000 mL of water. Adjust with glacial acetic acid to a pH of 3.5.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Acebutolol Hydrochloride RS quantitatively in methanol to obtain a solution having a known concentration of about 0.22 mg per mL. This is equivalent to about 0.2 mg of acebutolol per mL.

**Assay preparation**—Weigh and mix, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of acebutolol, to a 200-mL volumetric flask. Add about 180 mL of methanol, and stir by mechanical means for about 30 minutes. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

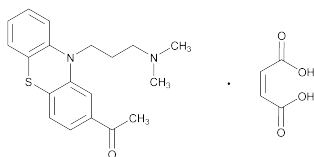
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of acebutolol (C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of Capsules taken by the formula:

$$(336.44/372.89)(1000C)(r_U / r_S)$$

in which 336.44 and 372.89 are the molecular weights of acebutolol and acebutolol hydrochloride, respectively; C is the concentration, in mg per mL, of USP Acebutolol Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acebutolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acepromazine Maleate



C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>OS · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> 442.53  
 Ethanone, 1-[10-[3-(dimethylamino)propyl]-10H-phenothiazin-2-yl]-, (Z)-2-butenedioate (1:1);  
 10-[3-(Dimethylamino)propyl]phenothiazin-2-yl methyl ketone maleate (1:1) [3598-37-6].

### DEFINITION

Acepromazine Maleate contains NLT 98.0% and NMT 101.0% of acepromazine maleate (C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>OS · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>), calculated on the anhydrous basis.

Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak for acepromazine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and *Buffer* (300:700)

**Standard stock solution:** 1 mg/mL of USP

Acepromazine Maleate RS in 0.05 N hydrochloric acid

**Standard solution:** 0.1 mg/mL of USP Acepromazine

Maleate RS in water from *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Acepromazine

Maleate in 0.05 N hydrochloric acid

**Sample solution:** 0.1 mg/mL of Acepromazine Maleate

in water from *Sample stock solution*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4-mm × 15-cm; 5-μm packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acepromazine maleate

(C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>OS · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Acepromazine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area response from the *Sample solution*

$r_S$  = peak area response from the *Standard solution*

$C_S$  = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.0% on the anhydrous basis

### IMPURITIES

- RESIDUE ON IGNITION** <281>: NMT 0.2%

#### ORGANIC IMPURITIES

Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.

**Diluent:** Methanol and diethylamine (19:1)

**Sample solution:** 20.0 mg/mL of Acepromazine Maleate in *Diluent*

**Standard solution:** 0.1 mg/mL of Acepromazine

Maleate in *Diluent* from the *Sample solution*

### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 μL

**Developing solvent system:** *n*-Heptane, isobutyl alcohol, and diethylamine (75:17:8)

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the chamber and allow to air dry. Examine the plate under short-wavelength UV light.

**Acceptance criteria:** 0.5%; no spot, other than the principal acepromazine spot and any at the origin, observed in the chromatogram of the *Sample solution* is more intense than the principal spot observed in the chromatogram of the *Standard solution*.

### SPECIFIC TESTS

- MELTING RANGE OR TEMPERATURE** <741>: 136°–139°

#### PH <791>

**Sample solution:** 10 mg/mL of Acepromazine Maleate in water

- Acceptance criteria: 4.0–5.5
- **WATER DETERMINATION, Method I** (921): NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
USP Acepromazine Maleate RS

**Acepromazine Maleate Injection****DEFINITION**

Acepromazine Maleate Injection is a sterile solution of Acepromazine Maleate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ). Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)  
**Sample:** To a volume of Injection, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.  
**Acceptance criteria:** Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**  
**Buffer:** Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.  
**Mobile phase:** Acetonitrile and *Buffer* (300:700)  
**Standard stock solution:** 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid  
**Standard solution:** 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*  
**Sample stock solution:** 1 mg/mL of Acepromazine Maleate in 0.05 N hydrochloric acid from an appropriately diluted volume of Injection  
**Sample solution:** Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 280 nm  
**Column:** 4-mm  $\times$  15-cm; 5- $\mu$ m packing L7  
**Flow rate:** 1 mL/min  
**Injection volume:** 10  $\mu$ L  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Column efficiency:** NLT 1500 theoretical plates  
**Tailing factor:** NMT 2.5  
**Relative standard deviation:** NMT 2.0%
- Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **pH** (791): 4.5–5.8
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 4.5 USP Endotoxin Units/mg of acepromazine maleate
- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections* (1).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-dose or multiple-dose containers for injections as described in *Injections* (1), *Containers for Injections*, preferably of Type I glass, and store at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
USP Acepromazine Maleate RS  
USP Endotoxin RS

**Acepromazine Maleate Tablets****DEFINITION**

Acepromazine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ). Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)  
**Sample:** To a quantity of powdered Tablets, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.  
**Acceptance criteria:** Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**  
**Buffer:** Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.  
**Mobile phase:** Acetonitrile and *Buffer* (300:700)  
**Standard stock solution:** 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid  
**Standard solution:** 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*  
**Sample stock solution:** Transfer NLT 10 Tablets to a 200-mL volumetric flask, add 100 mL of 0.05 N hydrochloric acid, and sonicate for 10 min. Shake by mechanical means for 30 min, and dilute with 0.05 N hydrochloric acid to volume.  
**Sample solution:** Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Column:** 4-mm × 15-cm; 5-μm packing L7**Flow rate:** 1 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 1500 theoretical plates**Tailing factor:** NMT 2.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acepromazine maleate

(C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>OS · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

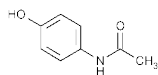
 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Acepromazine MaleateRS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Label the Tablets to indicate that they are for veterinary use only.
- **USP REFERENCE STANDARDS** <11>  
USP Acepromazine Maleate RS

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**Acetaminophen**


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C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> 151.16Acetamide, *N*-(4-hydroxyphenyl)-.

4'-Hydroxyacetanilide [103-90-2].

» Acetaminophen contains not less than 98.0 percent and not more than 101.0 percent of C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at room temperature. Protect from moisture and heat.

**USP Reference standards** <11>—

USP Acetaminophen RS

**Identification**—**A:** *Infrared Absorption* <197K>.**B:** *Ultraviolet Absorption* <197U>—*Solution:* 5 μg per mL.*Medium:* 0.1 N hydrochloric acid in methanol (1 in 100).

**C:** It responds to the *Thin-layer Chromatographic Identification Test* <201>, a test solution in methanol containing about 1 mg per mL and a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Melting range** <741>: between 168° and 172°.**Water, Method I** <921>: not more than 0.5%.**Residue on ignition** <281>: not more than 0.1%.

**Chloride** <221>—Shake 1.0 g with 25 mL of water, filter, and add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: the filtrate shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.014%).

**Sulfate** <221>—Shake 1.0 g with 25 mL of water, filter, add 2 mL of 1 N acetic acid, then add 2 mL of barium chloride TS: the mixture shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.02%).

**Sulfide**—Place about 2.5 g in a 50-mL beaker. Add 5 mL of alcohol and 1 mL of 3 N hydrochloric acid. Moisten a piece of lead acetate test paper with water, and fix to the underside of a watch glass. Cover the beaker with the watch glass so that part of the lead acetate paper hangs down near the pouring spout of the beaker. Heat the contents of the beaker on a hot plate just to boiling: no coloration or spotting of the test paper occurs.

**Heavy metals, Method II** <231>: 0.001%.

**Free *p*-aminophenol**—Transfer 5.0 g to a 100-mL volumetric flask, and dissolve in about 75 mL of a mixture of equal volumes of methanol and water. Add 5.0 mL of alkaline nitroferricyanide solution (prepared by dissolving 1 g of sodium nitroferricyanide and 1 g of anhydrous sodium carbonate in 100 mL of water), dilute with a mixture of equal volumes of methanol and water to volume, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of this solution and of a freshly prepared solution of *p*-aminophenol, similarly prepared at a concentration of 2.5 μg per mL, using the same quantities of the same reagents, in 1-cm cells, at the maximum at about 710 nm, with a suitable spectrophotometer, using 5.0 mL of alkaline nitroferricyanide solution diluted with a mixture of equal volumes of methanol and water to 100 mL as the blank: the absorbance of the test solution does not exceed that of the standard solution, corresponding to not more than 0.005% of *p*-aminophenol.

**Limit of *p*-chloroacetanilide**—Transfer 1.0 g to a glass-stoppered, 15-mL centrifuge tube, add 5.0 mL of ether, shake by mechanical means for 30 minutes, and centrifuge at 1000 rpm for 15 minutes or until a clean separation is obtained. Apply 200 μL of the supernatant, in 40-μL portions, to obtain a single spot not more than 10 mm in diameter to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Similarly apply 40 μL of a Standard solution in ether containing 10 μg of *p*-chloroacetanilide per mL, and allow the spots to dry. Develop the chromatogram in an unsaturated chamber, with a solvent system consisting of a mixture of solvent hexane and acetone (75:25), until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots in the chromatogram by examination under short-wavelength UV light: any spot obtained from the solution under test, at an  $R_f$  value corresponding to the principal spot from the Standard solution, is not greater in size or intensity than the principal spot obtained from the Standard solution, corresponding to not more than 0.001% of *p*-chloroacetanilide.

**Readily carbonizable substances** <271>—Dissolve 0.50 g in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

**Assay**—Dissolve about 120 mg of Acetaminophen, accurately weighed, in 10 mL of methanol in a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Acetaminophen RS, in the same medium, at a concentration of about 12 μg per mL in 1-cm cells, at the

wavelength of maximum absorbance at about 244 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_8H_9NO_2$  in the Acetaminophen taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Acetaminophen RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Acetaminophen and the Standard solution, respectively.

## Acetaminophen Capsules

» Acetaminophen Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Triturate an amount of the contents of the Capsules, equivalent to about 50 mg of acetaminophen, with 50 mL of methanol, and filter: the clear filtrate (test solution) responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of  $C_8H_9NO_2$  dissolved from UV absorption at the wavelength of maximum absorbance at about 249 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Acetaminophen RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_8H_9NO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Prepare a suitable degassed mixture of water and methanol (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Acetaminophen RS in *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg per mL.

**Assay preparation**—Weigh the contents of not fewer than 20 Capsules, and calculate the average weight of the contents of each Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 100 mg of acetaminophen, to a 200-mL volumetric flask. Add about 100 mL of *Mobile phase*, and shake by mechanical means for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a

filter having a 0.5- $\mu\text{m}$  or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Chromatographic system**—The liquid chromatograph is equipped with a 243-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Capsules taken by the formula:

$$10,000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen Oral Solution

» Acetaminophen Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Dilute a portion of Oral Solution with methanol to obtain a solution containing about 1 mg of acetaminophen per mL. This test solution responds to the *Thin-Layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.8 and 6.1.

**Alcohol content** (if present), *Method II* (611): between 90.0% and 115.0% of the labeled amount of  $C_2H_5OH$ , determined by the gas-liquid chromatographic procedure, acetone being used as the internal standard.

**Assay**—

**Mobile phase**, **Standard preparation**, and **Chromatographic system**—Proceed as directed in the *Assay* under *Acetaminophen Capsules*.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 500 mg of acetaminophen, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution

to a second 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 25.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Acetaminophen Capsules*. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each mL of the Oral Solution taken by the formula:

$$50,000(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Acetaminophen for Effervescent Oral Solution

» Acetaminophen for Effervescent Oral Solution contains, in each 100 g, not less than 5.63 g and not more than 6.88 g of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification**—

**A:** A 10-g portion dissolves, with effervescence, in 200 mL of water when performed as directed for the *Assay preparation* in the *Assay*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** Triturate about 0.4 g of the powder with 25 mL of methanol, and filter: this test solution responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Minimum fill** (755)—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Acetaminophen Capsules*.

*Assay preparation*—Dissolve about 10 g of Acetaminophen for Effervescent Oral Solution, accurately weighed, in about 200 mL of water in a 1000-mL volumetric flask, using gentle heat if necessary, until effervescence subsides, then dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 8.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Acetaminophen Capsules*. Calculate the quantity, in g, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Acetaminophen for Effervescent Oral Solution taken by the formula:

$$62.5C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Acetaminophen Suppositories

» Acetaminophen Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature or in a cool place.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer a portion of Suppositories, equivalent to about 20 mg of acetaminophen, to a beaker, add 20 mL of methanol, and heat on a steam bath until melted. Remove the beaker from the steam bath, allow to cool with occasional stirring, and filter: the clear filtrate (test solution) responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Acetaminophen Capsules*.

*Assay preparation*—Tare a small dish and a glass rod, place in the dish not fewer than 5 Suppositories, heat gently on a steam bath until melted, then stir, cool while stirring, and weigh. Transfer an accurately weighed portion of the mass, equivalent to about 100 mg of acetaminophen, to a separator, add 30 mL of solvent hexane, and mix to dissolve. Add 30 mL of water, shake gently, and allow the phases to separate. [NOTE—If an emulsion forms, allow sufficient time for it to separate.] Transfer the aqueous layer to a 200-mL volumetric flask, wash the solvent hexane in the separator with three 30-mL portions of water, adding the washings to the volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Acetaminophen Capsules*. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Suppository taken by the formula:

$$10,000C(A/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; A is the average weight, in mg, of each Suppository taken; W is the weight, in mg, of the Suppository mass taken; and  $r_U$  and  $r_S$  are the

acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen Oral Suspension

» Acetaminophen Oral Suspension is a suspension of Acetaminophen in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP 4-Aminophenol RS

**Identification**—Transfer a volume of Oral Suspension, equivalent to about 240 mg of acetaminophen, to a separator, add 50 mL of ethyl acetate, and shake. Filter the ethyl acetate extract through a funnel containing glass wool and about 10 g of anhydrous sodium sulfate. Collect the filtrate in a beaker, and evaporate on a steam bath to dryness. Dry the residue in vacuum over silica gel: the crystals so obtained respond to *Identification test A* under *Acetaminophen*.

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Limit of 4-aminophenol**—

*Diluent*—Prepare a mixture of water, methanol, and formic acid (425:75:2).

*Mobile phase*—Prepare a filtered and degassed mixture of 0.01 M sodium butanesulfonate in *Diluent*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP 4-Aminophenol RS, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 24 µg per mL.

*Test solution*—Transfer an accurately measured portion of Oral Suspension, equivalent to about 120 mg of acetaminophen, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 20-cm column that contains 10-µm packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution* and the *Test solution*, and record the peak areas as directed for *Procedure*.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks: the peak area of 4-aminophenol obtained from the *Test solution* is not greater than the corresponding peak area obtained from the *Standard solution*.

**pH** (791): between 4.0 and 6.9.

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Acetaminophen Capsules*.

*Assay preparation*—Transfer an accurately measured volume of Oral Suspension, previously well-shaken, equivalent to about 100 mg of acetaminophen, to a 200-mL volumet-

ric flask, add about 100 mL of *Mobile phase*, and shake by mechanical means for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Acetaminophen Capsules*. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each mL of the Oral Suspension taken by the formula:

$$10,000(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen Tablets

» Acetaminophen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label Tablets that must be chewed to indicate that they are to be chewed before swallowing.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Triturate an amount of powdered Tablets, equivalent to about 50 mg of acetaminophen, with 50 mL of methanol, and filter: the clear filtrate (test solution) responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of methylene chloride and methanol (4:1) being used.

**Dissolution** (711)—

*Medium:* pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_8H_9NO_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 243 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Acetaminophen RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_8H_9NO_2$  is dissolved in 30 minutes.

FOR TABLETS LABELED AS CHEWABLE—

*Medium:* pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

*Procedure*—Proceed as directed for *Procedure* for *Acetaminophen Tablets*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_8H_9NO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Acetaminophen Capsules*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 200-mL volumetric flask, add about 100 mL of *Mobile phase*, shake by mechanical means for 10 minutes, sonicate for about 5 minutes, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Acetaminophen Capsules*. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$10,000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen Extended-Release Tablets

» Acetaminophen Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where the Tablets are gelatin-coated, the label so states. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**USP Reference standards** (11)—

USP Acetaminophen RS

**Identification—**

**A: Infrared Absorption** (197K)—Use a portion of powdered Tablets.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution** (711)—

TEST 1—

*Medium:* simulated gastric fluid TS (without enzyme); 900 mL.

*Apparatus 2:* 50 rpm.

*Times:* 15 minutes, 1 hour, and 3 hours.

*Procedure*—Determine the amount of  $C_8H_9NO_2$  dissolved from UV absorbances at 280 nm, using a filtered portion of the solution under test in comparison with a *Standard solution* having a known concentration of USP Acetaminophen RS in the same *Medium*.

*Tolerances*—The percentages of the labeled amount of  $C_8H_9NO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time	Amount dissolved
15 minutes	between 45% and 65%
1 hour	between 60% and 85%
3 hours	not less than 85%

FOR GELATIN-COATED TABLETS—

*Medium, Apparatus, and Procedure*—Proceed as directed above.

*Times:* 30 minutes, 90 minutes, and 4 hours.

*Tolerances*—The percentage of the labeled amount of  $C_8H_9NO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time	Amount dissolved
30 minutes	between 40% and 60%
90 minutes	between 55% and 85%
4 hours	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Medium, Apparatus, and Procedure*—Proceed as directed for *Test 1*.

*Tolerances*—The percentages of the labeled amount of  $C_8H_9NO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time	Amount dissolved
15 minutes	between 40% and 60%
1 hour	between 55% and 75%
3 hours	not less than 80%

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Mobile phase*—Prepare a mixture of water and phosphoric acid (9:1). Combine 1 mL of this solution with a mixture of water and methanol (700:300). Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Acetaminophen RS in methanol, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.65 mg per mL.

*Assay preparation*—Transfer 10 Tablets into a 250-mL volumetric flask containing 50 mL of water and a magnetic stir bar. Stir at least 30 minutes or until the coating has dissolved. Add 150 mL of methanol, and stir for 45 minutes. Tablet cores should be disintegrated at least 15 minutes prior to ending the stirring. Remove the magnetic stir bar and rinse into the flask with methanol. Dilute with methanol to volume, mix well, and centrifuge. Transfer 5 mL of the clear supernatant to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 295-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-



tity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Tablet taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Aspirin Tablets

» Acetaminophen and Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acetaminophen RS

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—The relative retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution, Procedure for a Pooled Sample (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Mobile phase—Prepare as directed under *Assay*.

Solvent mixture—Prepare as directed under *Assay*.

Internal standard solution—Prepare a solution of benzoic acid in methanol having a concentration of about 1 mg per mL.

*Standard preparation I*—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Solvent mixture* to obtain a solution having a known concentration of about 70 µg per mL. Combine 4.0 mL of this solution and 1.0 mL of the *Internal standard solution*, and mix.

*Standard preparation II*—Dissolve accurately weighed quantities of USP Acetaminophen RS and USP Aspirin RS in the *Solvent mixture* to obtain a solution having known concentrations of about 360 µg of acetaminophen and about 360 µg of aspirin per mL. Combine 4.0 mL of this solution and 1.0 mL of the *Internal standard solution*, and mix.

*Test preparation*—Combine 4.0 mL of a filtered portion of the solution under test and 1.0 mL of the *Internal standard solution*, and mix.

Chromatographic system—Proceed as directed under *Assay*.

*Procedure*—Separately inject equal volumes (about 20 µL) of the two *Standard preparations* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.3 for acetaminophen, 0.4 for salicylic acid, 0.6 for aspirin, and 1.0 for benzoic acid. Determine the amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved by the formula:

$$90(C/W)(R_U / R_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Acetaminophen RS in *Standard preparation II*;  $R_U$  and  $R_S$  are the relative peak response ratios obtained from the *Test preparation* and *Standard preparation II*, respectively; and  $W$  is the

labeled amount, in mg, of acetaminophen. Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved by the formula:

$$[90C_1(R_{U1} / R_{S1})] + [90C_2(R_{U2} / R_{S2})(1.3044)] / W$$

in which  $C_1$  and  $C_2$  are the concentrations, in µg per mL, of USP Aspirin RS in *Standard preparation II* and USP Salicylic Acid RS in *Standard preparation I*, respectively;  $R_{U1}$  and  $R_{S1}$  are the relative peak response ratios for the aspirin peak and the internal standard peak obtained from the *Test preparation* and *Standard preparation II*, respectively;  $R_{U2}$  and  $R_{S2}$  are the relative peak response ratios for the salicylic acid peak and the internal standard peak obtained from the *Test preparation* and *Standard preparation I*, respectively; and  $W$  is the labeled amount, in mg, of aspirin.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$  and  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to acetaminophen and to aspirin.

### Limit of salicylic acid—

*Solvent mixture, Mobile phase, Internal standard solution, and Chromatographic system*—Prepare as directed in the *Assay*.

*Procedure*—Dissolve a suitable quantity of USP Salicylic Acid RS, accurately weighed, in *Solvent mixture* to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 1.0-mL, 5.0-mL, and 10.0-mL portions, respectively, of this solution to separate 100-mL volumetric flasks, add 10.0 mL of *Internal standard solution* to each flask, dilute with *Solvent mixture* to volume, and mix. Chromatograph these three *Standard solutions* as directed in the *Assay*. Plot the ratios of the peak responses for salicylic acid and benzoic acid for each of the *Standard solutions* versus concentrations, in mg per mL, of salicylic acid, and draw the straight line best fitting the three plotted points. From the graph so obtained, and from the ratio of the peak responses for salicylic acid and benzoic acid in the chromatogram of the *Assay preparation* as obtained in the *Assay*, determine the concentration, in mg per mL, of salicylic acid ( $C_7H_6O_3$ ) in the *Assay preparation*, and calculate the percentage of salicylic acid in relation to the concentration of aspirin in the *Assay preparation*, as determined in the *Assay*. Not more than 3.0% is found.

**Assay**—[NOTE—Use clean, dry glassware. Inject the *Standard preparation* and the *Assay preparation* promptly after preparation.]

*Solvent mixture*—Prepare a mixture of chloroform, methanol, and glacial acetic acid (78:20:2).

*Mobile phase*—Transfer 225 mg of tetramethylammonium hydroxide pentahydrate to a 1000-mL flask, and add 750 mL of water, 125 mL of methanol, 125 mL of acetonitrile, and 1.0 mL of glacial acetic acid. Stir for 3 minutes, pass through a membrane filter having a 0.5-µm or finer porosity, and degas.

*Internal standard solution*—Dissolve benzoic acid in *Solvent mixture* to obtain a solution having a concentration of about 20 mg per mL.

*Standard preparation*—Transfer about 325 mg of USP Acetaminophen RS and about 325 mg of USP Aspirin RS, each accurately weighed, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of acetaminophen, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution* and about 50 mL of *Solvent mixture*, and sonicate for about 3 minutes. Dilute with *Solvent mixture* to volume, and mix. Pass a portion of this solution through a filter having a 2.5-µm or finer porosity, and use the filtrate as the *Assay preparation*.

**Chromatographic system**—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph four replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for either analyte is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention times are about 2, 3, 5, and 8 minutes for acetaminophen, salicylic acid (if present), aspirin, and benzoic acid, respectively. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of acetaminophen and benzoic acid obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the same formula, except to read "USP Aspirin RS" where "USP Acetaminophen RS" is specified, and "aspirin" where "acetaminophen" is specified.

## Acetaminophen, Aspirin, and Caffeine Tablets

» Acetaminophen, Aspirin, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acetaminophen RS  
USP Aspirin RS  
USP Caffeine RS  
USP Salicylic Acid RS

**Identification**—The relative retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 100 rpm.

Time: 60 minutes.

**Mobile phase, Internal standard solution, Solvent mixture, Standard stock solution, and Chromatographic system**—Proceed as directed in the *Assay*.

**Standard preparation**—Transfer 20.0 mL of *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, mix, and allow to stand for about 30 seconds. Dilute with *Solvent mixture* to volume, and mix. Use within 8 hours.

**Test preparation**—Transfer 20.0 mL of a filtered portion of the solution under test to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution* and 20 mL of *Solvent mixture*, mix, and allow to stand for 30 seconds. Dilute with *Solvent mixture* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the quantities, in mg, of acetaminophen

( $C_8H_9NO_2$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the formula:

$$2250C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the corresponding analyte and internal standard peaks of the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$ ,  $C_9H_8O_4$ , and  $C_8H_{10}N_4O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units (905)**: meet the requirements for *Content Uniformity* with respect to acetaminophen, aspirin, and caffeine.

### Limit of salicylic acid—

**Mobile phase and Solvent mixture**—Prepare as directed in the *Assay*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL. Transfer 2.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This solution contains about 0.02 mg of USP Salicylic Acid RS per mL.

**Test preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of aspirin, to a 100-mL volumetric flask. Add about 75 mL of *Solvent mixture*, and shake by mechanical means for 30 minutes. Dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system**—Proceed as directed for *Chromatographic system* in the *Assay*, except to use a 302-nm detector. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor is not more than 1.6; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the salicylic acid peaks. Calculate the percentage of salicylic acid in the portion of Tablets taken by the formula:

$$10,000(C/a)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*; a is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $r_U$  and  $r_S$  are the salicylic acid peak responses of the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

### Assay—

**Mobile phase**—Prepare a suitable mixture of water, methanol, and glacial acetic acid (69:28:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of benzoic acid in methanol containing about 6 mg per mL.

**Solvent mixture**—Prepare a mixture of methanol and glacial acetic acid (95:5).

**Standard stock solution**—Dissolve accurately weighed quantities of USP Acetaminophen RS, USP Aspirin RS, and USP Caffeine RS in *Solvent mixture* to obtain a solution having known concentrations of about 0.25 mg of USP Acetaminophen RS per mL, 0.25 mg of USP Aspirin RS per mL, and 0.25 mg of USP Caffeine RS per mL, / being the ratio of the labeled amount, in mg, of aspirin to the labeled amount, in mg, of acetaminophen per Tablet; and /' being

the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet.

**Standard preparation**—Transfer 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This solution contains about 0.1 mg of USP Acetaminophen RS, 0.1/ mg of USP Aspirin RS, and 0.1/ mg of USP Caffeine RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of acetaminophen, to a 100-mL volumetric flask. Add about 75 mL of *Solvent mixture*, and shake by mechanical means for 30 minutes. Dilute with *Solvent mixture* to volume, and mix. Transfer 2.0 mL of this solution and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 10-cm column that contains 5-μm packing L1, and is maintained at 45 ± 1°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for each analyte peak is not more than 1.2; the resolution, *R*, between any of the analyte and internal standard peaks is not less than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.3 for acetaminophen, 0.5 for caffeine, 0.8 for aspirin, 1.0 for benzoic acid, and 1.2 for salicylic acid. Calculate the quantities, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>), and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Tablets taken by the formula:

$$2500C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses of the corresponding analyte and internal standard peaks of the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Caffeine Tablets

» Acetaminophen and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acetaminophen RS

USP Caffeine RS

**Identification**—The retention times of the major peaks for acetaminophen and caffeine in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation*, relative to the internal standard, obtained as directed in the *Assay*.

### Dissolution (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 100 rpm.

*Time*: 60 minutes.

*Mobile phase*, *Internal standard solution*, *Solvent mixture*, *Standard stock solution*, and *Chromatographic system*—Prepare as directed in the *Assay*.

**Standard solution**—Transfer 20.0 mL of *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, mix, and allow to stand for about 30 seconds. Dilute with *Solvent mixture* to volume, and mix. Use within 8 hours.

**Test solution**—Transfer an aliquot of a filtered portion of the solution under test to a 50-mL volumetric flask in order to obtain an expected concentration of about 0.1 mg per mL of acetaminophen and 0.1/ mg per mL of caffeine, where *J* is defined for the *Standard stock solution*. Add 3.0 mL of *Internal standard solution* and 20 mL of *Solvent mixture*, mix, and allow to stand for 30 seconds. Dilute with *Solvent mixture* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*, except to inject the *Standard solution* and the *Test solution*. Calculate the quantities, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) dissolved by the formula:

$$(45,000/V_d)C(R_U / R_S)$$

in which *V<sub>d</sub>* is the volume, in mL, of *Test solution* that is transferred to the volumetric flask; *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses of the corresponding analyte and internal standard peaks obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 75% (*Q*) of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

**Mobile phase**—Prepare a suitable mixture of water, methanol, and glacial acetic acid (69:28:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of benzoic acid in methanol containing about 6 mg per mL.

**Solvent mixture**—Prepare a mixture of methanol and glacial acetic acid (95:5).

**Standard stock solution**—Dissolve accurately weighed quantities of USP Acetaminophen RS and USP Caffeine RS in *Solvent mixture* to obtain a solution having known concentrations of about 0.25 mg of USP Acetaminophen RS per mL and 0.25/ mg of USP Caffeine RS per mL, *J* being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet.

**Standard preparation**—Transfer 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This solution contains about 0.1 mg of USP Acetaminophen RS and 0.1/ mg of USP Caffeine RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Acetaminophen and Caffeine Tablets. Transfer an accurately weighed quantity of the well-mixed powder, equivalent to about 250 mg of acetaminophen, to a 100-mL volumetric flask. Add about 75 mL of *Solvent mixture*, and shake by mechanical means for 30 minutes. Dilute with *Solvent mixture* to volume, and mix. Transfer 2.0 mL of this solution and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 10-cm column that contains 5-μm packing L1, and is maintained at 45 ± 1°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for each analyte peak is not more than 1.2; the resolution, *R*, between any of the analyte and internal standard peaks is not less than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.3 for acetaminophen, 0.5 for caffeine, and 1.0 for benzoic acid. Calculate the quantities, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Tablets taken by the formula:

$$2500C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses of the corresponding analyte and internal standard peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine

» Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>), dextromethorphan hydrobromide (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O), and phenylpropanolamine hydrochloride (C<sub>9</sub>H<sub>13</sub>NO · HCl).

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Acetaminophen RS

USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS

USP Phenylpropanolamine Hydrochloride RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active

ingredient and indicates its function (or purpose) in the article.

#### Identification—

**A:** If phenylpropanolamine hydrochloride is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for phenylpropanolamine hydrochloride*, exhibits a major peak for phenylpropanolamine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** If acetaminophen is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

#### Dissolution, Procedure for a Pooled Sample (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 50 rpm.

**Time:** 45 minutes.

**Test solution**—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

**Procedure**—Determine the amounts of phenylpropanolamine hydrochloride, acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for phenylpropanolamine hydrochloride*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of phenylpropanolamine hydrochloride (C<sub>9</sub>H<sub>13</sub>NO · HCl), acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>), and dextromethorphan hydrobromide (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O) are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay for phenylpropanolamine hydrochloride (if present)—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride* under *Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Capsules that contain either all four ingredients or a combination of three including chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Capsules that contain no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Transfer not less than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and

gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with water to obtain a solution having a concentration of about 0.05 mg of phenylpropanolamine hydrochloride per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the phenylpropanolamine peaks. Calculate the quantity, in mg, of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ) in the Capsules taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenylpropanolamine Hydrochloride RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of phenylpropanolamine hydrochloride in each Capsule; *D* is the concentration, in mg per mL, of phenylpropanolamine hydrochloride in the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of phenylpropanolamine hydrochloride in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the phenylpropanolamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 25 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Add 0.2 mL of phosphoric acid, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.25 mg of acetaminophen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of acetaminophen in each Capsule; *D* is the concentration, in mg per mL, of acetaminophen in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of acetaminophen in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are

the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride* under *Oral Solution Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 8  $\mu$ g of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule; *D* is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride* under *Oral Solution Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.06 mg of dextromethorphan hydrobromide per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan

hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each Capsule taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule;  $D$  is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine

» Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ).

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS

USP Phenylpropanolamine Hydrochloride RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

**Identification**—

A: If phenylpropanolamine hydrochloride is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for phenylpropanolamine hydrochloride*, exhibits a major peak for phenylpropanolamine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

B: If acetaminophen is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

C: If chlorpheniramine maleate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*. The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeast count does not exceed 10 cfu per g.

**pH** (791): between 2.6 and 7.5.

**Alcohol content** (if present), *Method II* (611): between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Assay for phenylpropanolamine hydrochloride**—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Phenylpropanolamine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8.0 mL of *Mobile phase*, dilute with water to volume, and mix.

*Chlorpheniramine standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

*Dextromethorphan standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

*System suitability solution 1* (for Oral Solution that contains either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

*System suitability solution 2* (for Oral Solution that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

*Assay preparation*—Transfer an accurately measured volume of the Oral Solution, equivalent to 2.5 mg of phenylpropanolamine hydrochloride, to a 50-mL volumetric flask, add about 40 mL of *Mobile phase*, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the phenylpropanolamine peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10  $\mu$ L of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution,  $R$ , between phenylpropanolamine and chlorphen-

iramine or between phenylpropanolamine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the phenylpropanolamine peaks. Calculate the quantity, in mg per mL, of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ), in the volume of Oral Solution taken by the formula:

$$50(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Phenylpropanolamine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of the Oral Solution taken; and  $r_U$  and  $r_S$  are the phenylpropanolamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a suitable degassed and filtered mixture of water, methanol, and glacial acetic acid (79:20:1). Make any necessary adjustments (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 16.5 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 2.5 mL of methanol, and mix until solution is complete. Dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.165 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 33 mg of acetaminophen, to a 200-mL volumetric flask, add 5 mL of methanol, and mix. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg per mL, of acetaminophen ( $C_8H_9NO_2$ ) in the volume of Oral Solution taken by the formula:

$$200(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; V is the volume, in mL, of the Oral Solution taken; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.08 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 0.4 mg of chlorpheniramine maleate, to a 50-mL volumetric flask. Add 40 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure

the responses for the chlorpheniramine peaks. Calculate the quantity, in mg per mL, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the volume of Oral Solution taken by the formula:

$$50(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; V is the volume, in mL, of the Oral Solution taken; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.4 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 2 mg of dextromethorphan hydrobromide, to a 50-mL volumetric flask, add 40 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg per mL, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the volume of Oral Solution taken by the formula:

$$(370.33/352.32)50(C/V)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine

» Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ).

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the

official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS

USP Phenylpropanolamine Hydrochloride RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

**Identification**—

**A:** If phenylpropanolamine hydrochloride is claimed in the labeling to be present, the retention time of the major peak for phenylpropanolamine in the chromatogram of the *Phenylpropanolamine assay preparation* corresponds to that in the chromatogram of the *Phenylpropanolamine standard preparation*, as obtained in the *Assay for phenylpropanolamine hydrochloride*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Chlorpheniramine assay preparation* corresponds to that in the chromatogram of the *Chlorpheniramine standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Dextromethorphan assay preparation* corresponds to that in the chromatogram of the *Dextromethorphan standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Dissolution, Procedure for a Pooled Sample** (711)—

**Medium:** 0.1 M hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Test solution**—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

**Procedure**—Determine the amounts of phenylpropanolamine hydrochloride, acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for phenylpropanolamine hydrochloride*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ), acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay for phenylpropanolamine hydrochloride**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monoba-

sic potassium phosphate, 0.05 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Phenylpropanolamine standard preparation**—Dissolve an accurately weighed quantity of USP Phenylpropanolamine Hydrochloride RS in water to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

**Chlorpheniramine standard preparation**—Prepare as directed in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Tablets that contain either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Phenylpropanolamine standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Tablets that contain no chlorpheniramine salt)—Mix equal volumes of the *Phenylpropanolamine standard preparation* and the *Dextromethorphan standard preparation*.

**Phenylpropanolamine assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 2.5 mg of phenylpropanolamine hydrochloride, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Phenylpropanolamine standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the phenylpropanolamine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 20  $\mu$ L of *System suitability solution 1* or *System suitability solution 2*, as appropriate: the resolution, *R*, between phenylpropanolamine and chlorpheniramine or between phenylpropanolamine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Phenylpropanolamine standard preparation* and the *Phenylpropanolamine assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the phenylpropanolamine peaks. Calculate the quantity, in mg, of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$50C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Phenylpropanolamine Hydrochloride RS in the *Phenylpropanolamine standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Phenylpropanolamine assay preparation* and the *Phenylpropanolamine standard preparation*, respectively.

**Assay for acetaminophen** (if present)—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until dissolved. Dilute with 0.1% phosphoric acid to volume, and mix.



**Assay preparation**—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 50-mL volumetric flask. Add about 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, mix, and filter. Transfer 25.0 mL of the filtered solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate** (if present)—

*Mobile phase*, *Phenylpropanolamine standard preparation*, *System suitability solution 1*, and *Chromatographic system*—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride*.

**Chlorpheniramine standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

**Chlorpheniramine assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.4 mg of chlorpheniramine maleate, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Add 0.2 mL of phosphoric acid, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Chlorpheniramine standard preparation* and the *Chlorpheniramine assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Chlorpheniramine standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Chlorpheniramine assay preparation* and the *Chlorpheniramine standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide** (if present)—

*Mobile phase*, *Phenylpropanolamine standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride*.

*System suitability solution 1* or *System suitability solution 2* (as appropriate)—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride*.

**Dextromethorphan standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan

Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

**Dextromethorphan assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 3 mg of dextromethorphan hydrobromide, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Add 0.2 mL of phosphoric acid, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Tablets taken by the formula:

$$(370.33/352.32)50C(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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### Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

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» Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ]. NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** <11>—

USP Acetaminophen RS

USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS

USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients contained in the article. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

**Identification**—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is purported to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Dissolution**, Procedure for a Pooled Sample (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

*Test preparation*—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

*Procedure*—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ], acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.

*Chlorpheniramine standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

*Dextromethorphan standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

*System suitability solution 1* (for Capsules that contain either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

*System suitability solution 2* (for Capsules that contain no chlorpheniramine)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

*Assay preparation*—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with water to obtain a solution having a concentration of about 0.12 mg of pseudoephedrine hydrochloride per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the Capsules taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; L is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule; D is the concentration, in mg per mL, of pseudoephedrine hydrochloride in the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule and the extent of dilution; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

*Mobile phase*, *System suitability solutions*, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.

*Chlorpheniramine standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

*Dextromethorphan standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

*Assay preparation*—Proceed as directed for the *Assay preparation* in the *Assay for pseudoephedrine hydrochloride* to obtain a solution having a concentration of about 0.24 mg of pseudoephedrine sulfate per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride*. Calculate the quantity, in mg, of pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)—**

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 25 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Add 0.2 mL of phosphoric acid, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.25 mg of acetaminophen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for Procedure: the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of acetaminophen in each Capsule;  $D$  is the concentration, in mg per mL, of acetaminophen in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of acetaminophen in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)—**

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 8 µg of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule;  $D$  is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)—**

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.04 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.04 mg of dextromethorphan hydrobromide per mL.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each Capsule taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule;  $D$  is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acetaminophen RS

USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS

USP Pseudoephedrine Hydrochloride RS

USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

### Identification—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Minimum fill** (755): meets the requirements.

### Uniformity of dosage units (905)—

FOR ORAL POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

*Chlorpheniramine standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

*Dextromethorphan standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

*System suitability solution 1* (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

*System suitability solution 2* (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

*Assay preparation*—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2.0 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.24 mg of pseudoephedrine hydrochloride per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ), in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container; *D* is the concentration, in mg per mL, of pseudoephedrine hydrochloride in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container, and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the Assay for chlorpheniramine maleate.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the Assay for dextromethorphan hydrobromide.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 6.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

**System suitability solution 1** (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Proceed as directed for the Assay preparation in the Assay for pseudoephedrine hydrochloride to obtain a solution having a concentration of about 0.48 mg of pseudoephedrine sulfate per mL.

**Procedure**—Proceed as directed for Procedure in the Assay for pseudoephedrine hydrochloride. Calculate the quantity, in mg, of pseudoephedrine sulfate  $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$  in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.50 mg of acetaminophen per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; L is the labeled quantity, in mg, of acetaminophen in each unit-dose container; D is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of acetaminophen in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of 8  $\mu$ g of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; L is the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container; D is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of 0.08 mg per mL.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. If necessary, quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a concentration of 0.08 mg of dextromethorphan hydrobromide per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan

hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container;  $D$  is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

#### Identification—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Uniformity of dosage units (905)**—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH (791):** between 3.7 and 7.5.

**Alcohol content (if present), Method II (611):** between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Microbial enumeration tests (61) and Absence of specified microorganisms (62)**—The total bacterial count does not exceed 100 cfu per g, the total combined molds and yeasts count does not exceed 10 cfu per g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography (621)*).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Oral Solution that contains either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Oral Solution that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Transfer an accurately measured volume of the Oral Solution, equivalent to 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask, add

80.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10 µL of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution, *R*, between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

**Mobile phase, System suitability solutions, and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 4.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to 30 mg of pseudoephedrine sulfate, to a 100-mL volumetric flask, add 80.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride*. Calculate the quantity, in mg, of pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a suitable degassed and filtered mixture of water, methanol, and glacial acetic acid (79:20:1). Make any necessary adjustments (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 16.5 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 2.5 mL of methanol, and mix until solution is complete. Dilute with water to volume, and mix to obtain

a solution having a known concentration of about 0.165 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 33 mg of acetaminophen, to a 200-mL volumetric flask, add 5 mL of methanol, and mix. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each mL of the Oral Solution taken by the formula:

$$200(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 7.5 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask,

add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each mL of the Oral Solution taken by the formula:

$$(370.33/352.32)(100C/V)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*;  $V$  is the volume, in mL, of the Oral Solution taken; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ]. NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### Identification—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the chromat-

ogram of the *Assay preparation*, obtained as directed in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*, exhibits a major peak for pseudoephedrine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** If acetaminophen is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

#### Dissolution, Procedure for a Pooled Sample <711>—

##### TEST 1—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Test preparation**—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

**Procedure**—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ], acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) are dissolved in 45 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** water; 900 mL.

**Apparatus, Time, Test preparation, Procedure, and Tolerances**—Proceed as directed for *Test 1*.

**Uniformity of dosage units <905>**: meet the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.



**Chlorpheniramine standard preparation**—Prepare as directed for the *Standard preparation* in the Assay for chlorpheniramine maleate.

**Dextromethorphan standard preparation**—Prepare as directed for the *Standard preparation* in the Assay for dextromethorphan hydrobromide.

**System suitability solution 1** (for Tablets that contain either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Tablets that contain no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 6 mg of pseudoephedrine hydrochloride, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10 µL of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution, *R*, between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

**Mobile phase, Chlorpheniramine standard preparation, Dextromethorphan standard preparation, System suitability solutions, and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 12 mg of pseudoephedrine sulfate, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

**Procedure**—Proceed as directed for *Procedure* in the Assay for pseudoephedrine hydrochloride. Calculate the quantity, in

mg, of pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Dilute with 0.1% phosphoric acid to volume, and mix.

**Assay preparation**—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 50-mL volumetric flask. Add about 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, mix, and filter. Transfer 25.0 mL of the filtered solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of chlorpheniramine maleate, to a 250-mL volumetric flask. Add 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate

( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for chlorpheniramine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide** (if present)—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Tablets taken by the formula:

$$(370.33 / 352.32)(100C)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets

» Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**USP Reference standards** (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS

USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS

**Identification**—

**A:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**C:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**Dissolution**, Procedure for a Pooled Sample (711)—

TEST 1—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

*Test solution*—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

*Procedure*—Determine the amounts of acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

*Tolerances*—Not less than 75% (Q) of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) are dissolved in 45 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Medium:* 0.1 M hydrochloric acid; 900 mL.

*Apparatus, Time, Test solution, Procedure, and Tolerances*—Proceed as directed for *Test 1*.

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

*Medium:* pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus, Time, Test solution, Procedure, and Tolerances*—Proceed as directed for *Test 1*.

**Uniformity of dosage units** (905): meet the requirements.

**Assay for acetaminophen**—

*Mobile phase*—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Dilute with 0.1% phosphoric acid to volume, and mix.

*Assay preparation*—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 50-mL volumetric flask. Add about 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, mix, and filter. Transfer 25.0 mL of the filtered solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7.

The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for chlorpheniramine maleate—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*Pseudoephedrine hydrochloride stock preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

*System suitability solution*—Mix equal volumes of the *Pseudoephedrine hydrochloride stock preparation* and the *Standard preparation*.

*Assay preparation*—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of chlorpheniramine maleate, to a 250-mL volumetric flask. Add about 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, mix, and filter.

*Chromatographic system*—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%. Inject about 10  $\mu$ L of the *System suitability solution*; the resolution,  $R$ , between pseudoephedrine and chlorpheniramine is not less than 2.0.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for chlorpheniramine

obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for dextromethorphan hydrobromide—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*Pseudoephedrine hydrochloride stock preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

*System suitability solution*—Mix equal volumes of the *Pseudoephedrine hydrochloride stock preparation* and the *Standard preparation*.

*Assay preparation*—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, mix, and filter.

*Chromatographic system*—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%. Inject about 10  $\mu$ L of the *System suitability solution*; the resolution,  $R$ , between pseudoephedrine and dextromethorphan is not less than 2.0.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Tablets taken by the formula:

$$(370.33 / 352.32)(100C)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Codeine Phosphate Capsules

» Acetaminophen and Codeine Phosphate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts

of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) and codeine phosphate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Codeine Phosphate RS

**Identification**—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer a portion of Capsule contents, equivalent to about 12 mg of codeine phosphate, to a separator, add 5 mL of water, 1 mL of ammonium hydroxide, and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare a Standard solution of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol containing 12 mg of each per mL. Apply 10  $\mu\text{L}$  of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methanol and ammonium hydroxide (49:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wave-length UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amounts of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) and codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) dissolved by employing the procedure set forth in the *Assay*, except to use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution* and to make any other necessary volumetric adjustments.

*Tolerances*—Not less than 75% (Q) of the labeled amounts of  $\text{C}_8\text{H}_9\text{NO}_2$  and  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

*Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Sample preparation*—Transfer the contents of 1 Capsule to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0-mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu\text{m}$  filter.

*Procedure*—Separately inject equal volumes (about 30  $\mu\text{L}$ ) of the *Standard preparation* and the *Sample preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) in the Capsule taken by the formula:

$$1000C_A(r_U / r_S)$$

in which  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$

are the peak responses obtained from the *Sample preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) in the Capsule taken by the formula:

$$(406.37/397.37)1000C_U(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C_U$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and the other terms are as defined herein.

**Assay**—

*Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Acetaminophen and Codeine Phosphate Tablets*.

*Assay preparation*—Remove as completely as possible the contents of not fewer than 20 Capsules, weigh, and mix. Transfer an accurately weighed portion of the combined contents, equivalent to about 300 mg of acetaminophen, to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0-mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu\text{m}$  filter.

*Procedure*—Separately inject equal volumes (about 30  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) in the portion of Capsules taken by the formula:

$$(LC_A / C_U)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of acetaminophen in each Capsule;  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based upon the labeled quantity per Capsule and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) in the portion of Capsules taken by the formula:

$$(406.37/397.37)(LC_C / C_U)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $L$  is the labeled quantity, in mg, of codeine phosphate hemihydrate in each Capsule;  $C_C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of codeine phosphate hemihydrate in the *Assay preparation*, based upon the labeled quantity per Capsule and the extent of dilution; and the other terms are as defined herein.

## Acetaminophen and Codeine Phosphate Oral Solution

» Acetaminophen and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) and co-

deine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Codeine Phosphate RS

**Identification**—

**A:** The retention times of the major peaks in the chromatograms of the *Assay preparation* correspond to those in the chromatograms of the *Standard preparations*, as obtained in the *Assay for acetaminophen* and the *Assay for codeine phosphate*, respectively.

**B:** Transfer a volume of Oral Solution, equivalent to about 12 mg of codeine phosphate, to a separator, add 1 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare a Standard solution of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol containing 12 mg of each per mL. Apply 10  $\mu\text{L}$  of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methanol and ammonium hydroxide (49:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 6.1.

**Alcohol content** (if present), *Method II* (611): between 90.0% and 120.0% of the labeled amount of  $\text{C}_2\text{H}_5\text{OH}$ , acetone being used as the internal standard.

**Assay for acetaminophen**—

*Mobile phase*—Prepare a suitable mixture of water and methanol (7:3), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Acetaminophen RS in *Mobile phase* to obtain a solution having a known concentration of about 0.48 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 120 mg of acetaminophen, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1000 theoretical plates, the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate

the quantity, in mg, of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) in each mL of the Oral Solution taken by the formula:

$$250(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses of acetaminophen obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for codeine phosphate**—

*Mobile phase*—Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol, water, tetrahydrofuran, and phosphoric acid (600:360:40:1) with stirring, and pass through a membrane filter having a 0.45- $\mu\text{m}$  or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Solvent mixture*—Mix water and methanol (7:3).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in *Solvent mixture* to obtain a solution having a known concentration of about 0.12 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 12 mg of codeine phosphate hemihydrate, to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the codeine peaks. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.37)(100C/V)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses of codeine phosphate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Codeine Phosphate Oral Suspension

» Acetaminophen and Codeine Phosphate Oral Suspension is a suspension of Acetaminophen and Codeine Phosphate in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) and codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS  
USP Codeine Phosphate RS

**Identification**—

**A:** The retention times of the major peaks in the chromatograms of the *Assay preparations* correspond to those in the chromatograms of the *Standard preparation*, as obtained in the *Assay*.

**B:** Using the Oral Suspension, proceed as directed for *Identification test B* under *Acetaminophen and Codeine Phosphate Oral Solution*: the indicated results are obtained.

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 6.1.

**Assay**—

**Mobile phase**—Dissolve 4.9 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.9, add 216 mg of sodium 1-octanesulfonate, and mix. Add 100 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of 0.01 N sodium hydroxide and methanol (70:30).

**Codeine phosphate standard stock solution**—Prepare a solution of USP Codeine Phosphate RS, accurately weighed, in *Diluent* having a known concentration of about 0.5 mg per mL.

**Standard stock preparation**—Transfer an accurately weighed quantity of about 5/*J* mg of USP Acetaminophen RS, *J* being the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of codeine phosphate hemihydrate, and 10.0 mL of *Codeine phosphate standard stock solution* to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Standard preparation**—Transfer 10.0 mL of *Standard stock preparation* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.01 mg of USP Codeine Phosphate RS and 0.01/*J* mg of USP Acetaminophen RS per mL.

**Assay preparation**—Transfer an accurately measured volume of well-mixed Oral Suspension, equivalent to about 50 mg of acetaminophen, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and mix by mechanical means for 30 minutes. Dilute with *Diluent* to volume, and mix. [NOTE—Foaming may be minimized by adding a few drops of acetonitrile before diluting with *Diluent* to volume.] Centrifuge a portion of this mixture, and transfer 10.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Resolution solution**—Prepare a solution in *Diluent* containing about 0.02 mg of sodium benzoate and 0.03 mg of methylparaben per mL. Transfer 10.0 mL of this solution and 10.0 mL of *Standard stock preparation* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and measure the peak responses as directed for *Procedure*: the relative retention times are about 0.25 for acetaminophen, 0.5 for benzoate, 1.0 for codeine, and 1.3 for methylparaben; and the resolution, *R*, between each pair of adjacent peaks is not less than 2. Chromatograph the *Standard preparation*, and record the

peak responses as directed for *Procedure*: the tailing factor for each analyte peak is not more than 2, the peak efficiency is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) in each mL of the Oral Suspension taken by the formula:

$$500(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Suspension taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O) in each mL of the Oral Suspension taken by the formula:

$$(406.37/397.37)(500C/V)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Suspension taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the codeine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Codeine Phosphate Tablets

» Acetaminophen and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and codeine phosphate hemihydrate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS  
USP Codeine Phosphate RS

**Identification**—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** A quantity of finely powdered Tablets, equivalent to about 12 mg of codeine phosphate, meets the requirements of *Identification test B* under *Acetaminophen and Codeine Phosphate Capsules*.

**Dissolution** (711)—

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and codeine phosphate hemihydrate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O) dissolved by employing the procedure set forth in the *Assay*, except to use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution* and to make any other necessary volumetric adjustments.

**Tolerances**—Not less than 75% (*Q*) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**PROCEDURE FOR CONTENT UNIFORMITY—**

**Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system**—Prepare as directed in the Assay.

**Sample preparation**—Transfer 1 Tablet to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Procedure**—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Sample preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Tablet taken by the formula:

$$1000C_A(r_U / r_S)$$

in which  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Sample preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in each Tablet taken by the formula:

$$(406.37/397.37)1000C_C(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C_C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and the other terms are as defined herein.

**Assay—**

**Buffer solution**—Dissolve 2.04 g of monobasic potassium phosphate in about 950 mL of water. Add 2 mL of triethylamine, adjust with phosphoric acid to a pH of 2.35, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (92:8). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Codeine phosphate standard stock solution**—Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL.

**Standard preparation**—Transfer about 30 mg of USP Acetaminophen RS and 100  $\mu$ L of *Codeine phosphate standard stock solution*,  $J$  being the ratio of the labeled amount, in mg, of codeine phosphate hemihydrate to that of acetaminophen, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.3 mg of acetaminophen and 0.3 $J$  mg of codeine phosphate hemihydrate per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 300 mg of acetaminophen, to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector

and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between acetaminophen and codeine phosphate is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0% and 3.0%, respectively.

**Procedure**—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$(LC_A / C_U)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of acetaminophen in each Tablet;  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(406.37/397.37)(LC_C / C_U)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $L$  is the labeled quantity, in mg, of codeine phosphate hemihydrate in each Tablet;  $C_C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of codeine phosphate hemihydrate in the *Assay preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and the other terms are as defined herein.

## Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution

» Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Acetaminophen ( $C_8H_9NO_2$ ), Dextromethorphan Hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), Doxylamine Succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ), and Pseudoephedrine Hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS  
USP Dextromethorphan Hydrobromide RS  
USP Doxylamine Succinate RS  
USP Pseudoephedrine Hydrochloride RS

**Identification—**

**A:** The retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**B:** The retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**C:** The retention time of the major peak for doxylamine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for doxylamine succinate*.

**D:** The retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride*.

#### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.5 and 6.3.

**Alcohol content** (if present), *Method II* (611): between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—The total bacterial count does not exceed 100 cfu per g, the total combined molds and yeasts count does not exceed 10 cfu per g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

#### Assay for acetaminophen—

**Mobile phase**—Prepare a filtered and degassed mixture of water and methanol (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Acetaminophen RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Dissolve an accurately measured volume of Oral Solution, equivalent to about 200 mg of acetaminophen, in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.2 mg of acetaminophen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, determined from the analyte peak, is not less than 500 theoretical plates; the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each mL of the Oral Solution taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *L* is the labeled quantity, in mg per mL, of acetaminophen in the Oral Solution; *D* is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for dextromethorphan hydrobromide—

**0.05 M Buffer solution**—Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water.

**Mobile phase**—Prepare a filtered and degassed mixture of 0.05 M Buffer solution and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Dextromethorphan Hydrobromide RS, USP Doxylamine Succinate RS, and USP Pseudoephedrine Hydrochloride RS in *Mobile phase*, and, dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1, 0.04, and 0.2 mg per mL, respectively.

**Assay preparation**—Dissolve an accurately measured volume of Oral Solution, equivalent to about 5 mg of dextromethorphan hydrobromide, in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution having a concentration of about 0.1 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L9. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.38 for pseudoephedrine, 0.65 for dextromethorphan, and 1.0 for doxylamine; the tailing factors for the dextromethorphan, doxylamine, and pseudoephedrine peaks are not more than 2.5; the column efficiencies for the dextromethorphan, doxylamine, and pseudoephedrine peaks are not less than 500; and the relative standard deviations of the dextromethorphan, doxylamine, and pseudoephedrine responses for replicate injections are not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the dextromethorphan peak responses. Calculate the quantity, in mg per mL, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Oral Solution taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; *C* is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; *L* is the labeled quantity, in mg per mL, of dextromethorphan hydrobromide in the Oral Solution; *D* is the concentration, in mg per mL, of dextromethorphan hydrobromide in the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for doxylamine succinate—

**0.05 M Buffer solution, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for dextromethorphan hydrobromide*.

**Assay preparation**—Dissolve an accurately measured volume of Oral Solution, equivalent to about 2 mg of doxylamine succinate, in *Mobile phase*, and dilute with *Mobile phase* to obtain a solution having a concentration of about 0.04 mg per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for dextromethorphan hydrobromide*. Calculate the quantity, in mg per mL, of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ) in the portion of Oral Solution taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Doxylamine Succinate RS in the *Standard preparation*; *L* is the



labeled quantity, in mg per mL, of doxylamine succinate in the Oral Solution;  $D$  is the concentration, in mg per mL, of doxylamine succinate in the Assay preparation; and  $r_U$  and  $r_S$  are the doxylamine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

#### Assay for pseudoephedrine hydrochloride—

*0.05 M Buffer solution, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay for dextromethorphan hydrobromide.

*Assay preparation*—Dissolve an accurately measured volume of Oral Solution, equivalent to about 10 mg of pseudoephedrine hydrochloride, in *Mobile phase*, and dilute with *Mobile phase* to obtain a solution having a concentration of about 0.2 mg per mL.

*Procedure*—Proceed as directed for Procedure in the Assay for dextromethorphan hydrobromide. Calculate the quantity, in mg per mL, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the Standard preparation;  $L$  is the labeled amount, in mg per mL, of pseudoephedrine hydrochloride in the Oral Solution;  $D$  is the concentration, in mg per mL, of pseudoephedrine hydrochloride in the Assay preparation; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Acetaminophen and Diphenhydramine Citrate Tablets

» Acetaminophen and Diphenhydramine Citrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS

USP Diphenhydramine Citrate RS

**Identification**—The retention times of the major peaks in the chromatograms of the Assay preparations, obtained in the Assay for acetaminophen and in the Assay for diphenhydramine citrate, relative to the retention times of the respective internal standards, correspond to those in the chromatogram of the respective Standard preparation.

#### Dissolution, Procedure for a Pooled Sample (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

*Procedure*—Determine the amount of acetaminophen ( $C_8H_9NO_2$ ) and of diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ) dissolved, employing the procedures set forth in the Assay for acetaminophen and the Assay for diphenhydramine citrate, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{17}H_{21}NO \cdot C_6H_8O_7$  are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for Content Uniformity with respect to acetaminophen and to diphenhydramine citrate.

#### Assay for acetaminophen—

*Mobile phase*—Prepare a suitable degassed and filtered mixture of water and methanol (60:40), making adjustments if necessary (see System Suitability under Chromatography (621)).

*Internal standard solution*—Prepare a solution of guaifenesin in a mixture of water and methanol (4:1) to obtain a solution containing 8.0 mg per mL.

*Standard preparation*—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in 2.5 mL of methanol, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetaminophen, to a 100-mL volumetric flask, add 25 mL of methanol, and shake by mechanical means for 10 minutes. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

*Chromatographic system* (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about  $35 \pm 0.5^\circ$ . Chromatograph the Standard preparation, and record the responses as directed for Procedure: the column efficiency as determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 6.0; and the relative standard deviation of the peak response ratios for replicate injections is not more than 2.5%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for acetaminophen and 1.0 for guaifenesin. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$10W_S(R_U / R_S)$$

in which  $W_S$  is the weight, in mg, of USP Acetaminophen RS taken; and  $R_U$  and  $R_S$  are the ratios of the peak response of acetaminophen to that of the internal standard obtained from the Assay preparation and the Standard preparation, respectively.

#### Assay for diphenhydramine citrate—

*Mobile phase*—Prepare a suitable degassed and filtered mixture of methanol, water, and glacial acetic acid (61:38:1) containing 1.0813 g of sodium 1-octanesulfonate in each 1000 mL of solution, making adjustments if necessary (see System Suitability under Chromatography (621)).

*Solvent mixture*—Prepare a mixture of methanol and water (1:1).

*Internal standard solution*—Prepare a solution of xylometazoline hydrochloride in water having a concentration of about 8 mg per mL.

*Standard preparation*—Transfer about 38 mg of USP Diphenhydramine Citrate RS, accurately weighed, to a 100-mL volumetric flask containing 500 mg of acetaminophen. Add 5.0 mL of Internal standard solution and about 50 mL of Solvent mixture, and mix until solution is complete. Dilute with Solvent mixture to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of

the powder, equivalent to about 38 mg of diphenhydramine citrate, to a 100-mL volumetric flask, add about 65 mL of *Solvent mixture*, and shake by mechanical means for about 15 minutes. Add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at about 35 ± 0.5°. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the column efficiency as determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 1.7; the resolution, *R*, between the analyte and internal standard peaks is not less than 2.5; and the relative standard deviation of the peak response ratios for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the diphenhydramine citrate and xylometazoline hydrochloride peaks. The relative retention times are about 0.3 for acetaminophen, 0.7 for diphenhydramine citrate, and 1.0 for xylometazoline hydrochloride, respectively. Calculate the quantity, in mg, of diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ) in the portion of Tablets taken by the formula:

$$W_s(R_U / R_s)$$

in which  $W_s$  is the weight, in mg, of USP Diphenhydramine Citrate RS taken; and  $R_U$  and  $R_s$  are the ratios of the peak response of diphenhydramine citrate to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets

» Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards <11>—

USP Acetaminophen RS  
USP Diphenhydramine Hydrochloride RS  
USP Pseudoephedrine Hydrochloride RS

### Identification—

**A:** The retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**B:** The retention time of the major peak for diphenhydramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for diphenhydramine hydrochloride*.

**C:** The retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride*.

as obtained in the *Assay for pseudoephedrine hydrochloride*.

### Dissolution, Procedure for a Pooled Sample <711>—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amounts of acetaminophen ( $C_8H_9NO_2$ ), diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by employing the following method.

**Buffer solution, Diluting solvent, Mobile phase, and Chromatographic system**—Proceed as directed in the *Assay for acetaminophen*.

**Standard solution**—Prepare as directed for the *Standard preparation* in the *Assay for acetaminophen*.

**Test solution 1**—Combine equal volumes of the filtered solutions under test, and use the pooled sample.

**Test solution 2**—Transfer 5.0 mL of *Test solution 1* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Using *Test solution 1* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay for diphenhydramine hydrochloride* and the *Assay for pseudoephedrine hydrochloride*, and determine the amounts of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved. Using *Test solution 2* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay for acetaminophen*, and determine the amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$ ,  $C_{17}H_{21}NO \cdot HCl$ , and  $C_{10}H_{15}NO \cdot HCl$  is dissolved in 45 minutes.

FOR TABLETS LABELED AS CHEWABLE—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 45 minutes.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$ ,  $C_{17}H_{21}NO \cdot HCl$ , and  $C_{10}H_{15}NO \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay for acetaminophen—

**Buffer solution**—Transfer 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask, and add water to dissolve. Add 2.0 mL of triethylamine, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 4.0.

**Diluting solvent**—Prepare a mixture of *Buffer solution* and acetonitrile (89:11).

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (94:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve accurately weighed quantities of USP Acetaminophen RS, USP Diphenhydramine Hydrochloride RS, and USP Pseudoephedrine Hydrochloride RS in *Diluting solvent*, and dilute quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having known concentrations of about 0.025 mg per mL, 0.0125 mg per mL, and 0.03 mg per mL, respectively.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetaminophen, to a 100-mL volumetric flask. Add about 75 mL of *Diluting solvent*, shake, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluting solvent* to volume,

and mix. Dilute an accurately measured volume of the solution quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having a concentration of about 25 µg of acetaminophen per mL.

**Chromatographic system** (see *Chromatography* <621>).—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiencies, determined from the acetaminophen, diphenhydramine, and pseudoephedrine peaks, are not less than 3000 theoretical plates; the tailing factors for the acetaminophen, diphenhydramine, and pseudoephedrine peaks are not more than 2.0; and the relative standard deviations determined from the acetaminophen, diphenhydramine hydrochloride, and pseudoephedrine hydrochloride responses for replicate injections are not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the acetaminophen peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$20C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for diphenhydramine hydrochloride—

**Buffer solution, Diluting solvent, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for acetaminophen*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Add about 75 mL of *Diluting solvent*, shake, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluting solvent* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having a concentration of about 12.5 µg of diphenhydramine per mL.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the diphenhydramine peak responses. Calculate the quantity, in mg, of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the diphenhydramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for pseudoephedrine hydrochloride—

**Buffer solution, Diluting solvent, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for acetaminophen*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask, add about 75 mL of *Diluting solvent*, shake, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluting solvent* to volume, and mix. Dilute an accurately measured volume

of this solution quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having a concentration of about 30 µg of pseudoephedrine hydrochloride per mL.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the pseudoephedrine peak responses. Calculate the quantity, in mg per mL, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Pseudoephedrine Hydrochloride Tablets

» Acetaminophen and Pseudoephedrine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Acetaminophen RS

USP Pseudoephedrine Hydrochloride RS

**Identification**—The retention times of the acetaminophen and pseudoephedrine peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution, Procedure for a Pooled Sample <711>—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by employing the following method.

**Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Prepare a solution in *Dissolution Medium* having known concentrations of about  $L/900$  mg of USP Pseudoephedrine Hydrochloride RS and  $L/900$  mg of USP Acetaminophen RS per mL, in which L is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet; and J is the ratio of the labeled quantity, in mg, of acetaminophen to the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet.

**Test solution**—Use a filtered portion of the solution under test.

**Chromatographic system**—Proceed as directed in the *Assay*, except to inject the *Standard solution*.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen and pseudoephedrine peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ )

and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by the formula:

$$900C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{10}H_{15}NO \cdot HCl$  is dissolved in 45 minutes.

FOR TABLETS LABELED AS CHEWABLE—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 45 minutes.

**Standard solution, Test solution, Chromatographic system, and Procedure**—Proceed as directed above in *Procedure for a Pooled Sample*.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{10}H_{15}NO \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Diluent**—Prepare a mixture of water and acetonitrile (90:10).

**Mobile phase**—Prepare a solution of 0.005 M ethanesulfonic acid and 0.05 M monobasic potassium phosphate. Prepare a filtered and degassed mixture of this solution and acetonitrile (900:100), and adjust with 5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.6. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Pseudoephedrine hydrochloride stock standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.6 mg per mL.

**Standard preparation**—Transfer about 6/ $j$  mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask,  $j$  being the ratio of the labeled quantity, in mg, of acetaminophen to the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet. Add 2.0 mL of 1 N hydrochloric acid and about 20 mL of *Diluent*, and mix to dissolve. Add 10.0 mL of *Pseudoephedrine hydrochloride stock standard solution*, dilute with *Diluent* to volume, and mix. This solution contains about 0.06/ $j$  mg of USP Acetaminophen RS and 0.06 mg of USP Pseudoephedrine Hydrochloride RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of pseudoephedrine hydrochloride, to a 500-mL volumetric flask, add 10.0 mL of 1 N hydrochloric acid and about 100 mL of *Diluent*, and sonicate for 30 minutes, with occasional shaking. Allow to cool, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a glass fiber filter, and use the filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  25-cm column containing base-deactivated or end-capped packing L1. The flow rate is about 3 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*; the retention time for the acetaminophen peak is not less than 2 minutes and the relative retention times are about 0.55 for acetaminophen and 1.0 for pseudoephedrine; the resolution  $R$ , between acetaminophen and pseudoephedrine is not less

than 3.5; the tailing factor for the pseudoephedrine peak is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen and pseudoephedrine peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Tramadol Hydrochloride Tablets

### DEFINITION

Acetaminophen and Tramadol Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ).

### IDENTIFICATION

- The retention time of the major peaks in the *Tramadol sample solution* and the *Acetaminophen sample solution* corresponds to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Tetrahydrofuran, triethylamine, water, and trifluoroacetic acid (8:0.1:92:0.1). [NOTE—The apparent pH of the final solvent mixture should be between 2.2 and 2.4.]

**Diluent:** Methanol and water (1:9)

**Standard solution:** 0.065 mg/mL of USP Acetaminophen RS and 0.075 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*. [NOTE—Sonication may be used to aid dissolution.]

**Sample stock solution:** Weigh NLT 20 Tablets, and determine the average Tablet weight. Grind the Tablets into a fine powder, and transfer an amount equivalent to one Tablet to a 50-mL volumetric flask. Add 30 mL of *Diluent* with continuous shaking to disperse the powder. Sonicate for 15 min with intermittent shaking, and shake the flask on a mechanical shaker for 30 min. Dilute with *Diluent* to volume, and mix well. Centrifuge the suspension, and use the supernatant for subsequent dilutions.

**Tramadol sample solution:** 75  $\mu$ g/mL of tramadol hydrochloride in *Diluent* from the *Sample stock solution*

**Acetaminophen sample solution:** 65  $\mu$ g/mL of acetaminophen in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** 216 nm for tramadol hydrochloride and 249 nm for acetaminophen

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L11

**Column temperature:** 50°

**Flow rate:** 1.0 mL/min

**Injection size:** 20  $\mu$ L

**Run time:** Four times the retention time of acetaminophen

**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 10.0 between acetaminophen and tramadol hydrochloride**Column efficiency:** NLT 2000 theoretical plates for each analyte**Tailing factor:** NMT 2.0 for each analyte**Relative standard deviation:** NMT 2.0% for each analyte**Analysis****Samples:** *Standard solution, Tramadol sample solution, and Acetaminophen sample solution*Calculate the percentage of the labeled amount of tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Tramadol sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of tramadol hydrochloride in the *Tramadol sample solution* (mg/mL)Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Acetaminophen sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of acetaminophen in the *Acetaminophen sample solution* (mg/mL)**Acceptance criteria:** NLT 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION <711>****Test 1****Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 50 rpm**Time:** 30 min**Standard solution:** 0.36 mg/mL of USP Acetaminophen RS and 0.04 mg/mL of USP Tramadol Hydrochloride RS in *Medium***Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.**Buffer solution:** 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.50.**Mobile phase:** Acetonitrile and *Buffer solution* (1:4)**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 272 nm**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7**Column temperature:** 25°**Flow rate:** 1.0 mL/min**Injection size:** 25  $\mu$ L**System suitability****Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and tramadol hydrochloride are about 0.5 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 5.0 between the peaks for acetaminophen and tramadol hydrochloride**Relative standard deviation:** NMT 2.0% for both the acetaminophen and tramadol hydrochloride peaks**Analysis****Samples:** *Standard solution and Sample solution*Record the chromatograms for two times the retention time of tramadol hydrochloride. Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U \times C_S \times V \times 100)/(r_S \times L)$$

 $r_U$  = peak response of acetaminophen or tramadol hydrochloride from the *Sample solution* $C_S$  = concentration of USP Acetaminophen RS or USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 900 mL $r_S$  = peak response of acetaminophen or tramadol hydrochloride from the *Standard solution* $L$  = label claim for acetaminophen or tramadol hydrochloride (mg/Tablet)**Tolerances:** NLT 80% (Q) of the labeled amounts of acetaminophen and tramadol hydrochloride is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 50 rpm**Time:** 20 min**Standard solution, Sample solution, Buffer solution, Mobile phase, Chromatographic system, and Analysis:** Proceed as directed in *Dissolution Test 1*.**Tolerances:** NLT 80% (Q) of the labeled amounts of acetaminophen and tramadol hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**OTHER COMPONENTS****Change to read:****• LIMIT OF *p*-AMINOPHENOL**[NOTE—All Standards, the *Sample solution*, and the *Blank solution* must be mixed with the *Basic ferricyanide solution* and analyzed as soon as possible after a 30-min waiting period.]**Diluent:** Methanol and water (1:1)**Basic ferricyanide solution:** Dissolve 1 g of sodium nitroferricyanide (ERR 1-May-2012) and 1 g of anhydrous sodium carbonate in 100 mL of water.**Standard solution:** Dissolve USP *p*-Aminophenol RS in *Diluent* to obtain a solution having a known concentration of 0.05 mg/mL. Sonicate if necessary to dissolve. Transfer 5 mL of the resulting solution to a 100-mL volumetric flask, and add 50 mL of *Diluent* and 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and mix. Let stand for 30 min. Pass the solution through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate.**Sample solution:** Weigh NLT 20 Tablets. Grind the Tablets into a fine powder. Accurately transfer an amount of powder, equivalent to about 5 g of acetaminophen based on the label claim, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate for 15 min with intermittent shaking, followed by mechanical shaking for 30 min. Add 6 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, mix, and let stand for 30 min. Centrifuge a portion of the solution, and pass the clear supernatant through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.**Blank solution:** Add 50 mL of *Diluent* to a 100-mL volumetric flask. Add 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and let stand for 30 min. Pass a portion of the solution through a nylon mem-

brane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV-Vis

Analytical wavelength: 710 nm

Cell: 1 cm

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Relative standard deviation: NMT 6.0%

[NOTE—The percent difference between the initial and final absorbance readings of the *Standard solution* differs by NMT 10%.]

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *p*-aminophenol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = absorbance from the *Sample solution*

$r_S$  = absorbance from the *Standard solution*

$C_S$  = concentration of USP *p*-Aminophenol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.01%

#### IMPURITIES

##### • ORGANIC IMPURITIES

Mobile phase, Diluent, and Sample stock solution: Proceed as directed in the *Assay*.

Standard solution: 0.75  $\mu$ g/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent*

Sample solution: Pass a suitable volume of *Sample stock solution* through a nylon membrane filter of 0.45- $\mu$ m pore size. Use the filtrate after discarding the first 4 mL of filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 216 nm

Column: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L11

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection size: 30  $\mu$ L

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Resolution: NLT 2.0 between tramadol related compound A and tramadol hydrochloride

Column efficiency: NLT 2000 theoretical plates for tramadol hydrochloride

Relative standard deviation: NMT 6.0% for tramadol hydrochloride

#### Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*  
[NOTE—Disregard the peaks due to the *Diluent*.]

Calculate the percentage of each known and unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of tramadol hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of tramadol hydrochloride in the *Sample solution* ( $\mu$ g/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
O-Desmethyl-tramadol <sup>a</sup>	0.60	0.2
Tramadol related compound A <sup>b</sup>	0.80	0.2
Tramadol hydrochloride	1.0	—
Acetaminophen	0.38	—
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.8

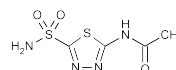
<sup>a</sup> 3-[(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]phenol.

<sup>b</sup> (*RS*,*SR*)-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
  - USP Acetaminophen RS
  - 4'-Hydroxyacetanilide.
  - C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> 151.16
  - USP *p*-Aminophenol RS
  - 4-Amino-1-hydroxybenzene.
  - C<sub>6</sub>H<sub>7</sub>NO 109.13
  - USP Tramadol Hydrochloride RS
  - (±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride.
  - C<sub>16</sub>H<sub>25</sub>NO<sub>2</sub> · HCl 299.84
  - USP Tramadol Related Compound A RS
  - RS*,*SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride.

## Acetazolamide



C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub> 222.25

Acetamide, *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-

*N*-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide [59-66-5].

» Acetazolamide contains not less than 98.0 percent and not more than 102.0 percent of C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, and store at room temperature.

#### USP Reference standards (11)—

USP Acetazolamide RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** Dissolve about 100 mg in 5 mL of 1 N sodium hydroxide. Add 5 mL of a solution made by dissolving 100 mg of hydroxylamine hydrochloride and 80 mg of cupric sulfate in 10 mL of water. Mix, and heat the resulting pale yellow solution on a steam bath for 5 minutes: a clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.

**Water**, *Method I* (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Digest 1.5 g with 75 mL of water at about 70° for 5 minutes. Cool to room temperature, and filter: a 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

**Sulfate** (221)—A 25-mL portion of the filtrate prepared in the test for *Chloride* shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

**Selenium** (291): 0.003%, a 200-mg specimen being used.

**Heavy metals**, *Method II* (231): 0.002%.

**Silver-reducing substances**—Thoroughly wet 5.0 g with alcohol. Add 125 mL of water, 10 mL of nitric acid, and 5.0 mL of 0.1 N silver nitrate VS. Stir with a mechanical stirrer for 30 minutes. Filter, add 5 mL of ferric ammonium sulfate TS to the filtrate, and titrate with 0.1 N ammonium thiocyanate VS to a reddish-brown endpoint: not less than 4.8 mL of 0.1 N ammonium thiocyanate is required.

**Ordinary impurities** (466)—

*Test solution*: a mixture of acetone and methanol (1:1).

*Standard solution*: a mixture of acetone and methanol (1:1).

*Eluent*: a mixture of *n*-propyl alcohol and 1 N ammonium hydroxide (88:12).

*Visualization*: 1.

**Assay**—Dissolve about 200 mg of Acetazolamide, accurately weighed, in a small volume of pyridine in a 10-mL volumetric flask, add the solvent to volume, and mix. Similarly, dissolve an accurately weighed quantity of USP Acetazolamide RS in pyridine to obtain a Standard solution having a known concentration of about 20 mg per mL. Concomitantly determine the absorbances of both solutions in 0.1-mm cells at the wavelength of maximum absorbance at about 7.38  $\mu\text{m}$  (1350  $\text{cm}^{-1}$ ), with a suitable IR spectrophotometer, using pyridine as the blank. Calculate the quantity, in mg, of  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$  in the portion of Acetazolamide taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Acetazolamide RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Acetazolamide and the Standard solution, respectively.

## Acetazolamide for Injection

» Acetazolamide for Injection is prepared from Acetazolamide with the aid of Sodium Hydroxide. It is suitable for parenteral use. The contents of each container, when constituted as directed in the labeling, yield a solution containing not less than 95.0 percent and not more than 110.0 percent of the labeled amount of acetazolamide ( $\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), preferably of Type III glass, and store at room temperature.

**USP Reference standards** (11)—

USP Acetazolamide RS

USP Endotoxin RS

**Completeness of solution** (641)—A 1.0-g portion dissolves in 10 mL of carbon dioxide-free water to yield a clear solution.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—

**A**: Dissolve about 500 mg in 5 mL of water, add 2 drops of hydrochloric acid, and allow the mixture to stand for about 15 minutes. Filter through a fine sintered-glass funnel, wash with several small portions of water, and dry in vacuum over silica gel for 3 hours: the crystals so obtained respond to the *Identification* tests under *Acetazolamide*.

**B**: It responds to the tests for *Sodium* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of acetazolamide.

**pH** (791): between 9.0 and 10.0, in a freshly prepared solution (1 in 10).

**Other requirements**—It meets the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and *Labeling* under *Injections* (1).

**Assay**—Dissolve the contents of 1 container of Acetazolamide for Injection in an accurately measured volume of water corresponding to the volume of solvent specified in the labeling. Dilute a portion of the solution quantitatively and stepwise with water to obtain a solution having a concentration of about 500  $\mu\text{g}$  of acetazolamide per mL. Pipet 5 mL of the solution into a 250-mL volumetric flask, add 25 mL of 1 N hydrochloric acid, then add water to volume, and mix. Dissolve an accurately weighed quantity of USP Acetazolamide RS in sodium hydroxide solution (1 in 100) to obtain a Standard solution having a known concentration of about 100  $\mu\text{g}$  per mL. Dilute 10.0 mL of this solution with 0.1 N hydrochloric acid to 100 mL. Concomitantly determine the absorption of both solutions at the wavelength of maximum absorbance at about 265 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in  $\mu\text{g}$ , of  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$  in the 5.0-mL portion of the solution of Acetazolamide for Injection taken by the formula:

$$25C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Acetazolamide RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Acetazolamide for Injection and the Standard solution, respectively.

## Acetazolamide Oral Suspension

### DEFINITION

Acetazolamide Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acetazolamide ( $\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$ ).

Prepare Acetazolamide Oral Suspension, 25 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795); see also *Acetazolamide Oral Solution*).

Acetazolamide	2.5 g
Vehicle: a mixture of Vehicle for Oral Solution, <i>NF</i> (regular or sugar-free), and Vehicle for Oral Suspension, <i>NF</i> (1:1), or Cherry Syrup, <i>NF</i> , a sufficient quantity to make	100 mL

[NOTE—If tablets are used instead of bulk powder, the preparation becomes a suspension and should be labeled as such.]

If using tablets, place in a mortar and comminute to a fine powder, or add *Acetazolamide* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add

enough liquid *Vehicle* to bring to final volume, and mix well.

## ASSAY

### • PROCEDURE

**Mobile phase:** Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, and add 20 mL of methanol and 30 mL of acetonitrile. Adjust with glacial acetic acid to a pH of 4.0.

**Standard stock solution:** Transfer about 25 mg of USP Acetazolamide RS, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume, and mix.

**Standard solution:** 250 µg/mL from the *Standard stock solution* in water

**Sample solution:** 250 µg/mL of acetazolamide from Oral Suspension in *Mobile phase*. Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^{\circ}$  until analyzed. At the time of analysis, remove the sample from the freezer, allow to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20 µL

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for the acetazolamide peak is about 3 min.]

### Suitability requirements

**Relative standard deviation:** NMT 1.1% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of acetazolamide in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of acetazolamide in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

## SPECIFIC TESTS

- **pH** <791>: 4.0–5.0 (*Vehicle* for Oral Solution and *Vehicle* for Oral Suspension), 3.1–3.9 (*Cherry Syrup*)

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded
- **USP REFERENCE STANDARDS** <11>  
USP Acetazolamide RS

## Acetazolamide Tablets

» Acetazolamide Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards <11>—

USP Acetazolamide RS

**Identification**—Extract a quantity of finely powdered Tablets, equivalent to about 500 mg of acetazolamide, with 50 mL of acetone. Filter, and add sufficient solvent hexane to the filtrate to cause formation of a heavy, white precipitate. Collect the precipitate on a medium-porosity, sintered-glass funnel, and dry with suction: the acetazolamide so obtained responds to the *Identification* tests under *Acetazolamide*.

### Dissolution <711>—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 60 minutes.

**Procedure**—Determine the amount of  $C_4H_6N_4O_3S_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Acetazolamide RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_4H_6N_4O_3S_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

### Assay—

**Mobile phase**—Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of  $4.0 \pm 0.05$ . Filter and degas the solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard acetazolamide stock solution**—Transfer about 25 mg of USP Acetazolamide RS, accurately weighed, to a 25-mL volumetric flask, add 2.5 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume, and mix.

**Internal standard solution**—Transfer about 100 mg of sulfadiazine to a 100-mL volumetric flask, add 10 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume, and mix.

**Standard preparation**—Transfer 10.0 mL of *Standard acetazolamide stock solution* and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask, add 10 mL of 0.5 N sodium hydroxide, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Acetazolamide RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetazolamide, to a 100-mL volumetric flask, add 10 mL of 0.5 N sodium hydroxide, and sonicate for 5 minutes. Cool to room temperature, dilute with water to volume, and mix. Filter a portion of this solution, discarding the first 20 mL of the filtrate. Transfer 10.0 mL of the clear filtrate to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution* and 10 mL of 0.5 N sodium hydroxide, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector



and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R_s$ , between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation of the ratios of the analyte peak response to the internal standard peak response for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for acetazolamide and 1.0 for sulfadiazine. Calculate the quantity, in mg, of acetazolamide ( $C_4H_6N_4O_3S_2$ ) in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Acetazolamide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the analyte peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glacial Acetic Acid



$C_2H_4O_2$  60.05  
Acetic acid [64-19-7].

### DEFINITION

Glacial Acetic Acid contains NLT 99.5% and NMT 100.5%, by weight, of  $C_2H_4O_2$ .

### IDENTIFICATION

- **IDENTIFICATION TESTS—GENERAL, Acetate <191>**: Meets the requirements  
**Sample solution** (for lanthanum nitrate test): Glacial Acetic Acid and water (1:100)

### ASSAY

- **PROCEDURE**  
**Sample solution**: Measure 2 mL of Glacial Acetic Acid into a glass-stoppered flask, previously tared while containing about 20 mL of water, and weigh again to obtain the weight of the substance under assay.  
**Analysis**: Add 20 mL of water, then add phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of  $C_2H_4O_2$ .  
**Acceptance criteria**: 99.5%–100.5%

### IMPURITIES

#### Inorganic Impurities

- **LIMIT OF NONVOLATILE RESIDUE**: Evaporate 20 mL in a tared dish, and dry at 105° for 1 h: the weight of the residue does not exceed 1.0 mg.
- **HEAVY METALS <231>**: NMT 5 ppm  
**Sample solution**: To the residue obtained in the test for *Limit of Nonvolatile Residue* add 8 mL of 0.1 N hydrochloric acid, warm gently until solution is complete, dilute with water to 100 mL, and use 20 mL.

- **CHLORIDE AND SULFATE, Chloride <221>**  
**Sample solution**: Dilute 1.0 mL with 20 mL of water.  
**Analysis**: Add 5 drops of silver nitrate TS.  
**Acceptance criteria**: No opalescence is produced.
- **CHLORIDE AND SULFATE, Sulfate <221>**  
**Sample solution**: Dilute 1.0 mL with 10 mL of water.  
**Analysis**: Add 1 mL of barium chloride TS.  
**Acceptance criteria**: No turbidity is produced.

### Organic Impurities

#### PROCEDURE: READILY OXIDIZABLE SUBSTANCES

**Sample solution**: Dilute 2.0 mL in a glass-stoppered vessel with 10 mL of water.  
**Analysis**: Add 0.10 mL of 0.10 N potassium permanganate.  
**Acceptance criteria**: The pink color is not changed to brown within 2 h.

### SPECIFIC TESTS

- **CONGEALING TEMPERATURE <651>**: NLT 15.6°

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers, and store at room temperature.

## Acetic Acid Irrigation

### DEFINITION

Acetic Acid Irrigation is a sterile solution of Glacial Acetic Acid in Water for Injection. It contains, in each 100 mL, NLT 237.5 mg and NMT 262.5 mg of  $C_2H_4O_2$ .

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Acetate <191>**  
**Sample**: 100 mL of Acetic Acid Irrigation  
**Analysis**: Evaporate the *Sample* to about 10 mL.  
**Acceptance criteria**: The resulting solution meets the requirements.

### ASSAY

- **PROCEDURE**  
**Sample**: 50 mL of Acetic Acid Irrigation  
**Analysis**: Pipet the *Sample* into a 150-mL conical flask, add 2 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ( $C_2H_4O_2$ ).  
**Acceptance criteria**: 237.5–262.5 mg of  $C_2H_4O_2$  in each 100 mL of Acetic Acid Irrigation

### SPECIFIC TESTS

- **pH <791>**: 2.8–3.4
- **BACTERIAL ENDOTOXINS TEST <85>**: It contains NMT 0.5 USP Endotoxin Unit/mL.
- **OTHER REQUIREMENTS**: It meets the requirements under *Injections* (1), except that the container in which it is packaged may be designed to empty rapidly and may exceed 1000 mL in capacity.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature. It may be packaged in suitable plastic containers.
- **USP REFERENCE STANDARDS <11>**  
USP Endotoxin RS

## Acetic Acid Otic Solution

### DEFINITION

Acetic Acid Otic Solution is a solution of Glacial Acetic Acid in a suitable nonaqueous solvent. It contains NLT 85.0% and NMT 130.0% of the labeled amount of  $C_2H_4O_2$ .

### IDENTIFICATION

- **A.**  
**Sample solution:** Dilute 5 mL of Acetic Acid Otic Solution with 10 mL of water.  
**Analysis:** Adjust the *Sample solution* with 1 N sodium hydroxide to a pH of 7. Add ferric chloride TS.  
**Acceptance criteria:** A deep red color is produced, and it is destroyed by the addition of hydrochloric acid.
- **B.**  
**Analysis:** Warm it with sulfuric acid and alcohol.  
**Acceptance criteria:** Ethyl acetate, recognizable by its characteristic odor, is evolved.

### ASSAY

- **PROCEDURE**  
**Sample:** A quantity of Acetic Acid Otic Solution containing 100 mg of glacial acetic acid  
**Analysis:** Transfer the *Sample* to a 250-mL conical flask, and add 5 mL of saturated sodium chloride solution, 40 mL of water, and 3 drops of phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS to a faint pink endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ( $C_2H_4O_2$ ).  
**Acceptance criteria:** 85.0%–130.0%

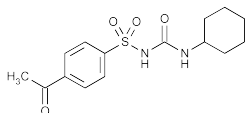
### SPECIFIC TESTS

- **pH** <791>  
**Sample solution:** Acetic Acid Otic Solution and water (1:1)  
**Acceptance criteria:** 2.0–4.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

## Acetohexamide



$C_{15}H_{20}N_2O_4S$  324.40  
 Benzenesulfonamide, 4-acetyl-N-[[cyclohexylamino]-carbonyl]-  
 1-[(p-Acetylphenyl)sulfonyl]-3-cyclohexylurea [968-81-0].

» Acetohexamide contains not less than 97.0 percent and not more than 101.0 percent of  $C_{15}H_{20}N_2O_4S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
 USP Acetohexamide RS

### Identification—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

*Medium:* 0.01 N sodium hydroxide.

Absorptivities at 247 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** <741>: between 182.5° and 187°.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Selenium** <291>: 0.003%, a 200-mg specimen mixed with 200 mg of magnesium oxide being used.

**Heavy metals, Method II** <231>: 0.002%.

**Assay**—Dissolve about 300 mg of Acetohexamide, accurately weighed, in 40 mL of dimethylformamide, add 5 drops of thymol blue TS, and titrate, using a magnetic stirrer, with 0.1 N sodium methoxide VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 32.44 mg of  $C_{15}H_{20}N_2O_4S$ .

## Acetohexamide Tablets

» Acetohexamide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{15}H_{20}N_2O_4S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
 USP Acetohexamide RS

**Identification**—Evaporate on a steam bath to dryness a 20-mL portion of the diluted chloroform solution prepared as directed in the *Assay*: the residue meets the requirements of *Identification* test A under *Acetohexamide*.

**Dissolution** <711>—

*Medium:* pH 7.6 phosphate buffer (see pH <791>); 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 60 minutes.

**Procedure**—Determine the amount of  $C_{15}H_{20}N_2O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 245 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, using *Medium* as the blank, in comparison with a Standard solution having a known concentration of USP Acetohexamide RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{15}H_{20}N_2O_4S$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetohexamide, to a 100-mL volumetric flask, add 60 mL of 0.1 N sodium hydroxide, and shake for 30 minutes. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Transfer 20.0 mL of the subsequent filtrate to a 125-mL separator, add 2 mL of 3 N hydrochloric acid, and extract with four 40-mL portions of chloroform, filtering each portion through chloroform-washed paper into a 200-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 20.0 mL of this solution to a suitable beaker, and evaporate on a steam bath to dryness. Transfer the residue, with the aid of 0.1 N sodium hydroxide, to a 100-mL volumetric

flask, add 0.1 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of the solution from the Tablets and a Standard solution prepared from USP Acetohexamide RS, in the same medium, at a concentration of about 10 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 247 nm, with a suitable spectrophotometer, using 0.01 N sodium hydroxide as the blank. Calculate the quantity, in mg, of  $C_{15}H_{20}N_2O_4S$  in the portion of Tablets taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Acetohexamide RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablets and the Standard solution, respectively.

## Acetohydroxamic Acid



$C_2H_5NO_2$  75.07

N-Acetyl hydroxyacetamide.

Acetohydroxamic acid [546-88-3].

» Acetohydroxamic Acid, dried over phosphorus pentoxide for 16 hours, contains not less than 98.0 percent and not more than 101.0 percent of  $C_2H_5NO_2$ .

**Packaging and storage**—Preserve in tight containers, and store in a cool, dry place.

**USP Reference standards** (11)—

USP Acetohydroxamic Acid RS

**Completeness of solution** (641)—A 1.0-g portion dissolves in 10 mL of water to yield a clear solution.

**Color of solution**—Dissolve 1.0 g in 5 mL of water: the absorbance, determined in a 1-cm cell in the wavelength range between 400 nm and 750 nm in a suitable spectrophotometer, water being used as the blank, is not greater than 0.050.

**Identification**—

**A: Infrared Absorption** (197K).

**B:** To 10 mL of a solution (1 in 50) add 2 drops of potassium permanganate TS: the pink color of the permanganate disappears.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231)—Dissolve 1 g in 23 mL of water, and add 2 mL of 1 N acetic acid: the limit is 0.002%.

**Limit of hydroxylamine**—

**Phosphate buffer**—Dissolve 1.36 g of monobasic potassium phosphate in about 950 mL of water, adjust with 1 M potassium hydroxide to a pH of 7.4, dilute with water to 1000 mL, and mix.

**Pyridoxal 5-phosphate solution**—Dissolve 50 mg of pyridoxal 5-phosphate monohydrate in 50 mL of *Phosphate buffer* in a low-actinic flask. Prepare fresh before use.

**Standard solutions**—Dissolve an accurately weighed quantity of hydroxylamine hydrochloride in water to obtain a final concentration of 2.0 mg per mL. To separate 100-mL volumetric flasks, transfer 5.0, 10.0, and 15.0 mL of the hy-

droxylamine stock solution, respectively, dilute each flask with water to volume, and mix.

**Test solution**—Transfer an accurately weighed quantity of about 1500 mg of Acetohydroxamic Acid, previously dried, to a 100-mL beaker, and dissolve in a sufficient amount of water to cover the electrode of a calibrated pH meter (about 60 mL). While stirring, adjust with 0.05 M potassium hydroxide to a pH of 7.4. Quantitatively transfer the contents of the beaker, with the aid of small portions of water, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Transfer 2.0 mL of each *Standard solution* and the *Test solution* into separate 100-mL volumetric flasks. Pipet 2.0 mL of water into a 100-mL volumetric flask for the reagent blank. To each flask, add 4.0 mL of *Pyridoxal 5-phosphate solution*, and mix. After 8 minutes, accurately timed, dilute the contents of each flask with *Phosphate buffer* to volume. Immediately determine the fluorescence intensities of the solutions from the *Standard solutions* and the *Test solution* in a fluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, setting the instrument to zero with the reagent blank. Determine the best-fit straight line from the fluorescence intensities of the three *Standard solutions* versus the hydroxylamine hydrochloride concentrations, in µg per mL. From the best-fit straight line, determine the concentration, in µg per mL, of hydroxylamine hydrochloride in the *Test solution*. Calculate the percentage of hydroxylamine in the portion of Acetohydroxamic Acid taken by the formula:

$$(33.03/69.50)(10C/W)$$

in which 33.03 and 69.50 are the molecular weights of hydroxylamine and hydroxylamine hydrochloride, respectively; C is the concentration, in µg per mL, of hydroxylamine hydrochloride in the *Test solution*; and W is the weight, in mg, of Acetohydroxamic Acid taken. Not more than 0.5% is found.

**Assay**—

**Ferric chloride solution**—Dissolve 4 g of ferric chloride in about 50 mL of 0.1 N hydrochloric acid, dilute with the same solvent to make 200 mL, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Acetohydroxamic Acid RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 500 µg per mL.

**Assay preparation**—Transfer about 250 mg of Acetohydroxamic Acid, previously dried and accurately weighed, to a 500-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Transfer 10.0 mL each of the *Standard preparation*, the *Assay preparation*, and 0.1 N hydrochloric acid to provide the blank, to separate 100-mL volumetric flasks. To each flask add about 50 mL of 0.1 N hydrochloric acid and 10.0 mL of *Ferric chloride solution*, mix, dilute with 0.1 N hydrochloric acid to volume, and mix. Without delay, concomitantly determine the absorbances of the solutions from the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 502 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_2H_5NO_2$  in the portion of Acetohydroxamic Acid taken by the formula:

$$0.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Acetohydroxamic Acid RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Acetohydroxamic Acid Tablets

» Acetohydroxamic Acid Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_2H_5NO_2$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acetohydroxamic Acid RS

**Identification**—Tablets produce a purple color when mixed with an acidic solution of ferric chloride.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the amount of  $C_2H_5NO_2$  dissolved, employing the procedure set forth in the *Assay*, using a filtered portion of the solution under test as the *Assay preparation* in comparison with a Standard solution having a known concentration of USP Acetohydroxamic Acid RS in the same *Medium*.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $C_2H_5NO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of hydroxylamine**—

*Phosphate buffer*, *Pyridoxal 5-phosphate solution*, and *Standard solutions*—Prepare as directed in the test for *Limit of hydroxylamine* under *Acetohydroxamic Acid*.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1500 mg of acetohydroxamic acid to a 50-mL stoppered centrifuge tube. Add 30.0 mL of water, shake for about 2 minutes, and centrifuge. Pipet 15.0 mL of the clear solution into a 50-mL beaker, add just enough water to cover the electrode of a calibrated pH meter, and while stirring, adjust with 0.5 M potassium hydroxide to a pH of 7.4. Quantitatively transfer the contents of the beaker, with the aid of small portions of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed in the test for *Limit of hydroxylamine* under *Acetohydroxamic Acid*, except to calculate the percentage of hydroxylamine ( $H_3NO$ ) in the portion of Tablets taken by the formula:

$$(33.03/69.50)(10CA/SL)$$

in which *A* is the average weight, in mg, of each Tablet; *S* is the weight, in mg, of the portion of Tablets taken to prepare the *Test solution*; *L* is the labeled amount, in mg, of acetohydroxamic acid per Tablet; and the other terms are as defined therein. Not more than 0.5% is found.

**Assay**—

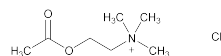
*Ferric chloride solution* and *Standard preparation*—Prepare as directed in the *Assay* under *Acetohydroxamic Acid*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetohydroxamic acid, to a 1000-mL volumetric flask, add about 500 mL of 0.1 N hydrochloric acid, and shake for 1 minute. Dilute with 0.1 N hydrochloric acid to volume, and mix. Filter, discarding the first 40 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Acetohydroxamic Acid*. Calculate the quantity, in mg, of  $C_2H_5NO_2$  in the portion of Tablets taken by the formula:

$$C(A_U / A_S).$$

## Acetylcholine Chloride



$C_7H_{16}ClNO_2$  181.66  
Ethanaminium, 2-(acetyloxy)-*N,N,N*-trimethyl-, chloride;  
Choline acetate (ester) chloride [60-31-1].

### DEFINITION

Acetylcholine Chloride contains NLT 98.0% and NMT 102.0% of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.**

**Sample solution**: 100 mg/mL in water

**Analysis**: To 5 mL of *Sample solution* add 5 mL of silver nitrate TS.

**Acceptance criteria**: A white, curdy precipitate, which is soluble in ammonium hydroxide but insoluble in nitric acid, is formed.

### ASSAY

#### • PROCEDURE

**Sample**: 400 mg of Acetylcholine Chloride

**Analysis**: Dissolve in 15 mL of water in a glass-stoppered conical flask, add 40.0 mL of 0.1 N sodium hydroxide VS, and heat on a steam bath for 30 min. Insert the stopper, allow to cool, add phenolphthalein TS, and titrate the excess alkali with 0.1 N sulfuric acid VS. Perform a blank determination (see *Titrimetry* (541), *Residual Titrations*). Each mL of 0.1 N sodium hydroxide is equivalent to 18.17 mg of  $C_7H_{16}ClNO_2$ .

**Acceptance criteria**: 98.0%–102.0% on the dried basis

### OTHER COMPONENTS

#### • CONTENT OF CHLORIDE

**Sample**: 280 mg of Acetylcholine Chloride

**Analysis**: Dissolve the *Sample* in 140 mL of water, and add 1 mL of dichlorofluorescein TS. Titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

**Acceptance criteria**: 19.3%–19.8% of Cl on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* (741): 149°–152°

#### • ACIDITY

**Sample**: 100 mg of Acetylcholine Chloride

**Analysis**: Dissolve the *Sample* in 10 mL of recently boiled water, and add at once 1 drop of bromothymol blue TS.

**Acceptance criteria**: NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.

- **LOSS ON DRYING** (731)  
Analysis: Dry a sample at 105° for 3 h.  
Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetylcholine Chloride RS

## Acetylcholine Chloride for Ophthalmic Solution

**DEFINITION**

Acetylcholine Chloride for Ophthalmic Solution is a sterile mixture of Acetylcholine Chloride with Mannitol or other suitable diluent, prepared by freeze-drying. Each container contains NLT 90.0% and NMT 115.0% of the labeled amount of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ).

**IDENTIFICATION**

- **A.**  
Standard solution: 10 mg/mL of USP Acetylcholine Chloride RS  
Sample solution: 10 mg/mL of acetylcholine chloride  
**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
Adsorbent: 0.25-mm layer of aluminum oxide  
Application volume: 2  $\mu$ L  
Developing solvent system: Mix butyl alcohol, glacial acetic acid, and water (40:10:50). Allow the layers to separate completely. Use the upper layer.  
Spray reagent A: Freshly prepared solution of 5 mg/mL of cobaltous chloride prepared as follows. Dissolve the required amount of cobaltous chloride in 50% of the final volume of water, and dilute with 50% alcohol. [NOTE—This solution is freshly prepared.]  
Spray reagent B: Freshly prepared potassium ferrocyanide solution prepared as follows. Dissolve 1.0 g of potassium ferrocyanide in 100 mL of water, and dilute with 50 mL of alcohol.  
**Analysis**  
Samples: Standard solution and Sample solution  
Develop the chromatogram, without delay, in a vapor-saturated chamber containing the *Developing solvent system*. Allow the solvent front to move about 10 cm beyond the initial spotting line. Dry the plate with a current of warm air. Immediately spray the plate with *Spray reagent A*. Dry the plate as before, and immediately spray the plate with *Spray reagent B*. Dry the plate with a current of warm air.  
Acceptance criteria: The  $R_f$  value and color of the principal spot from the *Sample solution* correspond to those from the *Standard solution*.
- **B.**  
Sample solution: Nominally 10 mg/mL of acetylcholine chloride  
Analysis: To 2 mL of *Sample solution* add 1 drop of nitric acid and 1 mL of silver nitrate TS.  
Acceptance criteria: A curdy, white precipitate, soluble in an excess of 6 N ammonium hydroxide, is formed.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Add 1.03 g of sodium 1-heptanesulfonate to a mixture of 900 mL of water and 10 mL of methanol. Adjust with ammonium hydroxide or glacial acetic acid to a pH of 4.0. Add 50 mL of acetonitrile. Dilute with water to 1 L. [NOTE—A slight variation of the amount of acetonitrile may be required to improve resolution or adjust retention time.]

**Standard solution:** A quantity of USP Acetylcholine Chloride RS in *Mobile phase*, to obtain a solution having a known concentration equal to that of the acetylcholine chloride in the *Sample solution*

**Sample solution:** Transfer the contents of 1 container of Acetylcholine Chloride for Ophthalmic Solution to a 10-mL volumetric flask with the aid of *Mobile phase*, and dilute with *Mobile phase* to volume.

**System suitability solution:** 0.2% each of acetylcholine chloride and choline chloride

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Samples:** Standard solution and System suitability solution

**Suitability requirements**

**Resolution:** NLT 2.0 between acetylcholine chloride and choline chloride, *System suitability solution*

**Relative standard deviation:** NMT 3.5%, *Standard solution*

**Analysis**

**Samples:** Standard solution and Sample solution  
Calculate the percentage of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ) in the container taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetylcholine Chloride RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Sample solution*, 10 mL

$L$  = label claim (mg/vial)

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Meets the requirements

• **ACIDITY**

**Analysis:** Dissolve an amount of Acetylcholine Chloride for Ophthalmic Solution equivalent to 100 mg of acetylcholine chloride in 10 mL of recently boiled water. Add at once 1 drop of bromothymol blue TS.

**Acceptance criteria:** NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.

- **WATER DETERMINATION, Method I** (921)

**Analysis:** Perform the titration in the original container, observing precautions against contact with water or moist atmosphere. Adjust the concentration of the reagent so that the titration volume approaches but does not exceed the capacity of the container. Titrate to an amber color that persists for 15 s after mixing.

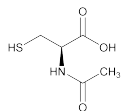
**Acceptance criteria:** NMT 1.0%

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* (1), *Constituted Solutions*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers as described in *Injections* (1), *Containers for Sterile Solids*, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetylcholine Chloride RS

## Acetylcysteine



$C_5H_9NO_3S$   
L-Cysteine, *N*-acetyl-;  
*N*-Acetyl-L-cysteine [616-91-1].

163.19

### DEFINITION

Acetylcysteine contains NLT 98.0% and NMT 102.0% of  $C_5H_9NO_3S$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>

### ASSAY

#### • PROCEDURE

**Mobile phase:** 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

**Sodium metabisulfite solution:** 0.5 mg/mL of sodium metabisulfite in water, freshly prepared

**Internal standard solution:** 5 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution*

**Standard stock solution:** 10 mg/mL of USP Acetylcysteine RS in *Sodium metabisulfite solution*

**Standard solution:** 0.5 mg/mL of USP Acetylcysteine RS and 0.25 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution* from *Standard stock solution* and *Internal standard solution*

**Sample stock solution:** 10 mg/mL of Acetylcysteine in *Sodium metabisulfite solution*

**Sample solution:** 0.5 mg/mL of Acetylcysteine and 0.25 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution* from *Sample stock solution* and *Internal standard solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 5 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetylcysteine and L-phenylalanine are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6 between acetylcysteine and L-phenylalanine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetylcysteine ( $C_5H_9NO_3S$ ) in the portion of Acetylcysteine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of acetylcysteine to L-phenylalanine from the *Sample solution*

$R_S$  = peak response ratio of acetylcysteine to L-phenylalanine from the *Standard solution*

$C_S$  = concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of acetylcysteine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.5%

- **HEAVY METALS**, *Method II* <231>

[**CAUTION**— Exercise care because explosion may occur.]

**Analysis:** In a dropwise manner, wet the sample with 2 mL of nitric acid, and proceed as directed for the *Test preparation*.

**Acceptance criteria:** NMT 10 ppm

### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* <781S>

**Buffer:** Mix 29.5 mL of 1 N sodium hydroxide, 50 mL of 1 M monobasic potassium phosphate, and sufficient water to make 100 mL. Adjust to a pH of  $7.0 \pm 0.1$  by adding more of either solution, as necessary.

**Sample solution:** In a 25-mL volumetric flask, mix 1.25 g with 1 mL of edetate disodium solution (1 in 100), add 7.5 mL of sodium hydroxide solution (1 in 25), and mix to dissolve. Dilute with *Buffer* to volume.

**Acceptance criteria:**  $+21^\circ$  to  $+27^\circ$

- **pH** <791>: 2.0–2.8 in a solution (1 in 100)

- **LOSS ON DRYING** <731>: Dry a sample at a pressure of about 50 mm of mercury at  $70^\circ$  for 4 h: it loses NMT 1.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS** <11>

USP Acetylcysteine RS

USP L-Phenylalanine RS

## Acetylcysteine Solution

» Acetylcysteine Solution is a sterile solution of Acetylcysteine in water, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetylcysteine ( $C_5H_9NO_3S$ ).

**Packaging and storage**—Preserve in single-unit or multi-unit tight containers that effectively exclude oxygen, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Acetylcysteine RS

USP L-Phenylalanine RS

**Identification**—Place about 10 mL in a suitable beaker, and adjust to a pH of about 2 (pH indicator paper) using 3 N hydrochloric acid. Add up to 2 g of finely powdered sodium chloride, in two portions of about 200 mg each initially, and then in smaller portions (about 25 mg), stirring after each addition until the sodium chloride dissolves and a precipitate is formed. [NOTE—The precipitate appears as a very fine powder, and the solution turns cloudy. If no precipitate forms, add an additional drop of 3 N hydrochloric acid, and stir until the precipitate forms.] Allow to stand at room temperature for 15 minutes, and collect the residue by suction filtration: the acetylcysteine so obtained, after being dried as directed in the test for *Loss on drying* under *Acetylcysteine*, responds to the *Identification* test under *Acetylcysteine*.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 6.0 and 7.5.

**Assay—**

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Acetylcysteine.

*Assay preparation*—Pipet a volume of Solution, equivalent to about 1000 mg of acetylcysteine, into a 100-mL volumetric flask, dilute with sodium bisulfite solution (1 in 2000) to volume, and mix. Pipet 10.0 mL of this solution and 10.0 mL of *Internal standard solution* into a 200-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Acetylcysteine. Calculate the quantity, in mg, of  $C_5H_9NO_3S$  in each mL of the Solution taken by the formula:

$$2000(C/V)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Acetylcysteine RS in the *Standard preparation*; V is the volume, in mL, of Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak response of acetylcysteine to that of DL-phenylalanine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution

» Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution is a sterile solution of Acetylcysteine and Isoproterenol Hydrochloride in water. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetylcysteine ( $C_5H_9NO_3S$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, tightly closed with a glass or polyethylene closure, and store at controlled room temperature.

**Labeling**—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** <11>—

USP Acetylcysteine RS

USP Isoproterenol Hydrochloride RS

USP L-Phenylalanine RS

**Color and clarity**—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

**Identification—**

**A:** Place 2 mL in a 10-mL beaker, and adjust with 3 N hydrochloric acid to a pH of about 3 (pH indicator paper). Add 500 mg to 1 g of finely powdered sodium chloride, in two portions of about 200 mg each initially, and then in smaller portions (about 25 mg), stirring after each addition, until a precipitate is formed. Allow to stand at room temperature for 15 minutes, and collect the residue by suction filtration: the acetylcysteine so obtained, after being dried as directed in the test for *Loss on drying* under Acetylcysteine, responds to the *Identification* test under Acetylcysteine.

**B:** *Ferro-Citrate Solution* and *Buffer Solution*—Prepare as directed under *Epinephrine Assay* <391>.

*Procedure*—Place a volume of Inhalation Solution, equivalent to about 0.26 mg of isoproterenol hydrochloride, in a test tube with 3 mL of 0.1 M mercuric chloride, and mix. Add 100  $\mu$ L of *Ferro-Citrate Solution* and 1.0 mL of *Buffer Solution*, and mix: the presence of isoproterenol hydrochloride is confirmed by the development of a purple color.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 6.0 and 7.0.

**Assay for acetylcysteine—**

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Acetylcysteine.

*Assay preparation*—Pipet a volume of Inhalation Solution, equivalent to about 1000 mg of acetylcysteine, into a 100-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix. Pipet 10 mL of this solution and 10 mL of *Internal standard solution* into a 200-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Acetylcysteine. Calculate the quantity, in mg, of  $C_5H_9NO_3S$  in each mL of the Inhalation Solution taken by the formula:

$$2000(C/V)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Acetylcysteine RS in the *Standard preparation*; V is the volume, in mL, of Inhalation Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak response of acetylcysteine to that of DL-phenylalanine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for isoproterenol hydrochloride—**

*Mobile phase*—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and pass through a membrane filter having a 0.45- $\mu$ m porosity. Add 20.0 mL of methanol, mix, and degas.

*Internal standard solution*—Place about 150 mg of acetaminophen in a 500-mL volumetric flask, add 5 mL of glacial acetic acid, dilute with water to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in 0.05 M sodium metabisulfite to obtain a solution having a known concentration of 0.15 mg per mL. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with 0.2 M acetic acid to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 1.5 mg of isoproterenol hydrochloride, and 10 mL of *Internal standard solution* to a 25-mL volumetric flask, add dilute glacial acetic acid (1 in 100) to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  40-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for isoproterenol hydrochloride and 1.0 for acetaminophen; the resolution,  $R$ , between isoproterenol hydrochloride and acetaminophen is not less than 6; and the relative standard deviation for replicate injections is not more than 2.0%.

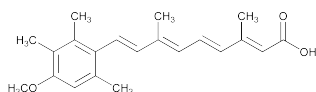
*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ) in each mL of the Inhalation Solution taken by the formula:

$$(25C/V)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Inhalation Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak responses of isoproterenol hydrochloride to those of acetaminophen obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acitretin



$C_{21}H_{26}O_3$  326.43  
2,4,6,8-Nonatetraenoic acid, 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-, (*all-E*)-; (*all-E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid [55079-83-9].

### DEFINITION

Acitretin contains NLT 98.0% and NMT 102.0% of  $C_{21}H_{26}O_3$ , calculated on the dried basis.

**[CAUTION]**—Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

[NOTE—Use low-actinic glassware and perform all tests under yellow and subdued light.]

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Store the solutions at 4° before injection.]

**Mobile phase:** Alcohol, glacial acetic acid, and water (92:0.3:8)

**System suitability stock solution:** 0.01 mg/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol.

[NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**System suitability solution:** 0.25 µg/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol, from *System suitability stock solution*

**Standard solution:** 0.1 mg/mL of USP Acitretin RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol. The final concentration of tetrahydrofuran in the preparation will be 2%.]

**Sample stock solution:** 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**Sample solution:** 0.1 mg/mL of Acitretin in alcohol, from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 4-mm × 25-cm; packing L1

**Flow rate:** 0.6 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for tretinoin and acitretin are 0.84 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between tretinoin and acitretin, *System suitability solution*

**Relative standard deviation:** NMT 1.0% of acitretin, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{21}H_{26}O_3$  in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Acitretin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS**, *Method II* (231): NMT 20 ppm

#### Organic Impurities

[NOTE—Store the solutions at 4° before injection.]

#### PROCEDURE

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.8 µg/mL each of USP Acitretin RS, USP Acitretin Related Compound A RS, and USP Acitretin Related Compound B RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**Sample solution:** 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

#### System suitability

(See *Chromatography* (621), *System Suitability*.)

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between acitretin related compound A and acitretin; NLT 1.5 between acitretin related compound B and acitretin

**Relative standard deviation:** NMT 10.0% for acitretin related compound A and NMT 10.0% for acitretin related compound B

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acitretin related compound A and acitretin related compound B in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the relevant impurity from the *Sample solution*



- $r_s$  = peak response from the relevant impurity from the *Standard solution*  
 $C_s$  = concentration of USP Acitretin Related Compound A RS or USP Acitretin Related Compound B RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Acitretin in the *Sample solution* ( $\mu\text{g/mL}$ )  
 Calculate the percentage of impurities other than acitretin related compounds A and B in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_s) \times (C_s/C_U) \times 100$$

- $r_U$  = peak response of each individual unspecified impurity from the *Sample solution*  
 $r_s$  = peak response of USP Acitretin RS in the *Standard solution*  
 $C_s$  = concentration of USP Acitretin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Acitretin in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acitretin related compound A	0.78	0.3
Acitretin	1.0	—
Acitretin related compound B	1.61	0.3
Any unspecified impurity	—	0.1
Total unspecified impurities	—	0.4

**SPECIFIC TESTS**

- LOSS ON DRYING (731):** Dry a sample in a vacuum at a pressure not exceeding 19 mm of mercury at 100° for 4 h: it loses NMT 0.2% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Acitretin RS
  - USP Acitretin Related Compound A RS  
(2Z,4E,6E,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.  
 $\text{C}_{21}\text{H}_{26}\text{O}_3$  326.43
  - USP Acitretin Related Compound B RS  
Ethyl (all-E)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate.  
 $\text{C}_{23}\text{H}_{30}\text{O}_3$  354.48
  - USP Tretinoin RS

**Acitretin Capsules****DEFINITION**

Acitretin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acitretin ( $\text{C}_{21}\text{H}_{26}\text{O}_3$ ).

**[CAUTION—**Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

**[NOTE—**Use low-actinic glassware and perform all tests under yellow and subdued light. Make all injections within 1 h of *Sample solution* preparation.]

**IDENTIFICATION**

- THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 10 mg/mL of USP Acitretin RS in tetrahydrofuran

**Sample solution:** Equivalent to 20 mg of acitretin from Capsules. Grind to a fine powder, then triturate for 30 s with 2 mL of tetrahydrofuran. Transfer the suspension to a 12-mL conical centrifuge tube, and centrifuge to obtain a clear supernatant.

**Application volume:** 10  $\mu\text{L}$

**Developing solvent system:** Chloroform and methanol (4:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, and then air-dry.

Spray the plate with a saturated solution of antimony trichloride in chloroform (250 mg/mL) followed by concentrated sulfuric acid, and then locate the spots.

**ASSAY**

- PROCEDURE**

**Diluent:** Methanol and tetrahydrofuran (13:10)

**Mobile phase:** Methanol, alcohol, glacial acetic acid, and water (74:5:0.5:21)

**Standard solution:** 0.1 mg/mL of USP Acitretin RS in a mixture of *Diluent* and water (23:2). Dissolve USP Acitretin RS in *Diluent* equivalent to 80% of the final volume, sonicate for 5 min, add water equivalent to 8% of the final volume, and dilute with *Diluent* to volume.

**System suitability solution:** Transfer 2 mL of the *Standard solution* to a clear 4-mL glass vial. After sealing the vial with a teflon-lined silicone septum and cap, place the vial on its side in a light chamber, expose it to 400 foot-candles of fluorescent light for 5 min, and then completely wrap the vial with aluminum foil.

[NOTE—Exposure to the fluorescent light allows for the formation of two degradation products: acitretin related compound A and the 9-*cis* isomer]

**Sample solution:** 0.1 mg/mL of acitretin in a mixture of *Diluent* and water (23:2). Open NLT 20 Capsules, composite the Capsule fill, and mix well. Transfer the Capsule fill to a volumetric flask, add water equivalent to 8% of the final volume to wet the sample, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a suitable filter of 0.5- $\mu\text{m}$  pore size, and use the clear filtrate. [NOTE—Inject the *Sample solution* within 1 h of preparation.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 365 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L1

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu\text{L}$

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between acitretin related compound A and acitretin; NLT 1.8 between the 9-*cis* isomer and acitretin, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $\text{C}_{21}\text{H}_{26}\text{O}_3$  in the portion of Capsules taken:

$$\text{Result} = (r_U/r_s) \times (C_s/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Medium:** 3% sodium lauryl sulfate in deaerated water, pH 9.6 to 10.0; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

Determine the amount of  $C_{21}H_{26}O_3$  dissolved using the following method.

**Standard solution:** Transfer about 14 mg of USP Acitretin RS to a 500-mL volumetric flask. Dissolve in 50 mL of alcohol, and dilute with *Medium* to volume.

**For Capsules labeled to contain 10 mg:** Transfer 20 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

**Sample solution:** Use portions of the solution under test passed through a suitable filter of 0.45- $\mu$ m pore size.

**Capsule shell solution:** Dissolve 6 clean empty-shell Capsules in 900 mL of *Medium*.

### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Capsule shell solution*

**Analytical wavelength:** 347 nm

**Cell length:** 2 mm

**Blank:** *Medium*

Calculate the amount of  $C_{21}H_{26}O_3$  dissolved:

$$\text{Result} = [(A_U - A_{CS})/A_S] \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_{CS}$  = Capsule shell correction, calculated as shown below

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the appropriate *Standard solution* (mg/mL)

$L$  = Capsule label claim (mg)

$V$  = volume of *Medium* (mL), 900

The Capsule shell correction,  $A_{CS}$ , is calculated as follows:

$$A_{CS} = A_{CSS}/N$$

$A_{CSS}$  = absorbance of the *Capsule shell solution*

$N$  = number of Capsule shells used to prepare the *Capsule shell solution*

**Tolerances:** NLT 85% (Q) of the labeled amount of  $C_{21}H_{26}O_3$  is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE: LIMIT OF DEGRADATION PRODUCTS

**Diluent, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each individual impurity

$r_T$  = sum of the responses of all the peaks

## Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acitretin related compound $A^a$	0.84	0.5
Acitretin	1.0	—
9- <i>cis</i> isomer <sup>b</sup>	1.09	—
Any unspecified impurity	—	0.4
Total unspecified impurities	—	0.8

<sup>a</sup> [(2Z,4E,6E,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid] ( $C_{21}H_{26}O_3$  326.43).

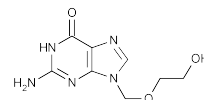
<sup>b</sup> (E,E,Z,E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS <11>**  
USP Acitretin RS

## Acyclovir



$C_8H_{11}N_5O_3$  225.20

6H-Purin-6-one, 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-.

9-[(2-Hydroxyethoxy)methyl]guanine [59277-89-3].

» Acyclovir contains not less than 98.0 percent and not more than 101.0 percent of  $C_8H_{11}N_5O_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store at room temperature. Protect from light and moisture.

**USP Reference standards <11>**—

USP Acyclovir RS

### Identification—

**A:** *Infrared Absorption* <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay and limit for guanine*.

**Water, Method I <921>:** not more than 6.0%.

### Ordinary impurities <466>—

**Test solution:** dimethyl sulfoxide.

**Standard solution:** dimethyl sulfoxide.

**Eluant:** a mixture of chloroform, methanol, and ammonium hydroxide (80:20:2).

**Visualization:** 1.

**Application volume:** 5  $\mu$ L.

**Limit:** 1%.

### Assay and limit for guanine—

**Mobile phase**—Prepare a filtered and degassed solution of glacial acetic acid in water (1 in 1000). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution 1**—Dissolve accurately weighed quantities of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having known concentrations of about 0.1 mg of each per mL.

**System suitability solution 2**—Dissolve an accurately weighed quantity of guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.7 µg per mL.

**Guanine standard preparation**—Transfer about 8.75 mg of guanine, accurately weighed, to a 500-mL volumetric flask. Dissolve in 50 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix to obtain a solution having a concentration of about 0.7 µg per mL.

**Standard preparation**—Dissolve about 25 mg of USP Acyclovir RS, accurately weighed, in 5 mL of 0.1 N sodium hydroxide in a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Acyclovir RS per mL.

**Assay preparation**—Dissolve about 100 mg of Acyclovir, accurately weighed, in 20 mL of 0.1 N sodium hydroxide in a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph *System suitability solution 1*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between acyclovir and guanine is not less than 2.0; the tailing factor for the analyte peak is not more than 2; and the relative standard deviation for replicate injections for the acyclovir peak is not more than 2.0%. Chromatograph *System suitability solution 2*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation*, the *Guanine standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in µg, of guanine in the portion of Acyclovir taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of guanine in the *Guanine standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to guanine in the *Assay preparation* and the *Guanine standard preparation*, respectively: not more than 0.7% of guanine is found. Calculate the quantity, in mg, of  $C_8H_{11}N_5O_3$  in the portion of Acyclovir taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Acyclovir RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to acyclovir in the *Assay preparation* and the *Standard preparation*, respectively.

## Acyclovir Capsules

### DEFINITION

Acyclovir Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**System suitability solution B:** 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer the contents of Capsules equivalent to 10 mg of acyclovir (NLT 10 Capsules) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute to volume with water, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between guanine and acyclovir, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for the acyclovir peak, *System suitability solution A*

**Relative standard deviation:** NMT 2.0%, *System suitability solution B*

**Analysis:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Detector:** UV 254 nm

**Standard solution:** USP Acyclovir RS in *Medium*

**Sample solutions:** Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

**Analysis:** Determine the amount of acyclovir ( $C_8H_{11}N_5O_3$ ) dissolved from UV absorption at the wavelength of maximum absorption on filtered portions of the solution under test.

**Tolerances:** NLT 75% ( $Q$ ) of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements for *Content Uniformity*

**IMPURITIES****• PROCEDURE**

Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Analysis:** *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses for all of the peaks

**Acceptance criteria**

Guanine: NMT 2.0%

Any individual impurity: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.
- USP REFERENCE STANDARDS** <11>  
USP Acyclovir RS

**Acyclovir for Injection****DEFINITION**

Acyclovir for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

Mobile phase: 0.02 M acetic acid

System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Constitute 1 vial of Acyclovir for Injection with water. Transfer an amount, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask, and dilute with water to volume.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.2-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

**System suitability**

Samples: *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are 0.6 and 1.0, respectively, in *System suitability solution A*.]

**Suitability requirements**

Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*

Relative standard deviation: NMT 2.0% for the acyclovir peak, *System suitability solution A*

Relative standard deviation: NMT 2.0%, *System suitability solution B*

**Analysis**

Calculate the percentage of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****• PROCEDURE**

**Solution A:** 0.17 M acetic acid and methanol (125:8)

**Solution B:** Methanol

Mobile phase: See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
45	65	35
46	100	0
56	100	0

**System suitability solution:** 0.5 µg/mL each of purine and USP Acyclovir RS in *Solution A*

**Acyclovir standard solution:** 5 µg/mL of USP Acyclovir RS in *Solution A*

**Guanine solution:** 0.05 mg/mL of guanine prepared as follows. Dissolve 25 mg of guanine in 50 mL of 0.1 N sodium hydroxide in a 500-mL volumetric flask, and bring the solution to volume with water.

**Standard solution A:** 0.5 µg/mL of *Acyclovir standard solution* in *Solution A*

**Standard solution B:** 5 µg/mL of *Guanine solution* in *Solution A*

**Sample solution:** Equivalent to 0.5 mg/mL of acyclovir from a mixture of NLT 10 reconstituted vials of Acyclovir for Injection in *Solution A*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

**System suitability**

Samples: *System suitability solution*, *Standard solution A*, and *Standard solution B*

[NOTE—Typical retention times for guanine and acyclovir of *Standard solution A* and *Standard solution B* are 5.8 and 14 min, respectively.]

**Suitability requirements**

Resolution: NLT 2.0 between purine and acyclovir, *System suitability solution*

Relative standard deviation: NMT 1% for the acyclovir and the guanine peaks, *Standard solution A* and *Standard solution B*

**Analysis 1**

Calculate the percentage of guanine in the Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for guanine, if present, in the *Sample solution*

$r_S$  = peak response of guanine in the *Standard solution*

$C_S$  = concentration of guanine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acyclovir in the  
Sample solution (mg/mL)

**Acceptance criteria 1:** NMT 1.0% guanine

#### Analysis 2

Calculate the percentage of each other impurity in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity

$r_S$  = peak response of acyclovir in the Standard solution

$C_S$  = concentration of USP Acyclovir RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

**Acceptance criteria 2:** NMT 0.15% for any peak having a relative retention time of about 0.7 compared to the acyclovir peak; NMT 0.5% for any other individual impurity; and NMT 1.0% for the total of all other impurities

#### SPECIFIC TESTS

- **PH** (791): 11.0–12.5, 50 mg/mL of acyclovir
- **WATER DETERMINATION, Method I** (921): NMT 5.5%
- **STERILITY TESTS** (71): Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.174 USP Endotoxin Unit/mg of acyclovir
- **OTHER REQUIREMENTS:** Meets the requirements for labeling in *Injections* (1), *Labeling*

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- **USP REFERENCE STANDARDS** (11)  
USP Acyclovir RS  
USP Endotoxin RS

## Acyclovir Ointment

#### DEFINITION

Acyclovir Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ), in a suitable ointment base.

#### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

**System suitability solution B:** 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of Ointment, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask. Dissolve in and dilute with 0.1 N sodium hydroxide to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 3 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** System suitability solution A and System suitability solution B

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in System suitability solution A.]

#### Suitability requirements

**Resolution:** NLT 2.0 between guanine and acyclovir, System suitability solution A

**Relative standard deviation:** NMT 2.0% for the acyclovir peak, System suitability solution A; NMT 2.0%, System suitability solution B

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Acyclovir RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

#### IMPURITIES

##### • LIMIT OF GUANINE

**Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 2.0 µg/mL of guanine in 0.1 M sodium hydroxide

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of guanine in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of guanine from the Sample solution

$r_S$  = peak response of guanine from the Standard solution

$C_S$  = concentration of guanine in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

**Acceptance criteria:** NMT 2.0%

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25° in a dry place.
- **USP REFERENCE STANDARDS** (11)  
USP Acyclovir RS

## Acyclovir Oral Suspension

### DEFINITION

Acyclovir Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

**System suitability solution B:** 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

**Sample stock solution:** Nominally 1 mg/mL of acyclovir prepared as follows. Transfer an amount of well-shaken Oral Suspension equivalent to 200 mg of acyclovir to a 200-mL volumetric flask. Add 100 mL of 0.1 N sodium hydroxide, shake by mechanical means for 15 min, and sonicate, if necessary, to dissolve the Oral Suspension completely. Dilute with 0.1 N sodium hydroxide to volume.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*).

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 3 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between guanine and acyclovir, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for replicate injections for the acyclovir peak, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for replicate injections, *System suitability solution B*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### IMPURITIES

#### LIMIT OF GUANINE

**Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 2.0 µg/mL of guanine in 0.1 M sodium hydroxide

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of guanine in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for guanine from the *Sample solution*

$r_S$  = peak response for guanine from the *Standard solution*

$C_S$  = concentration of guanine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 2.0%

### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Its total count does not exceed  $10^1$  cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

- PH** <791>: 4.5–7.0

### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements for Oral Suspension packaged in single-unit containers
- DELIVERABLE VOLUME** <698>: Meets the requirements for Oral Suspension packaged in multiple-unit containers

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- USP REFERENCE STANDARDS** <11>  
USP Acyclovir RS

## Acyclovir Tablets

### DEFINITION

Acyclovir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**System suitability solution B:** 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of finely powdered Tablets equivalent to 10 mg of acyclovir (NLT 10 Tablets) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute with water to volume, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*).

Mode: LC  
 Detector: UV 254 nm  
 Column: 4.6-mm × 25-cm; packing L1  
 Column temperature: 40°  
 Flow rate: 1.5 mL/min  
 Injection volume: 20 µL

**System suitability**

**Samples:** *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]

**Suitability requirements**

**Resolution:** NLT 2.0 between guanine and acyclovir, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for the acyclovir peak, *System suitability solution A*; NMT 2.0%, *System suitability solution B*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of acyclovir (C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Instrumental conditions**

**Mode:** UV

**Wavelength:** 254 nm

**Standard solution:** USP Acyclovir RS in *Medium*

**Sample solutions:** Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

**Analysis:** Determine the amount of acyclovir (C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub>) dissolved from UV absorption at the wavelength of maximum absorbance on filtered portions of the solution under test.

**Tolerances:** NLT 80% (Q) of the labeled amount of acyclovir (C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub>) is dissolved.

- UNIFORMITY OF DOSE UNITS <905>** Meet the requirements for *Weight Variation*

**IMPURITIES****• PROCEDURE**

**Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity  
 $r_T$  = sum of the responses for all of the peaks

**Acceptance criteria**

**Guanine:** NMT 2.0%

**Any other impurity:** NMT 0.5%

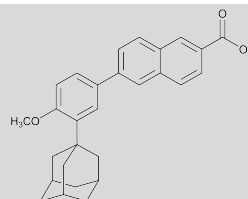
**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.

**• USP REFERENCE STANDARDS <11>**

USP Acyclovir RS

**Add the following:**

**• Adapalene**

C<sub>28</sub>H<sub>28</sub>O<sub>3</sub> 412.52  
 2-Naphthalenecarboxylic acid, 6-(4-methoxy-3-tricyclo[3.3.1.1.3,7]dec-1-ylphenyl)-;  
 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid.  
 [106685-40-9].

**DEFINITION**

Adapalene contains NLT 98.0% and NMT 102.0% of adapalene (C<sub>28</sub>H<sub>28</sub>O<sub>3</sub>), calculated on the dried basis.

**IDENTIFICATION****• A. INFRARED ABSORPTION <197K>**

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (21: 16: 0.01: 13)

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS in *Mobile phase*. Dissolve USP Adapalene RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Standard solution:** 40 µg/mL of USP Adapalene RS in *Mobile phase* from the *Standard stock solution*

**Sample stock solution:** 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Sample solution:** 40 µg/mL of Adapalene in *Mobile phase* from the *Sample stock solution*

**Chromatographic system**

(See *Chromatography <621>*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of adapalene (C<sub>28</sub>H<sub>28</sub>O<sub>3</sub>) in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Adapalene RS in the *Standard solution* (µg/mL)  
 $C_u$  = concentration of Adapalene in the *Sample solution* (µg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.20%

• **HEAVY METALS**, *Method II* (231): NMT 20 µg/g

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*.]

#### • ORGANIC IMPURITIES, PROCEDURE 1

*Procedure 1* is recommended if adapalene related compounds A and B may be present.

**Mobile phase:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS, 0.3 mg/mL of USP Adapalene Related Compound A RS, and 0.2 mg/mL of USP Adapalene Related Compound B RS in *Mobile phase*. Dissolve USP Adapalene RS, USP Adapalene Related Compound A RS, and USP Adapalene Related Compound B RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Standard solution:** 0.2 µg/mL of USP Adapalene RS, 0.3 µg/mL of USP Adapalene Related Compound A RS, and 0.2 µg/mL of USP Adapalene Related Compound B RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Chromatographic system:** Proceed as directed in the *Assay*, except use a run time of NLT two times the retention time of adapalene peak for *Standard solution* and NLT six times the retention time of adapalene peak for *Sample solution*.

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0% for the adapalene peak

**Column efficiency:** NLT 3000 theoretical plates for the adapalene peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of adapalene related compounds A and B in the portion of Adapalene taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak area of each impurity from the *Sample solution*  
 $r_s$  = peak area of corresponding adapalene related compound A or adapalene related compound B from the *Standard solution*  
 $C_s$  = concentration of corresponding USP Adapalene Related Compound A RS or USP Adapalene Related Compound B RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Adapalene in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Adapalene taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak area of each unspecified impurity from the *Sample solution*  
 $r_s$  = peak area of adapalene from the *Standard solution*

$C_s$  = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Adapalene in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peaks less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A <sup>a</sup>	0.52	0.10
Adapalene	1.0	—
Adapalene related compound B <sup>b</sup>	1.57	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

<sup>a</sup> Methyl 6-bromo-2-naphthoate.

<sup>b</sup> Methyl 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoate.

#### • ORGANIC IMPURITIES, PROCEDURE 2

*Procedure 2* is recommended if adapalene related compounds E, C, and D may be present.

**Solution A:** Glacial acetic acid and water (0.1:100)

**Solution B:** Acetonitrile and tetrahydrofuran (65:35)

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	50	50
2.5	50	50
40	28	72
42	28	72
42.1	50	50
50	50	50

**Diluent:** Acetonitrile, tetrahydrofuran, and water (37:20:43)

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS in tetrahydrofuran

**Standard solution:** 2.0 µg/mL of USP Adapalene RS in *Diluent* from the *Standard stock solution*

**System suitability solution:** 0.2 mg/mL of USP Adapalene RS and 1.2 µg/mL each of USP Adapalene Related Compound C RS, USP Adapalene Related Compound D RS, and USP Adapalene Related Compound E RS prepared by dissolving the standards in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume

**Sample solution:** 2.0 mg/mL of Adapalene prepared by dissolving in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11 with 7.5% carbon loading

**Column temperature:** 30°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 4.5 between the adapalene and adapalene related compound C peaks

**Signal-to-noise ratio:** NLT 10 for the adapalene related compound C peak



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of adapalene from the *Standard solution*

$C_S$  = concentration of adapalene in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)

$F$  = relative response factor for each individual impurity (see *Table 3*)

**Acceptance criteria:** See *Table 3*. Disregard any impurity peaks less than 0.05%.

**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Adapalene related compound E <sup>a</sup>	0.3	1.4	0.3
Hydroxyadapalene <sup>b</sup>	0.5	0.91	0.1
Adapalene related compound C <sup>c</sup>	0.9	0.14	0.1
Adapalene	1.0	—	—
Adapalene related compound D <sup>d</sup>	1.9	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

<sup>a</sup> 2,2'-Binaphthyl-6,6'-dicarboxylic acid.

<sup>b</sup> 6-[3-(3-Hydroxyadamant-1-yl)-4-methoxyphenyl]-2-naphthoic acid.

<sup>c</sup> 2-(Adamant-1-yl)methoxybenzene.

<sup>d</sup> 4,4'-Dimethoxy-3,3'-di(adamant-1-yl)biphenyl.

• **RESIDUAL SOLVENT: LIMIT OF TRIETHYLAMINE**

[NOTE—This test should be performed if triethylamine is used in the manufacturing process.]

**Diluent:** Dimethyl sulfoxide and 1 N sodium hydroxide solution (4:1)

**Standard solution:** 3.2 µg/mL of USP Triethylamine RS in *Diluent*

**Sample solution:** 40 mg/mL of Adapalene in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m × 0.53-mm; 3.0-µm coating of G27

**Temperatures**

**Injection port:** 250°

**Detector:** 300°

**Column:** See *Table 4*.

**Table 4**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	40	240	5

**Headspace operating parameters**

[NOTE—Headspace operating parameters can be modified in order to optimize the performance.]

**Equilibration temperature:** 95°

**Equilibration time:** 15 min

**Transfer line temperature:** 125°

**Pressurization time:** 3 min

**Carrier gas:** Nitrogen

**Flow rate:** 4.8 mL/min

**Injection volume:** 1 mL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 15%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the content, in ppm, of triethylamine in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

$r_U$  = peak response of triethylamine from the *Sample solution*

$r_S$  = peak response of triethylamine from the *Standard solution*

$C_S$  = concentration of triethylamine in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 80 ppm

**SPECIFIC TESTS**

• **LOSS ON DRYING** <731>

**Analysis:** Dry a sample at 105° for 4 h.

**Acceptance criteria:** NMT 0.6%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS** <11>

USP Adapalene RS

USP Adapalene Related Compound A RS

Methyl 6-bromo-2-naphthoate.

C<sub>12</sub>H<sub>9</sub>BrO<sub>2</sub> 265.10

USP Adapalene Related Compound B RS

Methyl 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoate.

C<sub>29</sub>H<sub>30</sub>O<sub>3</sub> 426.55

USP Adapalene Related Compound C RS

2-(Adamant-1-yl)methoxybenzene.

C<sub>17</sub>H<sub>22</sub>O 242.36

USP Adapalene Related Compound D RS

4,4'-Dimethoxy-3,3'-di(adamant-1-yl)biphenyl.

C<sub>34</sub>H<sub>42</sub>O<sub>2</sub> 482.70

USP Adapalene Related Compound E RS

2,2'-Binaphthyl-6,6'-dicarboxylic acid.

C<sub>22</sub>H<sub>14</sub>O<sub>4</sub> 342.34

USP Triethylamine RS

Triethylamine.

C<sub>6</sub>H<sub>15</sub>N 101.19 • (RB 1-Dec-2012)

## Adenine



C<sub>5</sub>H<sub>5</sub>N<sub>5</sub>

1H-Purin-6-amine;

1,6-Dihydro-6-iminopurine [73-24-5].

135.13

**DEFINITION**

Adenine contains NLT 98.0% and NMT 102.0% of  $C_5H_5N_5$ , calculated on the dried basis.

**IDENTIFICATION**

- **INFRARED ABSORPTION** <197K>

**ASSAY**

- **PROCEDURE**

**Sample:** 200 mg of Adenine

**Blank:** 80 mL of a mixture of 100 mL of glacial acetic acid and 300 mL of acetic anhydride

**Titrimetric System**

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS standardized as follows: Transfer 300 mg of potassium biphthalate to a 150-mL beaker and, by stirring, dissolve in 80 mL of a mixture of 100 mL of glacial acetic acid and 300 mL of acetic anhydride. Titrate with the perchloric acid solution. Each 20.42 mg of potassium biphthalate is equivalent to 1 mL of 0.1 N perchloric acid.

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 80 mL of a mixture of 100 mL of glacial acetic acid and 300 mL of acetic anhydride by stirring, and titrate with the *Titrant*. Calculate the percentage of adenine ( $C_5H_5N_5$ ) in the portion taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V = *Sample* titrant volume (mL)

B = *Blank* titrant volume (mL)

N = titrant normality (mEq/mL)

F = equivalency factor, 135.13 mg/mEq

W = weight of *Sample* (mg)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS**, *Method II* <231>: NMT 10 ppm

**Organic Impurities**

- **PROCEDURE**

**pH 7.0 phosphate buffer:** Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of the dibasic sodium phosphate solution. Adjust, if necessary, by the dropwise addition of the dibasic sodium phosphate solution to a pH of 7.0.

**Standard stock solution:** Dissolve a suitable quantity of USP Adenine RS in hot water, cool, and dilute quantitatively with water to obtain a solution having a known concentration of 0.19 mg/mL.

**Standard solutions:** Pipet 5-mL portions of the *Standard stock solution* into three 100-mL volumetric flasks, and dilute with 0.10 N hydrochloric acid, 0.010 N sodium hydroxide, and *pH 7.0 phosphate buffer*, respectively, to volume.

**Sample stock solution:** Dissolve a suitable quantity of Adenine in hot water, cool, and dilute quantitatively with water to obtain a solution having a known concentration of 0.19 mg/mL.

**Sample solutions:** Pipet 5-mL portions of the *Sample stock solution* into three 100-mL volumetric flasks, and dilute with 0.10 N hydrochloric acid, 0.010 N sodium hydroxide, and *pH 7.0 phosphate buffer*, respectively, to volume.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Wavelength range:** 220–320 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solutions* and *Sample solutions*

**Acceptance criteria:** The respective absorptivities, calculated on the dried basis, at the wavelengths of maximum absorbance, for each pair of corresponding solutions do not differ by more than 2.0%.

**SPECIFIC TESTS**

- **LOSS ON DRYING** <731>: Dry a sample at 110° for 4 h: it loses NMT 1.0% of its weight.
- **NITROGEN CONTENT**, *Method II* <461>: 50.2%–53.4%, calculated on the dried basis

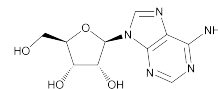
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Adenine RS

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**Adenosine**

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$C_{10}H_{13}N_5O_4$

6-Amino-9-β-D-ribofuranosyl-9H-purine;

9-β-D-Ribofuranosyladenine [58-61-7].

267.24

**DEFINITION**

Adenosine contains NLT 99.0% and NMT 101.0% of adenosine ( $C_{10}H_{13}N_5O_4$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197M>

**ASSAY**

- **ADENOSINE**

**Sample:** 200 mg of Adenosine previously dried at 105° for 2 h

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 50 mL of glacial acetic acid

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS. Calculate the percentage of adenosine ( $C_{10}H_{13}N_5O_4$ ) in the portion of Adenosine taken:

$$\text{Result} = [(V_S - V_B) \times N \times F \times 100]/W$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

N = normality of the *Titrant* (mEq/mL)

F = equivalency factor, 267.25 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, *Method II* (231): NMT 10 ppm

#### Delete the following:

##### ▲ LIMIT OF AMMONIA

**Sample solution:** Suspend 0.5 g in 10 mL of water. Stir for 30 s, and pass through a coarse filter. Dilute the filtrate with water to 15 mL, and use the filtrate.

**Standard solution:** 0.4 µg/mL of ammonium chloride in water

**Analysis:** To the *Sample solution* and the *Standard solution* add 0.3 mL of alkaline mercuric–potassium iodide TS, cap the test tubes, and allow to stand for 5 min.

**Acceptance criteria:** The *Sample solution* does not exhibit a more intense yellow color than that of the *Standard solution* (NMT 4 ppm of ammonia).▲USP36

#### Delete the following:

##### ▲ LIMIT OF CHLORIDE

**Sample solution:** Suspend 0.2 g in 10 mL of water. Stir for 30 s, pass through a coarse filter, and use the filtrate.

**Standard solution:** 2.3 µg/mL of sodium chloride in water

**Analysis:** To the *Sample solution* and 10 mL of the *Standard solution* add 1 mL of nitric acid and 1 mL of silver nitrate TS, and dilute each solution with water to 40 mL. Allow the solutions to stand for 5 min, protected from light.

**Acceptance criteria:** When viewed against a dark background, the *Sample solution* is not more turbid than the *Standard solution* (NMT 0.007% chloride).▲USP36

#### Delete the following:

##### ▲ LIMIT OF SULFATE

**Sample solution:** Suspend 0.75 g in 15 mL of water. Stir for 30 s, pass through a coarse filter, and use the filtrate.

**Standard solution:** Add 0.15 mL of 0.020 N sulfuric acid to 15 mL of water.

**Analysis:** To the *Sample solution* and the *Standard solution* add 2 mL of barium chloride TS and 1 mL of 3 N hydrochloric acid, dilute each solution with water to 30 mL, and mix. Allow the solutions to stand for 5 min.

**Acceptance criteria:** The *Sample solution* is not more turbid than the *Standard solution* (NMT 0.02% sulfate).

▲USP36

##### • ORGANIC IMPURITIES

**Solution A:** 6.8 g/L of potassium hydrogen sulfate and 3.4 g/L of tetrabutylammonium hydrogen sulfate in water. Adjust with 2 N potassium hydroxide to a pH of 6.5.

**Solution B:** 0.1 g/L of sodium azide solution

**Mobile phase:** *Solution A* and *Solution B* (60:40)

**System suitability solution:** 0.2 mg/mL each of Adenosine and inosine in *Mobile phase*

**Sample solution:** 1 mg/mL of Adenosine in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**Run time:** 2 times the retention time of adenosine

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 9.0 between adenosine and inosine

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Adenosine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the responses for all peaks from the *Sample solution*

**Acceptance criteria**

**Individual impurities:** NMT 0.1% each of guanosine, inosine, and uridine, and NMT 0.2% of adenine

**Total impurities:** NMT 0.5%

#### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 233°–238°

- **OPTICAL ROTATION**, *Specific Rotation* (7815)

**Sample solution:** 20 mg/mL in sodium hydroxide solution (1 in 20), from a sample previously dried at 105° for 2 h

**Acceptance criteria:** –68° to –72°

#### Delete the following:

- **ACIDITY OR ALKALINITY:** Suspend 1 g in 20 mL of carbon dioxide-free water. Stir for 30 s, and pass through a coarse filter. To each of two 10-mL portions of the filtrate add 0.1 mL of bromocresol purple TS.

**Acceptance criteria:** NMT 0.3 mL of 0.01 N sodium hydroxide is required to produce a blue-violet color in one portion. NMT 0.1 mL of 0.01 N hydrochloric acid is required to produce a yellow color in the other portion.

▲USP36

- **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Adenosine RS

## Adenosine Injection

#### DEFINITION

Adenosine Injection is a sterile solution of Adenosine in Water for Injection. It may contain Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of adenosine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>).

#### IDENTIFICATION

- The retention time of the adenosine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****Change to read:****PROCEDURE**

**Mobile phase:** Dissolve 2.0 g of monobasic potassium phosphate in 800 mL of water. Add 5 mL of 1.0 M tetrabutylammonium dihydrogen phosphate, dilute with water to 980 mL, and mix. Add 20 mL of acetonitrile.

**System suitability solution:** 0.03 mg/mL each of USP Adenosine RS and inosine dissolved in warm water (50° to 55°), and diluted with water

**Standard solution:** 0.03 mg/mL of USP Adenosine RS dissolved in warm water (50° to 55°), and diluted with water to volume. Before addition of the warm water, if sodium chloride is present in the Injection, add 0.01 mL of a solution of sodium chloride (0.9 in 100) per mL of the anticipated final volume of the *Standard solution*.

▲<sup>USP36</sup>

**Sample solution:** Nominally 0.03 mg/mL of adenosine, from a suitable volume of Injection in water

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.5 mL/min

**Injection volume:** 10 µL

**Run time:** 2.5 times the retention time of adenosine

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

▲[NOTE—The relative retention times of inosine and adenosine are 0.43 and 1.0, respectively.]▲<sup>USP36</sup>

**Suitability requirements**

**Resolution:** NLT 6.0 between adenosine and inosine, *System suitability solution*

**Tailing factor:** NMT 2.0 for the adenosine peak, *System suitability solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of adenosine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of adenosine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****Change to read:****ORGANIC IMPURITIES**

**Mobile phase, System suitability solution, Standard solution, ▲<sup>USP36</sup> Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Nominally 0.3 mg/mL of adenosine from a volume of Injection, in water

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the volume of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all of the peaks

**Acceptance criteria**

**Any individual impurity:** NMT 1.0%

**Total impurities:** NMT 1.5%

**SPECIFIC TESTS**

• **PH** <791>: 4.5–7.5

• **PARTICULATE MATTER IN INJECTIONS** <788>: It meets the requirements for small-volume injections.

• **BACTERIAL ENDOTOXINS TEST** <85>: When the product is used for rapid intravenous injection, it contains NMT 11.62 USP Endotoxin Units/mg of adenosine. When the product is used for continuous peripheral intravenous infusion, it contains NMT 5.95 USP Endotoxin Units/mg of adenosine.

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* <1>.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Adenosine RS

USP Endotoxin RS

**Medical Air**

» Medical Air is a natural or synthetic mixture of gases consisting largely of nitrogen and oxygen. It contains not less than 19.5 percent and not more than 23.5 percent, by volume, of O<sub>2</sub>.

**Packaging and storage**—Preserve in cylinders or in a low pressure collecting tank. Containers used for Medical Air are not to be treated with any toxic, sleep-inducing, or narcosis-producing compounds, and are not to be treated with any compound that would be irritating to the respiratory tract when the Medical Air is used.

[NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens. The various detector tubes called for in the respective tests are listed under *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*.]

**Labeling**—Where it is piped directly from the collecting tank to the point of use, label each outlet “Medical Air.”

**Water and oil**—Support 1 container in an inverted position (with the valve at the bottom) for 5 minutes. Cautiously open the valve slightly, maintaining the container in an inverted position. Vent the gas with a barely audible flow against a stainless steel mirror for a few seconds: no liquid is discernible on the mirror.

**Odor**—Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.

**Carbon dioxide**—Pass 1000 ± 50 mL through a carbon dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 0.05%.

**Carbon monoxide**—Pass 1000 ± 50 mL through a carbon monoxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 0.001%.

**Limit of nitric oxide and nitrogen dioxide**—Pass 550 ± 50 mL through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 2.5 ppm.

**Sulfur dioxide**—Pass  $1050 \pm 50$  mL through a sulfur dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 5 ppm.

**Assay**—Determine the oxygen concentration of Medical Air using an electrochemical cell analyzer readable to 0.1% of oxygen and calibrated with ambient air to an accuracy of  $\pm 0.2\%$  of oxygen. [NOTE—The instrument utilizes the variations of electric current produced by the interaction of oxygen with an electrochemical cell to display the oxygen strength of a confined sample or an in-line flow of the gas. This current generates a signal proportional to the oxygen concentration, which is displayed on a meter.]

## Alanine



$C_3H_7NO_2$   
L-Alanine [56-41-7].

89.09

### DEFINITION

Alanine contains NLT 98.5% and NMT 101.5% of  $C_3H_7NO_2$ , as L-alanine, calculated on the dried basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** <197K>

### ASSAY

#### • PROCEDURE

**Sample:** 80 mg of Alanine

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 3 mL of formic acid in 50 mL of glacial acetic acid

**Analysis:** Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS. Calculate the percentage of  $C_3H_7NO_2$  in the portion taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

V = *Sample* titrant volume (mL)

B = *Blank* titrant volume (mL)

N = titrant normality (mEq/mL)

F = equivalency factor: 89.09 mg/mEq

W = weight of the *Sample* (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** <281>: NMT 0.15%
- **CHLORIDE AND SULFATE**, *Chloride* <221>: A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).
- **CHLORIDE AND SULFATE**, *Sulfate* <221>: A 1.0-g portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (0.03%).
- **IRON** <241>: NMT 30 ppm
- **HEAVY METALS**, *Method I* <31>: NMT 15 ppm

#### Organic Impurities

##### • PROCEDURE

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Standard solution:** 0.05 mg/mL of USP L-Alanine RS.

[NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

**System suitability solution:** 0.4 mg/mL each of USP L-Alanine RS and USP Glycine RS

**Sample solution:** 10 mg/mL of Alanine

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:20:20)

**Application volume:** 5  $\mu$ L

#### Analysis

**Samples:** *Standard solution*, *System suitability solution*, and *Sample solution*

Proceed as directed for *Chromatography* <621>, *Thin-Layer Chromatography*. After air-drying the plate, repeat the development process. After air-drying a second time, spray with *Spray reagent*, and heat to  $100^\circ$ – $105^\circ$  for 15 min. Examine the plate under white light. The chromatogram obtained from the *System suitability solution* exhibits two clearly separated spots.

#### Acceptance criteria

**Individual impurities:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*, NMT 0.5%

**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* <781S>:  $+13.7^\circ$  to  $+15.1^\circ$

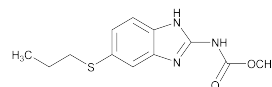
**Sample solution:** 100 mg/mL in 6 N hydrochloric acid

- **pH** <791>: 5.5–7.0, in a solution (1 in 20)
- **LOSS ON DRYING** <731>: Dry a sample at  $105^\circ$  for 3 h: it loses NMT 0.2% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP L-Alanine RS  
USP Glycine RS

## Albendazole



$C_{12}H_{15}N_3O_2S$  265.33

Carbamic acid, [5-(propylthio)-1H-benzimidazol-2-yl]-, methyl ester;

Methyl 5-(propylthio)-2-benzimidazolecarbamate [54965-21-8].

### DEFINITION

Albendazole contains NLT 98.0% and NMT 102.0% of albendazole ( $C_{12}H_{15}N_3O_2S$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197M>
- **B.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the principal spot of the *Standard solution*, as obtained in the test for *Organic Impurities*.

### ASSAY

#### • PROCEDURE

**Sample:** 250 mg of Albendazole

**Analysis:** Transfer the *Sample* to a suitable flask, and dissolve in 100 mL of glacial acetic acid, warming gently if necessary. Cool, and titrate with 0.1 N perchloric acid VS to a potentiometric endpoint (see *Titrimetry* <541>).

Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 26.53 mg of  $C_{12}H_{15}N_3O_2S$ .  
**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **ORGANIC IMPURITIES**

**Standard stock solution:** 5 mg/mL of USP Albendazole RS in glacial acetic acid

**Standard solution:** 0.05 mg/mL of USP Albendazole RS in glacial acetic acid from *Standard stock solution*

**Sample solution:** 10 mg/mL in glacial acetic acid

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbant:** 0.25-mm layer of silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Chloroform, ether, and glacial acetic acid (60:10:10)

**Analysis:** Proceed as directed for *Chromatography* (621), *Thin-Layer Chromatography*.

**Samples:** *Standard stock solution*, *Standard solution*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate from the plate, and examine the plate under short-wavelength UV light.

**Acceptance criteria:** 0.5%; no spot, other than the principal spot of the *Sample solution*, is larger or more intense than the principal spot of the *Standard solution*.

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 4 h.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Albendazole RS

**Sample stock solution:** Equivalent to 1 mg/mL of albendazole from a volume of Oral Suspension in *Solution A*

**Sample solution:** Nominally 100  $\mu$ g/mL of albendazole from *Sample stock solution* in *Mobile phase*. [NOTE—Filter, if necessary, to obtain a clear solution.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 308 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of albendazole ( $C_{12}H_{15}N_3O_2S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Albendazole RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of albendazole in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 4.5–5.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
USP Albendazole RS

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## Albendazole Oral Suspension

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**DEFINITION**

Albendazole Oral Suspension is Albendazole in an aqueous vehicle. It contains one or more preservatives and dispersing or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of albendazole ( $C_{12}H_{15}N_3O_2S$ ).

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION** (197U)

**Sample stock solution:** 1 mg/mL of albendazole from a quantity of Suspension, in a mixture of methanol and hydrochloric acid (99:1). Filter the mixture, if necessary, to obtain a clear solution.

**Sample solution:** 0.01 mg/mL of albendazole in 0.1 N sodium hydroxide from *Sample stock solution*

**Acceptance criteria:** Meets the requirements

**ASSAY**

- **PROCEDURE**

**Solution A:** Methanol and hydrochloric acid (99:1)

**Solution B:** 13.75 g/L of monobasic sodium phosphate

**Mobile phase:** Methanol and *Solution B* (60:40)

**Standard stock solution:** 1 mg/mL of USP Albendazole RS in *Solution A*

**Standard solution:** 100  $\mu$ g/mL of USP Albendazole RS from *Standard stock solution* in *Mobile phase*

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## Albendazole Tablets

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» Albendazole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of albendazole ( $C_{12}H_{15}N_3O_2S$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Tablets intended for veterinary use only are so labeled.

**USP Reference standards** (11)—

USP Albendazole RS

USP Parbendazole RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

**Solution:** Dilute a portion of the clear filtrate used to prepare the *Assay preparation* and a portion of the stock solution used to prepare the *Standard preparation* prepared in the *Assay* with *Acidified methanol*, prepared as directed for *Dissolution*, to obtain solutions containing about 10  $\mu$ g of albendazole per mL.

**B:** The retention time of the major peak for albendazole in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

Determine the amount of  $C_{12}H_{15}N_3O_2S$  dissolved using the following procedure.

*Acidified methanol*—To about 50 mL of methanol in a 100-mL volumetric flask add 2 mL of hydrochloric acid, dilute with methanol to volume, and mix.

*Standard solution*—Transfer about 90 mg of USP Albendazole RS, accurately weighed, to a 250-mL volumetric flask, add 10 mL of *Acidified methanol*, and shake to dissolve. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

*Procedure*—Transfer 10.0 mL of a filtered portion of the solution under test to a 250-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  dissolved by the formula:

$$22.5C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Albendazole RS in the *Standard solution*; and  $A_U$  and  $A_S$  are the differences in absorbance between 308 nm and 350 nm obtained from the solution under test and the *Standard solution*, respectively.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{12}H_{15}N_3O_2S$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—

*Acidified methanol and Standard solution*—Prepare as directed under *Dissolution*.

*Test solution*—Place 1 Tablet in a 500-mL volumetric flask, add about 300 mL of *Acidified methanol*, and shake by mechanical means for about 30 minutes. Dilute with *Acidified methanol* to volume, and mix. Filter a portion of this solution, discarding the first 20 mL of the filtrate. Transfer 4.0 mL of the clear filtrate to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  in the Tablet taken by the formula:

$$25C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Albendazole RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the differences in absorbance between 308 nm and 350 nm obtained from the *Test solution* and the *Standard solution*, respectively.

**Assay**—

*Mobile phase*—Dissolve 0.50 g of monobasic ammonium phosphate in 400 mL of water. Add 600 mL of methanol, mix, and filter, discarding the first 15 mL of the filtrate. Degass the clear filtrate before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Sulfuric acid in methanol*—Prepare a mixture of 1 mL of sulfuric acid and 99 mL of methanol.

*Internal standard solution*—Transfer about 150 mg of USP Parbendazole RS to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol*, 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix.

*Standard preparation*—Transfer about 100 mg of USP Albendazole RS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this stock solution and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of albendazole, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 20 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Transfer 5.0 mL of the clear filtrate and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; the resolution between the albendazole peak and the parbendazole peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  in the portion of Tablets taken by the formula:

$$500C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Albendazole RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the albendazole peak to the parbendazole peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Albumin Human

### DEFINITION

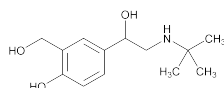
Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.80 to 640.86) (see *Biologics* (1041)). It is a sterile, nonpyrogenic preparation of serum albumin obtained by fractionating material (source blood, plasma, serum, or placentas) from healthy human donors, the source material being tested for the absence of hepatitis B surface antigen. It is made by a process that yields a product that is safe for intravenous use. NLT 96% of its total protein is albumin. It is a solution containing, in each 100 mL, either 25 g of serum albumin osmotically equivalent to 500 mL of normal human plasma, or 20 g equivalent to 400 mL, or 5 g equivalent to 100 mL, or 4 g equivalent to 80 mL, and contains NLT 93.75% and NMT 106.25% of the labeled amount in the case of the solution containing 4 g in each 100 mL, and NLT 94.0% and NMT 106.0% of the labeled amount in the other cases. It contains no added antimicrobial agent, but may contain sodium acetyltrypophanate with or without sodium caprylate as a stabilizing agent. It has a sodium content of NLT 130

mEq/L and NMT 160 mEq/L. It has a heme content such that the absorbance of a solution, diluted to contain 1% of protein, in a 1-cm holding cell, measured at a wavelength of 403 nm, is NMT 0.25. It meets the requirements of the test for heat stability and for pH.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at the temperature recommended by the manufacturer or indicated on the label.
- **EXPIRATION DATE:** The expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage at temperatures not higher than 37°; and not later than 10 years after date of manufacture if in a hermetically sealed metal container and labeling recommends storage between 2° and 10°.
- **LABELING:** Label it to state that it is not to be used if it is turbid and that it is to be used within 4 h after the container is entered. Label it also to state the osmotic equivalent in terms of plasma, the sodium content, and the type of source material (venous plasma, placental plasma, or both) from which it was prepared. Label it also to indicate that additional fluids are needed when the 20-g/100-mL or 25-g/100-mL product is administered to a markedly dehydrated patient.

## Albuterol



$C_{13}H_{21}NO_3$  239.31  
1,3-Benzenedimethanol,  $\alpha^1$ -[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-;  
 $\alpha^1$ -[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene- $\alpha,\alpha'$ -diol  
[18559-94-9].

#### DEFINITION

Albuterol contains NLT 98.5% and NMT 101.0% of albuterol ( $C_{13}H_{21}NO_3$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Sample solution: 80  $\mu$ g/mL in 0.1 N hydrochloric acid  
Acceptance criteria: Meets the requirements

#### ASSAY

- **PROCEDURE**  
Sample solution: 8 mg/mL of Albuterol in glacial acetic acid  
Analysis: To 50 mL of the *Sample solution* add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.93 mg of  $C_{13}H_{21}NO_3$ .  
Acceptance criteria: 98.5%–101.0% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ORGANIC IMPURITIES**  
Standard solution: 0.10 mg/mL of USP Albuterol RS in methanol  
Sample solution: 20 mg/mL of Albuterol in methanol

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)

**Visualization:** Iodine vapor

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, applying aliquots of the *Standard solution* and the *Sample solution*. Develop in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the developing chamber, air-dry, and expose it to iodine vapor.

**Acceptance criteria:** Any spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot produced by the *Standard solution* (0.5%), and the sum of the impurities is not greater than 2.0%.

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Albuterol RS

## Albuterol Sulfate

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$  576.70  
1,3-Benzenedimethanol,  $\alpha^1$ -[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-, sulfate (2:1) (salt).  
 $\alpha^1$ -[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene- $\alpha,\alpha'$ -diol sulfate (2:1) (salt) [51022-70-9].

» Albuterol Sulfate contains not less than 98.5 percent and not more than 101.0 percent of  $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Albuterol Related Compound A RS

4-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-methylphenol sulfate.

USP Albuterol Sulfate RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 80  $\mu$ g per mL.

*Medium:* 0.1 N hydrochloric acid.

**C:** Shake a quantity of it, equivalent to 4 mg of albuterol, with 10 mL of water, and filter: the filtrate so obtained meets the requirements of the tests for *Sulfate* (191).

**D:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.



**Water, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—It meets the requirements of the test for *Chromatographic purity* under *Albuterol*, except to read *Albuterol Sulfate* in place of *Albuterol* and to use water instead of methanol as the solvent to prepare the *Standard solution* and the *Test solution*.

**Assay—**

0.05 ± 0.01 M *Ammonium acetate solution*—Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and mix.

*Mobile phase*—Prepare a degassed mixture of water, 0.05 ± 0.01 M *Ammonium acetate solution*, and isopropanol [65:30: (5 ± 1)], and adjust dropwise with acetic acid to a pH of 4.5 ± 0.3.

*Resolution solution*—Dissolve accurately weighed quantities of USP *Albuterol Sulfate RS* and USP *Albuterol Related Compound A RS* in water, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.140 mg per mL and 0.030 mg per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP *Albuterol Sulfate RS* in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 0.6 mg per mL.

*Assay preparation*—Transfer about 60 mg of *Albuterol Sulfate*, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 20-cm column that contains packing L10. The flow rate is about 2.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between albuterol and albuterol related compound A is not less than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$  in the portion of *Albuterol Sulfate* taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP *Albuterol Sulfate RS* in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Albuterol Tablets

» *Albuterol Tablets* contain an amount of albuterol sulfate  $[(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4]$  equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of albuterol ( $C_{13}H_{21}NO_3$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP *Albuterol Sulfate RS*

**Identification—**

**A:** The  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from *Standard solution A* in the chromatograms obtained as directed in the test for *Related compounds*.

**B:** Shake a quantity of the powdered *Tablets*, equivalent to about 4 mg of *Albuterol*, with 10 mL of water and filter: the filtrate responds to the tests for *Sulfate* (191).

**Dissolution, Procedure for a Pooled Sample** (711)—

*Medium:* water; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

Determine the amount of  $C_{13}H_{21}NO_3$  dissolved using the following method.

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Procedure*—Inject a suitable volume (about 100 µL) of a portion of the solution under test, previously passed through a 0.45-µm nylon filter, into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of  $C_{13}H_{21}NO_3$  dissolved by comparing this peak response with the major peak response similarly obtained on chromatographing the *Standard preparation* previously diluted, if necessary, with a mixture of water and methanol (6:4) to obtain a *Standard solution* having a known concentration of USP *Albuterol Sulfate RS* approximately corresponding to the concentration of the solution under test.

*Tolerances*—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{13}H_{21}NO_3$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds—**

*Test preparation*—Place a quantity of finely powdered *Tablets*, equivalent to 48 mg of albuterol, into a suitable container. Add 60 mL of diluted alcohol (1 in 2), and shake by mechanical means for 30 minutes. Filter the mixture, and wash the filter with small portions of alcohol, combining this with the filtrate. Evaporate the filtrate to dryness under reduced pressure at a temperature below 40°. Dissolve the residue as completely as possible in 2 mL of water.

*Standard solutions*—Prepare solutions of USP *Albuterol Sulfate RS* in water having known concentrations of 0.580 mg per mL (*Solution A*), 0.218 mg per mL (*Solution B*), and 0.073 mg per mL (*Solution C*) equivalent to 0.483 mg, 0.183 mg, and 0.061 mg, respectively, of albuterol.

*Procedure*—Apply 10 µL aliquots (in two successive portions of 5 µL, allowing the solvent to evaporate between applications) of the *Test preparation* and each of *Standard solutions A*, *B*, and *C* to separate points to a suitable chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Air-dry, and place the plate in a saturated chromatographic chamber. Develop the chromatograms with a solvent system consisting of a mixture of methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, water, and ammonium hydroxide (50:45:35:18:3) until the solvent front has moved about 17 cm. Remove the plate from the developing chamber, air-dry, and spray first with 3-methyl-2-benzothiazolinone hydrazone hydrochloride TS, then with ammoniacal potassium ferricyanide TS, and finally again with 3-methyl-2-benzothiazolinone hydrazone hydrochloride TS. Examine the plate and estimate the responses of any secondary spots observed in the lane of the *Test preparation* by comparison with those of *Standard solutions A*, *B*, and *C*. No major secondary spot is greater in size or intensity than the principal spot produced by *Standard solution A* (2.0%). No other secondary spot is greater in size or intensity than the principal spot produced by *Standard solution B* (0.75%). No more than two other secondary spots are equal in size or intensity than the principal spot produced by *Standard solution C* (0.25%). The sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 3.5%.

**Assay—**

**1% Acetic acid**—Transfer a 20-mL portion of glacial acetic acid to a suitable volumetric flask, and dilute with water to 200 mL.

**Mobile phase**—Dissolve 1.13 g of sodium 1-hexane-sulfonate in 1200 mL of water, add 12 mL of glacial acetic acid, and mix. Prepare a filtered and degassed mixture of this solution and methanol (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 12 mg of USP Albuterol Sulfate RS, accurately weighed, to a 100-mL volumetric flask. Add 60 mL of 1% Acetic acid, sonicate for 5 minutes, dilute with methanol to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, dilute with a mixture of water and methanol (6:4) to volume, and mix.

**Assay preparation**—Transfer a number of whole Tablets, equivalent to about 50 mg of albuterol, to a 2000-mL volumetric flask. Add 1200 mL of 1% Acetic acid, shake by mechanical means for 45 minutes, sonicate for 10 minutes, allow to cool to room temperature, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.45-μm or finer porosity.

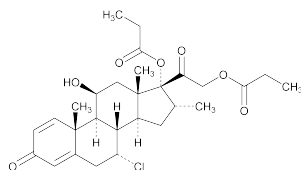
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 800 theoretical plates; the tailing factor for the analyte peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of albuterol (C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>) in the number of Tablets taken by the formula:

$$2000C(r_U / r_S)(239.31/576.70)$$

in which 2000 is the volume, in mL, of the *Assay preparation*; C is the concentration, in mg per mL, of USP Albuterol Sulfate RS in the *Standard preparation*;  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively; 2 is the number of molecules of albuterol released from each molecule of albuterol sulfate; and 239.31 and 576.70 are the molecular weights of albuterol and albuterol sulfate, respectively.

## Alclometasone Dipropionate



C<sub>28</sub>H<sub>37</sub>ClO<sub>7</sub> 521.04  
 Pregna-1,4-diene-3,20-dione, 7-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (7α,11β,16α)-; 7α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [66734-13-2].

**DEFINITION**

Alclometasone Dipropionate contains NLT 97.0% and NMT 102.0% of C<sub>28</sub>H<sub>37</sub>ClO<sub>7</sub>, calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197M>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Solution A:** 6.80 mg/mL of monobasic potassium phosphate (0.05 M)

**Mobile phase:** Methanol and *Solution A* (2:1)

**Internal standard solution:** 2 mg/mL of betamethasone dipropionate in methanol

**Standard stock solution:** 1.2 mg/mL of USP Alclometasone Dipropionate RS in methanol

**Standard solution:** 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution*. Dilute with methanol to 25 mL. [NOTE—This solution contains approximately 0.2 mg/mL of USP Alclometasone Dipropionate RS.]

**Sample stock solution:** 1.2 mg/mL of Alclometasone Dipropionate in methanol

**Sample solution:** 4 mL of *Sample stock solution* and 4 mL of *Internal standard solution*. Dilute with methanol to 25 mL.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm × 30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between the analyte and the internal standard peaks

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>28</sub>H<sub>37</sub>ClO<sub>7</sub> in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio from the *Sample solution*

$R_S$  = peak height ratio from the *Standard solution*

$C_S$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

- RESIDUE ON IGNITION** <281>: NMT 0.1%
- HEAVY METALS, Method II** <231>: NMT 30 ppm

**Organic Impurities****PROCEDURE**

**Mobile phase:** Acetonitrile and water (3:2)

**Diluent:** Acetonitrile and water (2:1)

**System suitability solution:** 1.5 mg/mL of USP Alclometasone Dipropionate RS and 0.015 mg/mL of USP Alclometasone Dipropionate Related Compound A RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Alclometasone Dipropionate in *Diluent*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 5 μL**Run time:** Three times the retention time of alclometasone**System suitability****Sample:** *System suitability solution***Suitability requirements****Tailing factor:** NMT 1.5 for alclometasone dipropionate**Relative standard deviation:** NMT 2.0% for alclometasone dipropionate**Resolution:** NLT 2.0 between alclometasone dipropionate and alclometasone dipropionate related compound A**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 $r_U$  = peak area for each impurity from the *Sample solution* $r_T$  = sum of all the peaks from the *Sample solution* $F$  = relative response factor (see *Impurity Table 1*)**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 2.0%**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alclometasone dipropionate	1.0	—	—
Alclometasone dipropionate related compound A <sup>a</sup>	1.2	0.93	1.0
2-Bromo alclometasone dipropionate <sup>b</sup>	1.7	0.91	0.5
Any individual, unspecified impurity	—	1.0	0.10

<sup>a</sup> 11β,17,21-Trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.<sup>b</sup> 2-Bromo-7α-chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.**SPECIFIC TESTS****• OPTICAL ROTATION, Specific Rotation <781S>****Sample solution:** 30 mg/mL in dioxane**Acceptance criteria:** +21° to +25°**• LOSS ON DRYING <731>:** Dry a sample in a vacuum at a pressure not exceeding 5 mm of mercury at 105° for 3 h: it loses NMT 0.5% of its weight.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.**• USP REFERENCE STANDARDS <11>**

USP Alclometasone Dipropionate RS

USP Alclometasone Dipropionate Related Compound A RS

11β,17,21-Trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.

C<sub>28</sub>H<sub>38</sub>O<sub>7</sub> 486.60**Alclometasone Dipropionate Cream****DEFINITION**Alclometasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate (C<sub>28</sub>H<sub>37</sub>ClO<sub>7</sub>) in a suitable cream base.**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

**• B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST <201>****Standard solution:** 0.08 mg/mL of USP Alclometasone Dipropionate RS in methanol**Sample solution:** Place a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, and add 15 mL of methanol. Insert a stopper securely into the tube, and place the tube in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice-methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5 min. Transfer the clear supernatant to a vial, and allow to equilibrate to room temperature.**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volume:** 20 μL**Developing solvent system:** Chloroform and acetone (7:1)**Analysis****Samples:** *Standard solution* and *Sample solution*

Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.**ASSAY****• PROCEDURE****Buffer:** 6.80 g/L of monobasic potassium phosphate (0.05 M)**Mobile phase:** Methanol and *Buffer* (2:1)**Internal standard solution:** 0.4 mg/mL of betametasone dipropionate in methanol**Standard stock solution:** 0.25 mg/mL of USP Alclometasone Dipropionate RS in methanol**Standard solution:** 0.08 mg/mL of USP Alclometasone Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution*, 5.0 mL of methanol, and 5.0 mL of *Internal standard solution***Sample solution:** Transfer a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Insert a stopper securely into the tube, and place it in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and return the tube to the 60° water bath until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice-methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5

min. Transfer the clear supernatant to a small stoppered flask, and allow to equilibrate to room temperature.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm × 30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between the analyte and internal standard peaks

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alclometasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** <755>: Meets the requirements

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Alclometasone Dipropionate RS

## Alclometasone Dipropionate Ointment

#### DEFINITION

Alclometasone Dipropionate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in a suitable ointment base.

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>  
**Standard solution:** 0.25 mg/mL USP Alclometasone Dipropionate RS in methanol

**Sample solution:** Place a quantity of Ointment, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of a solution of methanol in water (45 in 50), insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer to a stoppered vial.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20 µL

**Developing solvent system:** Chloroform and acetone (7:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 6.80 g/L of monobasic potassium phosphate (0.05 M)

**Solution A:** Dilute 450 mL of methanol with water to 500 mL.

**Mobile phase:** Methanol and *Buffer* (2:1)

**Internal standard solution:** 0.15 mg/mL of betamethasone dipropionate in *Solution A*

**Standard stock solution:** 0.1 mg/mL of USP Alclometasone Dipropionate RS in *Solution A*

**Standard solution:** 0.05 mg/mL of USP Alclometasone Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution*

**Sample solution:** Transfer a quantity of Ointment, equivalent to 0.5 mg of alclometasone dipropionate, to a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of *Internal standard solution* and 5.0 mL of *Solution A*, insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer this *Sample solution* to a stoppered vial.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm × 30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of alclometasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*  
 $R_S$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Alclometasone Dipropionate RS

## Alcohol



$C_2H_6O$  46.07  
Ethanol;  
Ethyl alcohol [64-17-5].

#### DEFINITION

Alcohol contains NLT 92.3% and NMT 93.8%, by weight, corresponding to NLT 94.9% and NMT 96.0%, by volume, at 15.56°, of  $C_2H_5OH$ .

#### IDENTIFICATION

- **A.** It meets the requirements of the test for *Specific Gravity* (841).
- **B. INFRARED ABSORPTION** (197F) or (197S): Neat

#### IMPURITIES

##### Inorganic Impurities

- **LIMIT OF NONVOLATILE RESIDUE:** Evaporate 100 mL in a tared dish on a water bath, and dry at 100°–105° for 1 h: the weight of the residue is NMT 2.5 mg.

##### Organic Impurities

##### PROCEDURE

**Sample solution A:** Alcohol (substance under test)  
**Sample solution B:** 300 ppm of 4-methylpentan-2-ol in *Sample solution A*  
**Standard solution A:** 200 ppm of methanol in *Sample solution A*  
**Standard solution B:** 10 ppm of methanol and 10 ppm of acetaldehyde in *Sample solution A*  
**Standard solution C:** 30 ppm of acetal in *Sample solution A*

**Standard solution D:** 2 ppm of benzene in *Sample solution A*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m fused silica capillary column bonded with a 1.8-μm layer of phase G43

**Split ratio:** 20:1

**Temperature**

**Detector:** 280°

**Injector:** 200°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

**Linear velocity:** 35 cm/s

**Carrier gas:** Helium

**Injection size:** 1.0 μL

#### System suitability

**Sample:** *Standard solution B*

#### Suitability requirements

**Resolution:** NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)

#### Analysis

**Samples:** *Sample solution A*, *Sample solution B*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Standard solution D*

#### Methanol calculation

$$\text{Result} = (r_U/r_S)$$

$r_U$  = peak area of methanol from *Sample solution A*

$r_S$  = peak area of methanol from *Standard solution A*

#### Acetaldehyde and Acetal calculation

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_S\} + \{[D_E/(D_T - D_E)] \times C_U\}$$

$A_E$  = area of the acetaldehyde peak from *Sample solution A*

$A_T$  = area of the acetaldehyde peak from *Standard solution B*

$C_S$  = concentration of acetaldehyde added in *Standard solution B*, 10 ppm

$D_E$  = area of the acetal peak from *Sample solution A*

$D_T$  = area of the acetal peak from *Standard solution C*

$C_U$  = concentration of acetal added in *Standard solution C*, 30 ppm

#### Benzene calculation

$$\text{Result} = [B_E/(B_T - B_E)] \times C_S$$

$B_E$  = area of the benzene peak from *Sample solution A*

$B_T$  = area of the benzene peak from *Standard solution D*

$C_S$  = concentration of benzene added in *Standard solution D*, 2 ppm

[NOTE— If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

#### Other impurities calculation

$$\text{Result} = (r_U/r_M) \times C_M$$

- $r_U$  = peak area of each impurity in *Sample solution B*  
 $r_M$  = peak area of 4-methylpentan-2-ol in *Sample solution B*  
 $C_M$  = concentration of 4-methylpentan-2-ol in *Sample solution B*

**Acceptance criteria:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 ppm
Acetaldehyde and Acetal	NMT 10 ppm, expressed as acetaldehyde
Benzene	NMT 2 ppm
Sum of all other impurities <sup>a</sup>	NMT 300 ppm

<sup>a</sup> Disregard any peaks of less than 9 ppm.

### SPECIFIC TESTS

- SPECIFIC GRAVITY (841):** 0.812–0.816 at 15.56°, indicating 92.3%–93.8%, by weight, or 94.9%–96.0%, by volume, of C<sub>2</sub>H<sub>5</sub>OH

- ULTRAVIOLET ABSORPTION**

**Analytical wavelength:** 235–340 nm

**Cell:** 5 cm

**Reference:** Water

**Acceptance criteria**

**Absorbance:** NMT 0.40 at 240 nm; NMT 0.30, between 250 nm and 260 nm; NMT 0.10, between 270 nm and 340 nm

**Curve:** The absorption curve is smooth

- CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Reference suspension A* and to water in diffused daylight 5 min after preparation of *Reference suspension A*.]

**Hydrazine solution:** 10 mg/mL of hydrazine sulfate in water. [NOTE—Allow to stand for 4–6 h.]

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

**Opalescence standard:** Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. [NOTE—This suspension should not be used beyond 24 h after preparation.]

**Reference suspension A:** *Opalescence standard* and water (1 in 20)

**Reference suspension B:** *Opalescence standard* and water (1 in 10)

**Sample solution A:** Substance to be examined

**Sample solution B:** Dilute 1.0 mL of *Sample solution A* with water to 20 mL, and allow to stand for 5 min before testing.

**Analysis:** Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension A*, *Reference suspension B*, and water to separate matching test tubes. Compare *Sample solution A*, *Sample solution B*, *Reference suspension A*, *Reference suspension B*, and water in diffused daylight, viewing vertically against a black background. (See *Spectrophotometry and Light-Scattering (851)*, *Visual Comparison*.)

[NOTE—The diffusion of light must be such that *Reference suspension A* can readily be distinguished from water, and that *Reference suspension B* can readily be distinguished from *Reference suspension A*.]

**Acceptance criteria:** *Sample solution A* and *Sample solution B* show the same clarity as that of water or their opalescence is not more pronounced than that of *Reference suspension A*.

- ACIDITY OR ALKALINITY**

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.

**Analysis:** To 20 mL of alcohol add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.

**Acceptance criteria:** The solution is pink (30 ppm, expressed as acetic acid).

- COLOR OF SOLUTION**

**Standard stock solution:** Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 g/L).

**Standard solution:** Transfer 1.0 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g/L). [NOTE—Prepare the *Standard solution* immediately before use.]

**Sample solution:** Substance to be examined

**Analysis:** Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution* and water to separate, matching test tubes. Compare the *Sample solution*, *Standard solution*, and water in diffused daylight, viewing vertically against a white background. (See *Spectrophotometry and Light-Scattering (851)*, *Visual Comparison*.)

**Acceptance criteria:** The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- USP REFERENCE STANDARDS (11)**  
USP Alcohol RS

## Dehydrated Alcohol



C<sub>2</sub>H<sub>6</sub>O

Ethanol;

Ethyl alcohol [64-17-5].

46.07

### DEFINITION

Dehydrated Alcohol contains NLT 99.2% by weight, corresponding to NLT 99.5% by volume, at 15.56°, of C<sub>2</sub>H<sub>5</sub>OH.

### IDENTIFICATION

- A. SPECIFIC GRAVITY (841):** NMT 0.7962 at 15.56°, indicating NLT 99.2% of C<sub>2</sub>H<sub>5</sub>OH by weight
- B. INFRARED ABSORPTION (197S) or (197F):** Neat

### IMPURITIES

- LIMIT OF NONVOLATILE RESIDUE**

**Sample:** 100 mL of Dehydrated Alcohol

**Analysis:** Evaporate the *Sample* in a tared dish on a water bath, and dry at 100°–105° for 1 h.

**Acceptance criteria:** The weight of the residue is NMT 2.5 mg.

• **VOLATILE IMPURITIES**

**Sample solution A:** Substance to be examined

**Sample solution B:** 300 µL/L of 4-methylpentan-2-ol in *Sample solution A*

**Standard solution A:** 200 µL/L of methanol in *Sample solution A*

**Standard solution B:** 10 µL/L of methanol and 10 µg/L of acetaldehyde in *Sample solution A*

**Standard solution C:** 30 µL/L of acetal in *Sample solution A*

**Standard solution D:** 2 µL/L of benzene in *Sample solution A*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m fused silica capillary; bonded with a 1.8-µm layer of phase G43

**Split ratio:** 1:20

**Temperatures**

**Injector:** 200°

**Detector:** 280°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

**Flow rate:** 35 cm/s

**Carrier gas:** Helium

**Injection volume:** 1.0 µL

**System suitability**

**Sample:** *Standard solution B*

**Suitability requirements**

**Resolution:** NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)

**Analysis**

**Samples:** *Sample solution A*, *Sample solution B*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Standard solution D*

**Methanol calculation**

$$\text{Result} = r_U/r_S$$

$r_U$  = peak area of methanol from *Sample solution A*

$r_S$  = peak area of methanol from *Standard solution A*

**Acetaldehyde calculation** (sum of acetaldehyde and acetal)

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_A\} + \{[D_E/(D_T - D_E)] \times C_D\}$$

$A_E$  = area of the acetaldehyde peak from *Sample solution A*

$A_T$  = area of the acetaldehyde peak from *Standard solution B*

$C_A$  = concentration of acetaldehyde in *Standard solution B* (µL/L)

$D_E$  = area of the acetal peak from *Sample solution A*

$D_T$  = area of the acetal peak from *Standard solution C*

$C_D$  = concentration of acetal in *Standard solution C* (µL/L)

**Benzene calculation**

$$\text{Result} = (B_E/(B_T - B_E)) \times C_B$$

$B_E$  = area of the benzene peak from *Sample solution A*

$B_T$  = area of the benzene peak from *Standard solution D*

$C_B$  = concentration of benzene in *Standard solution D* (µL/L)

[NOTE—If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

**Any other impurity calculation**

$$\text{Result} = (r_U/r_m) \times C_m$$

$r_U$  = peak area of each impurity from *Sample solution B*

$r_m$  = peak area of 4-methylpentan-2-ol from *Sample solution B*

$C_m$  = concentration of 4-methylpentan-2-ol in *Sample solution B* (µL/L)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 µL/L
Acetaldehyde and acetal	10 µL/L, expressed as acetaldehyde
Benzene	2 µL/L
Sum of all other impurities <sup>a</sup>	300 µL/L

<sup>a</sup> Disregard any peaks of less than 9 µL/L.

**SPECIFIC TESTS**

• **ULTRAVIOLET ABSORPTION**

**Analytical wavelength:** 200–400 nm

**Cell:** 5 cm

**Reference:** Water

**Acceptance criteria**

**Absorbance:** NMT 0.40 at 240 nm; NMT 0.30 between 250 and 260 nm; NMT 0.10 between 270 and 340 nm

**Curve:** Smooth between 235 and 340 nm

• **CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* and to water in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

**Opalescence standard:** Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.

**Standard suspension A:** Dilute 5.0 mL of *Standard stock suspension* with water to 100.0 mL.

**Standard suspension B:** Dilute 10.0 mL of *Standard stock suspension* with water to 100.0 mL.

**Sample solution A:** Substance to be examined

**Sample solution B:** 1.0 mL of *Sample solution A* diluted with water to 20 mL. Allow to stand for 5 min before testing.

**Blank:** Water

**Analysis**

**Samples:** *Standard suspension A*, *Standard suspension B*, *Sample solution A*, *Sample solution B*, and *Blank*. Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and *Blank* to separate matching test tubes. Compare samples in diffused daylight, viewing vertically against a black background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

**Acceptance criteria:** *Sample solution A* and *Sample solution B* show the same clarity as that of water, or their opalescence is not more pronounced than that of *Standard suspension A*. [NOTE—The diffusion of light must be such that *Standard suspension A* can be readily distinguished from water, and that *Standard suspension B* can be readily distinguished from *Standard suspension A*.]

• **ACIDITY OR ALKALINITY**

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL alcohol, and dilute with water to 100 mL.

**Sample solution:** 20 mL of Dehydrated Alcohol

**Analysis:** To the *Sample solution* add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.

**Acceptance criteria:** The solution is pink (30 µg/g, expressed as acetic acid).

• **COLOR OF SOLUTION**

**Standard stock solution:** Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 mg/mL).

**Standard solution:** 1.0 mL of *Standard stock solution*, diluted with dilute hydrochloric acid (10 mg/mL) to 100 mL. Prepare this immediately before use.

**Sample solution:** Substance to be examined

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and water. Transfer a sufficient portion of each of the *Samples* to individual test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Samples* in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

**Acceptance criteria:** The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **USP REFERENCE STANDARDS** (11)  
USP Dehydrated Alcohol RS

## Dehydrated Alcohol Injection

» Dehydrated Alcohol Injection is Dehydrated Alcohol suitable for parenteral use.

**Packaging and storage—**Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature. The container may contain an inert gas in the headspace.

**Identification—**

**A:** Mix 5 drops in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with a filter paper moistened with a solution recently prepared by dissolving 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

**B:** To 5 mL of a solution (1 in 10) add 1 mL of 1.0 N sodium hydroxide, then slowly (over a period of 3 minutes) add 2 mL of 0.1 N iodine: the odor of iodoform develops, and a yellow precipitate is formed within 30 minutes.

**Specific gravity** (841): not more than 0.8035 at 15.56°, indicating not less than 96.8%, by weight, of C<sub>2</sub>H<sub>5</sub>OH.

**Acidity—**To 50 mL, in a glass-stoppered flask, add 50 mL of recently boiled water. Add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 seconds: not more than 10.0 mL of 0.020 N sodium hydroxide is required for neutralization.

**Limit of nonvolatile residue—**Evaporate 40 mL in a tared dish on a water bath, and dry at 105° for 1 hour: the weight of the residue does not exceed 1 mg.

**Water-insoluble substances—**Dilute it with an equal volume of water: the mixture is clear and remains clear for 30 minutes after cooling to 10°.

**Aldehydes and other foreign organic substances—**

Place 20 mL in a glass-stoppered cylinder that has been thoroughly cleaned with hydrochloric acid, then rinsed with water and finally with the dehydrated alcohol to be tested. Cool the contents to approximately 15°, and add, by means of a carefully cleaned pipet, 0.10 mL of 0.10 N potassium permanganate, noting accurately the time of addition. Mix at once by inverting the stoppered cylinder, and allow it to stand at 15° for 5 minutes: the pink color does not entirely disappear.

**Amyl alcohol and nonvolatile, carbonizable substances—**

Allow 25 mL to evaporate spontaneously from a porcelain dish, carefully protected from dust, until the surface of the dish is barely moist: no red or brown color is produced immediately upon the addition of a few drops of sulfuric acid.

**Ultraviolet absorbance—**Record the UV absorption spectrum between 340 nm and 235 nm in a 1-cm cell, with water in a matched cell in the reference beam: the absorbance is not more than 0.08 at 240 nm, and 0.02 between 270 nm and 340 nm, and the curve drawn through these points is smooth.

**Limit of acetone and isopropyl alcohol—**To 1.0 mL add 1 mL of water, 1 mL of a saturated solution of dibasic sodium phosphate, and 3 mL of a saturated solution of potassium permanganate. Warm the mixture to 45° to 50°, and allow to stand until the permanganate color is discharged. Add 3 mL of 2.5 N sodium hydroxide, and pass, without washing, through a sintered-glass filter. Prepare a control containing 1 mL of the saturated solution of dibasic sodium phosphate, 3 mL of 2.5 N sodium hydroxide, and 80 µg of acetone in 9 mL. To each solution add 1 mL of furfural solution (1 in 100), and allow to stand for 10 minutes, then to 1.0 mL of each solution add 3 mL of hydrochloric acid: any pink color produced in the test solution is not more intense than that in the control.

**Methanol—**To 1 drop add 1 drop of water, 1 drop of dilute phosphoric acid (1 in 20), and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 minute, and add sodium metabisulfite solution (1 in 20), dropwise, until the permanganate color is discharged. If a brown color remains, add 1 drop of the dilute phosphoric acid. To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat on a water bath at 60° for 10 minutes: no violet color appears.



**Other requirements**—It meets the requirements under *Injections* <1>.

## Rubbing Alcohol

» Rubbing Alcohol and all preparations under the classification of Rubbing Alcohols are manufactured in accordance with the requirements of the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms, Formula 23-H (8 parts by volume of acetone, 1.5 parts by volume of methyl isobutyl ketone, and 100 parts by volume of ethyl alcohol) being used. It contains not less than 68.5 percent and not more than 71.5 percent by volume of dehydrated alcohol, the remainder consisting of water and the denaturants, with or without color additives, and perfume oils. Rubbing Alcohol contains, in each 100 mL, not less than 355 mg of sucrose octaacetate or not less than 1.40 mg of denatonium benzoate. The preparation may be colored with one or more color additives, listed by the FDA for use in drugs. A suitable stabilizer may be added. Rubbing Alcohol complies with the requirements of the Bureau of Alcohol, Tobacco, and Firearms of the U.S. Treasury Department.

NOTE—Rubbing Alcohol is packaged, labeled, and sold in accordance with the regulations issued by the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms.

**Packaging and storage**—Preserve in tight containers, remote from fire, and store at controlled room temperature.

**Labeling**—Label it to indicate that it is flammable.

**USP Reference standards** <11>—

USP Denatonium Benzoate RS

**Specific gravity** (841): between 0.8691 and 0.8771 at 15.56° (the U.S. Government standard temperature for alcohol determination), for Rubbing Alcohol manufactured with specially denatured alcohol Formula 23-H.

**Limit of nonvolatile residue**—

Where the denaturant is denatonium benzoate—Evaporate 200.0 mL of Rubbing Alcohol, transferred in convenient portions, in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 hour: the weight of the residue is not less than 2.8 mg. (Retain the residue for the Assay for denatonium benzoate.)

Where the denaturant is sucrose octaacetate—Evaporate 25.0 mL of Rubbing Alcohol in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 hour: the weight of the residue is not less than 89 mg. (Retain the residue for the Assay for sucrose octaacetate.)

**Methanol**—Dilute 0.50 mL of it with water to 1.0 mL. To 0.50 mL of the resulting solution add 1 drop of dilute phosphoric acid (1 in 20) and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 minute, and add sodium metabisulfite solution (1 in 20), dropwise, until the permanganate color is discharged. If a brown color remains, add 1 drop of dilute phosphoric acid (1 in 20). To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat in a water bath at 60° for 10 minutes: no violet color appears.

**Assay for denatonium benzoate**—

**Buffer solution**—Dissolve 9.23 g of anhydrous dibasic sodium phosphate in 800 mL of water, adjust with saturated citric acid solution to a pH of  $4 \pm 0.1$ , dilute with water to 1000 mL, and mix.

**Standard preparation**—Dissolve about 25 mg of USP Denatonium Benzoate RS, accurately weighed, in water to make 500 mL, and mix.

**Assay preparation**—Dissolve the residue obtained in the test for *Limit of nonvolatile residue* in 50.0 mL of water, and transfer to a suitable flask.

**Procedure**—Treat the *Standard preparation*, *Assay preparation*, and blank similarly and concomitantly. Transfer 10.0 mL each of the *Standard preparation*, *Assay preparation*, and *Buffer solution* to individual 250-mL separators, and add to each 40 mL of *Buffer solution*, 10 mL of a 1 in 1000 solution of bromophenol blue in chloroform, and 60 mL of chloroform. Shake the separators vigorously for 2 minutes, allow to stand for 15 minutes, then withdraw the chloroform layers through chloroform-washed cotton into 100-mL volumetric flasks. Repeat the extraction with 20 mL of chloroform, adding the filtered chloroform extracts to the respective volumetric flasks, dilute with chloroform to volume, and mix. Without delay, concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of denatonium benzoate ( $C_{28}H_{34}N_2O_3 \cdot H_2O$ ) in 100 mL of Rubbing Alcohol taken by the formula:

$$0.025C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Denatonium Benzoate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for sucrose octaacetate**—Using about 50 mL of 70% alcohol, transfer the residue obtained in the test for *Limit of nonvolatile residue* to a 500-mL conical flask. Neutralize the solution with 0.1 N sodium hydroxide VS, using phenolphthalein TS as the indicator. Add 25.0 mL of 0.1 N sodium hydroxide, attach an air condenser to the flask, and reflux on a steam bath for 1 hour. Remove from the steam bath, cool quickly, and titrate the excess alkali with 0.1 N sulfuric acid VS, using phenolphthalein TS as the indicator. Perform a blank determination (see *Residual Titrations* under *Titrimetry* <541>). Each mL of 0.1 N sodium hydroxide is equivalent to 8.483 mg of sucrose octaacetate ( $C_{28}H_{38}O_{19}$ ).

## Alcohol in Dextrose Injection

» Alcohol in Dextrose Injection is a sterile solution of Alcohol and Dextrose in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of alcohol ( $C_2H_5OH$ ), and not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ).

**Packaging and storage**—Preserve in tight, single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature.

**Labeling**—The label states the total osmolality of the solution expressed in mOsmol per L.

**USP Reference standards** <11>—

USP Endotoxin RS

**Identification**—It responds to the *Identification* tests under *Dextrose* and under *Dehydrated Alcohol*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 3.5 and 6.5, determined on a portion to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of dextrose.

**Heavy metals** (231)—Transfer a volume of Injection, equivalent to 4.0 g of dextrose, to a vessel, and adjust the volume to 25 mL by evaporation or by addition of water, as necessary: the limit is 0.0005C%, in which C is the labeled amount, in g, of  $C_6H_{12}O_6 \cdot H_2O$  per mL of Injection.

**Limit of 5-hydroxymethylfurfural and related substances**—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of  $C_6H_{12}O_6 \cdot H_2O$ , with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements under *Injections* (1).

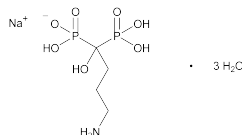
**Assay for alcohol**—Determine by *Method I—Distillation Method* under *Alcohol Determination* (611), using a 50.0-mL portion of Injection.

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing from 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; A is 100 mm divided by the length of the polarimeter tube, in mm; and R is the observed rotation, in degrees.

## Alendronate Sodium



$C_4H_{12}NNaO_7P_2 \cdot 3H_2O$  325.12

Phosphonic acid, (4-amino-1-hydroxybutylidene)bis-, monosodium salt, trihydrate.

Sodium trihydrogen (4-amino-1-hydroxybutylidene) diphosphonate, trihydrate [121268-17-5].

» Alendronate Sodium contains not less than 98.0 percent and not more than 102.0 percent of  $C_4H_{12}NNaO_7P_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at room temperature.

**USP Reference standards** (11)—

USP Alendronate Sodium RS

## Identification—

**A:** *Infrared Absorption* (197M).

**B:** It meets the requirements of the flame test for *Sodium* (191).

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at 140° to constant weight: it loses not less than 16.1% and not more than 17.1% of its weight.

**Heavy metals, Method III** (231): 0.001%.

## Chromatographic purity—

**Borate solution and Diluent**—Prepare as directed in the Assay.

**Buffer solution**—Transfer 5.88 g of sodium citrate dihydrate and 2.84 g of anhydrous dibasic sodium phosphate to a 2-L volumetric flask, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 8, and pass the solution through a filter having a 0.5-μm or finer porosity.

**9-Fluorenylmethyl chloroformate solution**—Prepare a solution in acetonitrile containing about 4 mg of 9-fluorenylmethyl chloroformate per mL. Prepare this solution fresh just prior to use.

**Solution A**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (17:3).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (7:3).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solution**—Prepare a solution of USP Alendronate Sodium RS in *Diluent* having a known concentration of about 0.6 mg per mL.

**Standard solution**—Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *9-Fluorenylmethyl chloroformate solution*, and shake for 45 seconds. Allow to stand at room temperature for 30 minutes. Add 20 mL of methylene chloride, and shake vigorously for 1 minute. Centrifuge for 5 to 10 minutes, and use a portion of the clear upper aqueous layer.

**Diluted standard solution**—Dilute a portion of the *Standard stock solution* with *Diluent* to obtain a solution having a known concentration of about 0.6 μg per mL. Using 5 mL of this solution, proceed as directed for the *Standard solution*, beginning with “to a 50-mL polypropylene, screw-cap centrifuge tube.”

**Reagent blank**—Using a 5.0-mL portion of *Diluent*, proceed as directed for *Standard solution*, beginning with “to a 50-mL polypropylene, screw-cap centrifuge tube.”

**Test solution**—Transfer about 30 mg of Alendronate Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Using a 5.0-mL volume of this solution, proceed as directed for *Standard solution*, beginning with “to a 50-mL polypropylene, screw-cap centrifuge tube.”

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 266-nm detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1.8 mL per minute. The column temperature is maintained at about 45°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–15	100→50	0→50	linear gradient
15–25	50→0	50→100	linear gradient
25–27	0→100	100→0	linear gradient
27–32	100	0	isocratic

Chromatograph the *Standard solution* and the *Diluted standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor for the main peak in the chromatogram of the *Standard solution* is not more than 2.0; and the peak at that locus in the chromatogram of the *Diluted standard solution* is detectable with a signal-to-noise ratio of not less than 3.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Test solution* and the *Reagent blank* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Disregard any peak corresponding to those obtained from the *Reagent blank*. Calculate the percentage of each impurity in the portion of Alendronate Sodium taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the area of each impurity peak, and  $r_s$  is the sum of all impurity peaks and the main peak: not more than 0.1% of any individual impurity is found, and not more than 0.5% of total impurities is found.

#### Assay—

*Buffer solution*—Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1-L volumetric flask, dilute with water to volume, mix, and adjust with phosphoric acid to a pH of 8.

*Diluent*—Dissolve 29.4 g of sodium citrate dihydrate in water in a 1-L volumetric flask, dilute with water to volume, and mix.

*Borate solution*—Dissolve 19.1 g of sodium borate in water in a 1-L volumetric flask, dilute with water to volume, and mix.

*9-Fluorenylmethyl chloroformate solution*—Prepare a solution in acetonitrile containing about 0.5 mg of 9-fluorenylmethyl chloroformate per mL. Prepare this solution fresh just prior to use.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution*, acetonitrile, and methanol (70:25:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard stock preparation*—Prepare a solution of USP Alendronate Sodium RS in *Diluent* having a known concentration of about 0.1 mg per mL. Calculate the concentration,  $C_s$ , of anhydrous alendronate sodium in this solution.

*Standard preparation*—Transfer 5.0 mL of the *Standard stock preparation* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *9-Fluorenylmethyl chloroformate solution*, and shake for 30 seconds. Allow to stand at room temperature for 25 minutes. Add 25 mL of methylene chloride, and shake vigorously for 1 minute. Centrifuge for 5 to 10 minutes. Use a portion of the clear upper aqueous layer.

*Reagent blank*—Using 5.0 mL of *Diluent*, proceed as directed for *Standard preparation*, beginning with “to a 50-mL polypropylene, screw-cap centrifuge tube.”

*Assay stock preparation*—Transfer about 25 mg of Alendronate Sodium, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

*Assay preparation*—Using 5.0 mL of the *Assay stock preparation*, proceed as directed for the *Standard preparation*, beginning with “to a 50-mL polypropylene, screw-cap centrifuge tube.”

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 266-nm detector and a 4.1-mm  $\times$  25-cm column that contains packing L21. The flow rate is about 1.2 mL per minute. The column temperature is maintained at about 35°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than

1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation*, the *Assay preparation*, and the *Reagent blank* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_4H_{12}NNaO_7P_2$  in the portion of Alendronate Sodium taken by the formula:

$$DC_s (r_u / r_s)$$

in which  $D$  is the dilution factor for the *Assay stock preparation*;  $C_s$  is as defined under the *Standard stock preparation*; and  $r_u$  and  $r_s$  are the peak area responses for alendronic acid obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Alendronate Sodium Tablets

### DEFINITION

Alendronate Sodium Tablets contain an amount of Alendronate Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alendronic acid ( $C_4H_{13}NO_7P_2$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate in water. [NOTE—Adjust with phosphoric acid to a pH of 8.0 before bringing the solution to volume.]

**Solution B:** 38.1 g/L of sodium borate in water

**Solution C:** 1 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (20:5:75)

**Diluent:** 29.4 g/L of sodium citrate dihydrate in water  
**Standard stock solution:** 0.03 mg/mL of anhydrous alendronate sodium in *Diluent*, from USP Alendronate Sodium RS

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

**Sample stock solution:** Transfer NLT 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Diluent*, shake by mechanical means for 30 min, and sonicate for 5 min. Dilute with *Diluent* to volume, and centrifuge a portion of this solution. Quantitatively dilute a portion of the clear supernatant to a concentration of 0.02–0.03 mg/mL of alendronic acid.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

**Blank:** Transfer 5 mL of *Diluent* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and

agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 266 nm

**Column:** 4.1-mm × 25-cm; packing L21

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Capacity factor:** NLT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Calculate the percentage of the label claim in the portion of  $C_4H_{13}NO_7P_2$  taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of anhydrous USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

$C_U$  = nominal concentration of alendronic acid in the *Sample stock solution* (mg/mL)

$M_{r1}$  = molecular weight of alendronic acid, 249.10

$M_{r2}$  = molecular weight of anhydrous alendronate sodium, 271.09

**Acceptance criteria:**  $C_4H_{12}NNaO_7P_2$  equivalent to 90.0%–110.0% of the labeled amount of  $C_4H_{13}NO_7P_2$

## PERFORMANCE TESTS

### • DISSOLUTION <711>

#### Test 1

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

Determine the amount of  $C_4H_{13}NO_7P_2$  dissolved by using the following method.

**Solution A and Mobile phase:** Proceed as directed in the *Assay*.

**Diluent:** 176.4 g/L of sodium citrate in *Medium*

**Solution B:** Dissolve 6.2 g of boric acid in approximately 950 mL of water. Adjust with 1 N sodium hydroxide to a pH of 9.0, and dilute with water to 1 L.

**Solution C:** 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh.]

**Standard stock solution:** USP Alendronate Sodium RS in *Medium* to make a concentration equivalent to dissolving 1 Tablet in 900 mL of the same *Medium*. Calculate the concentration,  $C$  (mg/mL), of anhydrous alendronate sodium in this solution.

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

**Blank:** Transfer 5 mL of water to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for

40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

**Sample solution:** Withdraw a portion of the solution under test, and centrifuge immediately. Transfer 5.0 mL of the supernatant to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_4H_{13}NO_7P_2$  dissolved:

$$\text{Result} = (r_U/r_S) \times C \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C$  = defined under the *Standard stock solution*

$M_{r1}$  = molecular weight of alendronic acid, 249.10

$M_{r2}$  = molecular weight of alendronate sodium, 271.09

$V$  = volume of the *Medium*, 900 mL

$L$  = Tablet label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_4H_{13}NO_7P_2$  is dissolved; for tablets labeled for weekly dosing, NLT 75% (Q) of the labeled amount of  $C_4H_{13}NO_7P_2$  is dissolved.

#### Test 2

If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of  $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$  dissolved using the following method.

**Solution B and Solution C:** Proceed as directed in the *Assay*.

**0.6 M citrate buffer:** 176.4 g/L of sodium citrate dihydrate in water

**0.05 M buffer:** Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with phosphoric acid to a pH of 8.0, and dilute with water to volume.

**Mobile phase:** 0.05 M buffer, acetonitrile, and methanol (76:19:5)

**Standard stock solution:** Prepare a solution of USP Alendronate Sodium RS in *Medium* with a final concentration corresponding to the concentration obtained by dissolving 1 tablet in 900 mL of *Medium*. Calculate the concentration,  $C$  (mg/mL), of anhydrous alendronate sodium in this solution.

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL screw-cap polypropylene centrifuge tube containing 1.0 mL of 0.6 M citrate buffer and 5.0 mL of *Solution B*, and mix for about 3 min. Add 4.0 mL of *Solution C*, and agitate for about 30 s. Allow the solution to stand at room temperature for about 30 min. Add 25 mL of methylene chloride, and agitate vigorously for about 40 s. Centrifuge the mixture for 10 min. Use a portion of the clear upper aqueous layer.

**Blank:** Using 5 mL of water, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

#### Sample solution

**For Tablets labeled to contain 5 mg, 10 mg, 35 mg, or 40 mg:** After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45-µm filter, discarding the first 10 mL. Using 5.0 mL of the

filtrate, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

**For Tablets labeled to contain 70 mg:** After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45- $\mu$ m filter, discarding the first 10 mL. Transfer 6.0 mL of the filtrate to a 10-mL volumetric flask, and dilute with water to volume. Using 5.0 mL of this dilution, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*.

**Analysis:** Proceed as directed in *Test 1*.

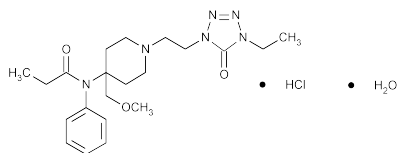
**Tolerances:** NLT 80% (Q) of the labeled amount of alendronate sodium ( $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°.
- **LABELING:** The labeling indicates weekly dosing where appropriate. When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
USP Alendronate Sodium RS

## Alfentanil Hydrochloride



$C_{21}H_{32}N_6O_3 \cdot HCl \cdot H_2O$  470.99

Propanamide, *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-*N*-phenyl, monohydrochloride, monohydrate.

*N*-[1-[2-(4-Ethyl-5-oxo-2-tetrazolin-1-yl)-ethyl]-4-(methoxymethyl)-4-piperidinyl]propionanilide monohydrochloride monohydrate [70879-28-6; 69049-06-5].

» Alfentanil Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{21}H_{32}N_6O_3 \cdot HCl$ , calculated on the anhydrous basis.

**Caution—Handle Alfentanil Hydrochloride with great care since it is a potent opioid analgesic. Great care should be taken to prevent inhaling particles of Alfentanil Hydrochloride and exposing the skin to it.**

**Packaging and storage—**Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Alfentanil Hydrochloride RS

**Identification, Infrared Absorption** (197K).

**Water, Method I** (921): not more than 4.0%.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity—**

**Mobile phase—**Prepare a filtered and degassed mixture of 0.01 M tetrabutylammonium hydrogen sulfate and acetonitrile (86:14). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution—**Dissolve an accurately weighed quantity of USP Alfentanil Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.54 mg per mL.

**Test solution—**Transfer about 54 mg of Alfentanil Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm  $\times$  25-cm column that contains spherical 5- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 5400 theoretical plates, the tailing factor is not more than 1.3, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure—**Inject a volume (about 25  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Alfentanil Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each impurity peak, and  $r_s$  is the sum of all of the peaks: not more than 0.5% of any single impurity is found, and the sum of all impurities is not more than 1.0%.

**Assay—**Dissolve about 350 mg of Alfentanil Hydrochloride, accurately weighed, in 30 mL of glacial acetic acid. Add 3 mL of mercuric acetate TS and 3 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 45.298 mg of  $C_{21}H_{32}N_6O_3 \cdot HCl$ .

## Alfentanil Injection

» Alfentanil Injection is a sterile solution of Alfentanil Hydrochloride in Water for Injection. It contains an amount of Alfentanil Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{32}N_6O_3$ .

**Caution—Handle Alfentanil Injection with great care since it is a potent opioid analgesic.**

**Packaging and storage—**Preserve in tight single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Alfentanil Hydrochloride RS

USP Endotoxin RS

**Identification—**

**A:** It responds to the *Thin-layer Chromatographic Identification Test* (201), a test solution of it diluted, if necessary, with water, to obtain a concentration of 0.5 mg of alfentanil per mL. Prepare a Standard solution in water to obtain a concentration of 0.54 mg per mL of USP Alfentanil Hydrochloride RS. Apply 200  $\mu$ L each of the Standard solution and the test solution, develop the plate using a solvent system consisting of a mixture of chloroform, methanol, and formic acid (85:10:5), and visualize the spots using Dragendorff's reagent.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the

chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 10 USP Endotoxin Units per mL.

**pH** (791): between 4.0 and 6.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

#### Chromatographic purity—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Alfentanil Hydrochloride*.

*Standard solution*—Use the *Standard preparation* as obtained in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Inject a volume (about 25  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each impurity peak, and  $r_s$  is the sum of all of the peaks: the sum of all the impurities is not more than 2.0%.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Alfentanil Hydrochloride*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Alfentanil Hydrochloride RS in saline TS, and dilute quantitatively, and stepwise if necessary, with saline TS to obtain a solution having a known concentration of about 0.54 mg per mL.

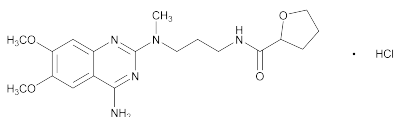
*Assay preparation*—Transfer an accurately measured volume of Injection to a suitable container, and dilute with saline TS, if necessary, to obtain a concentration of about 0.50 mg per mL of alfentanil.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{32}N_6O_3$  in each mL of the Injection taken by the formula:

$$(416.52/452.98)C(r_U / r_S)$$

in which 416.52 and 452.98 are the molecular weights of alfentanil and alfentanil hydrochloride, respectively; C is the concentration, in mg per mL, of USP Alfentanil Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Alfuzosin Hydrochloride



$C_{19}H_{27}N_5O_4 \cdot HCl$  425.91  
2-Furancarboxamide, N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-, monohydrochloride ( $\pm$ );

( $\pm$ )-N-[3-[(4-Amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-2-furamide monohydrochloride [81403-68-1].

#### DEFINITION

Alfuzosin Hydrochloride contains NLT 99.0% and NMT 101.0% of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

#### ASSAY

##### PROCEDURE

**Sample:** 300 mg

**Diluent:** Glacial acetic acid and acetic anhydride (1:1)

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 80 mL of *Diluent*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid VS is equivalent to 42.59 mg of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ).

**Acceptance criteria:** 99.0%–101.0% on the anhydrous basis

#### IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%

##### ORGANIC IMPURITIES

**Buffer:** Dilute 5 mL of perchloric acid in 900 mL of water, adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (20:1:80)

**System suitability solution:** 0.4 mg/mL of USP Alfuzosin System Suitability Mixture RS in *Mobile phase*

**Sample solution:** 0.40 mg/mL of Alfuzosin Hydrochloride in *Mobile phase*

**Reference solution:** 0.40  $\mu$ g/mL of Alfuzosin Hydrochloride in *Mobile phase* from the *Sample solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Peak-to-valley ratio:** The ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and alfuzosin is NLT 5.

##### Analysis

**Samples:** *Sample solution* and *Reference solution*  
Calculate the percentage of each impurity in the portion of Alfuzosin Hydrochloride taken:

$$\text{Result} = (r_U / r_S) \times (1/D) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of alfuzosin from the *Reference solution*

D = dilution factor between the *Sample solution* and the *Reference solution*, 1000

**Acceptance criteria:** See *Table 1*. [NOTE—Disregard any peak with an area less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity D <sup>a</sup>	0.5	0.20
Alfuzosin	1.0	—
Impurity A <sup>b</sup>	1.2	— <sup>c</sup>
Any other individual, unidentified impurity	—	0.10
Total impurities	—	0.30

<sup>a</sup> N-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-N-methylpropane-1,3-diamine.

<sup>b</sup> N-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide.

<sup>c</sup> Impurity A, a component of USP Alfuzosin System Suitability Mixture RS, is not a specified impurity.

### SPECIFIC TESTS

#### • OPTICAL ROTATION <781>

**Sample solution:** 20 mg/mL in carbon dioxide-free water

**Acceptance criteria:** −0.10° to +0.10°

#### Change to read:

#### • WATER DETERMINATION, Method I <921>: •NMT 2.0%• (RB)

1-May-2012

#### • PH <791>

**Sample solution:** 20 mg/mL in carbon dioxide-free water

**Acceptance criteria:** 4.0–5.5

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight containers. Protect from light and humidity, and store at room temperature.

#### • USP REFERENCE STANDARDS <11>

USP Alfuzosin Hydrochloride RS

USP Alfuzosin System Suitability Mixture RS

Alfuzosin Hydrochloride containing approximately 0.4% of impurity A (N-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide), and about 0.4% of impurity D (N-(4-amino-6,7-dimethoxyquinazolin-2-yl)-N-methylpropane-1,3-diamine).

#### Add the following:

## ▲Alfuzosin Hydrochloride Extended-Release Tablets

### DEFINITION

Alfuzosin Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alfuzosin hydrochloride (C<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> · HCl).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION <197> [NOTE—Methods described under *Infrared Absorption* <197K> or <197A> may be used.]

**Sample:** Grind 4 Tablets, and add 20 mL of water.

[NOTE—When analyzing multi-layer Tablets, isolate the layer containing alfuzosin hydrochloride using a suitable tool.] Add 20 mL of strong ammonia solution. Extract with 20 mL of methylene chloride, and separate the organic layer. Repeat the extraction successively with 20 mL, then with 10 mL of methylene chloride. Wash

the combined organic layers with 20 mL of water. Dry the organic solution using a phase separation filter. Take 2.0 mL of the dried organic solution, and mix with 200 mg of finely ground potassium bromide. Evaporate the methylene chloride at 60°, then at 105° for 30 min. Make a disc. Alternatively, evaporate methylene chloride from the dried organic solution at 60°, then at 105° for 30 min. Perform the IR spectrum.

**Acceptance criteria:** The maxima of the spectrum obtained from the *Sample* correspond in position and relative intensity to those obtained from USP Alfuzosin Hydrochloride RS, treated in the same manner as the *Sample*, beginning with “Add 20 mL of water.”

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** 5.0 mL of perchloric acid in 900 mL of water. Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Solution A* (20:1:80)

**Diluent:** 0.01 N hydrochloric acid

**Standard stock solution:** 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol

**Standard solution:** 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*

**Sample stock solution:** Place a suitable number of Tablets into a suitable volumetric flask to obtain a solution having a concentration of 0.16 mg/mL of alfuzosin hydrochloride. Add 80% of the flask volume of methanol, and stir for at least 1 h using a magnetic stirrer. Add 10% of the flask volume of *Diluent*, mix, and allow it to cool to room temperature. Dilute the resulting suspension with methanol to volume, stir, and allow to settle for 30 min.

**Sample solution:** 0.03 mg/mL of alfuzosin hydrochloride in *Diluent* from the *Sample stock solution* supernatant. Pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** 0.8–1.5 for alfuzosin

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of alfuzosin hydrochloride (C<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> · HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 2:** 100 rpm, with Tablet holder (see *Figure 1*)

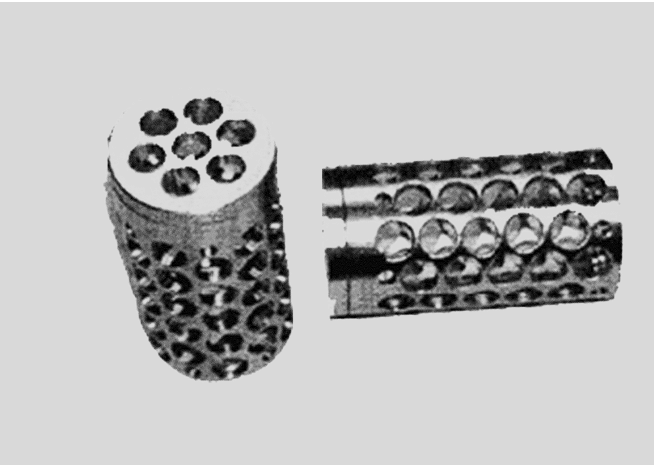


Figure 1. 37.5-mm (l) × 20-mm (d) stainless steel cylinders are used as sample holders. The cylinders contain screw caps drilled with seven 4.5-mm holes. Seven 4.5-mm holes are drilled in the bottom, and 12 longitudinal series of five 5-mm holes are drilled on the cylinders, alternatively starting and ending with one 1.7-mm hole.

**Times:** 1, 6, 12, and 20 h  
**Sample solution:** Pass a portion of the solution under test through a suitable filter.  
**Standard solution:** L/500 (mg/mL) of USP Alfuzosin Hydrochloride RS in Medium, where L is the Tablet label claim in mg  
**Detector:** UV 330 nm  
**Blank:** Medium  
**Path length:** 1 cm  
**Tolerances:** See Table 1.

Table 1

Level	Time (h)	Amount Dissolved
L1		Each Tablet:
	1	10%–20%
	6	40%–55%
	12	65%–85%
	20	NLT 85%
L2		Average of 12 Tablets complies with L1 and each Tablet:
	1	9%–22%
	6	36%–61%
	12	59%–94%
	20	NLT 77%
L3		Average of 24 Tablets complies with L1, NMT 2 Tablets outside L2, and all Tablets within:
	1	8%–24%
	6	32%–66%
	12	52%–102%
	20	NLT 68%

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.  
**Medium:** 0.01 N hydrochloric acid; 900 mL  
**Apparatus 2:** 100 rpm  
**Times:** 1, 3, 12, and 24 h  
**Buffer:** Dilute 5.0 mL of perchloric acid in 900 mL of water, adjust with diluted sodium hydroxide (0.1 g/mL) to a pH of 3.5 ± 0.5, and dilute with water to 1 L.  
**Mobile phase:** Acetonitrile and Buffer (25:75)  
**Standard stock solution:** 0.28 mg/mL of USP Alfuzosin Hydrochloride RS, prepared as follows. In a 200-mL volumetric flask dissolve 55.5 mg of USP Alfuzosin Hy-

drochloride RS in 5 mL of methanol, sonicate to dissolve, and dilute with Medium to volume.  
**Standard solution:** 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in Medium from the Standard stock solution  
**Sample solution:** Pass a portion of the solution under test through a suitable filter.  
**Chromatographic system**  
(See Chromatography <621>, System Suitability.)  
**Mode:** LC  
**Detector:** UV 244 nm  
**Column:** 4.6-mm × 15-cm; 5-μm packing L7  
**Column temperature:** 30°  
**Flow rate:** 1.2 mL/min  
**Injection volume:** 20 μL  
**System suitability**  
**Sample:** Standard solution  
**Suitability requirements**  
**Tailing factor:** NMT 2.0  
**Capacity factor:** NLT 3000 theoretical plates  
**Relative standard deviation:** NMT 2.0%  
**Analysis**  
**Samples:** Standard solution and Sample solution  
Calculate the concentration (C<sub>i</sub>) of alfuzosin hydrochloride (C<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> · HCl) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r<sub>U</sub> = peak response from the Sample solution  
r<sub>S</sub> = peak response from the Standard solution  
C<sub>S</sub> = concentration of the Standard solution (mg/mL)  
Calculate the percentage of the labeled amount of alfuzosin hydrochloride (C<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> · HCl) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times V] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C<sub>i</sub> = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)  
V = volume of medium, 900 mL  
L = label claim (mg/Tablet)  
V<sub>S</sub> = volume of the Sample solution withdrawn at each time point and replaced with Medium (mL)  
**Tolerances:** See Table 2.

Table 2

Time point (i)	Time (h)	Amount Dissolved
1	1	NMT 20%
2	3	15%–35%
3	12	50%–70%
4	24	NLT 80%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements
- **IMPURITIES**
  - **ORGANIC IMPURITIES**  
**Solution A, Mobile phase, Diluent, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.



**System suitability stock solution:** 0.4 mg/mL of USP Alfuzosin System Suitability Mixture A RS in methanol

**System suitability solution:** 0.03 mg/mL of USP Alfuzosin System Suitability Mixture A RS in *Diluent* from the *System suitability stock solution*

**Standard stock solution:** 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol

**Standard solution:** 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.0 between alfuzosin and the furamide analog, and NLT 1.0 between deacylated alfuzosin and the *N*-formyl analog, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for alfuzosin from the *Sample solution*

$C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alfuzosin hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 3*.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacylated alfuzosin <sup>a</sup>	0.46	0.40
<i>N</i> -Formyl analog <sup>b</sup>	0.50	0.30
Alfuzosin	1.0	—
Furamide analog <sup>c</sup>	1.18	— <sup>d</sup>
Any individual unspecified impurity	—	0.20
Total impurities	—	0.80

<sup>a</sup> *N*-(3-Aminopropyl)-6,7-dimethoxy-*N*-(2-methylquinazolin-2-yl)-2,4-diamine.

<sup>b</sup> *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]formamide.

<sup>c</sup> *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide.

<sup>d</sup> Furamide analog, a component of USP Alfuzosin System Suitability Mixture A RS, is not a specified impurity.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Protect from light and moisture. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### • USP REFERENCE STANDARDS (11)

USP Alfuzosin Hydrochloride RS

USP Alfuzosin System Suitability Mixture A RS

Furamide analog: *N*-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide.

$C_{19}H_{23}N_5O_4$  385.42

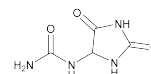
Deacylated alfuzosin: *N*-(3-Aminopropyl)-6,7-dimethoxy-*N*-(2-methylquinazolin-2-yl)-2,4-diamine.

$C_{14}H_{21}N_5O_2$  291.35

*N*-Formyl analog: *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]formamide.

$C_{15}H_{21}N_5O_3$  319.36<sup>▲</sup><sub>USP36</sub>

## Allantoin



$C_4H_6N_4O_3$

Urea, (2,5-dioxo-4-imidazolidinyl)-;

Allantoin [97-59-6].

158.12

#### DEFINITION

Allantoin contains NLT 98.5% and NMT 101.0% of  $C_4H_6N_4O_3$ .

#### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201): The  $R_f$  value of the principal spot from *Sample solution A* corresponds to that from *Standard solution A*, as described in the test for *Organic Impurities*.

#### ASSAY

##### • PROCEDURE

**Sample:** 120 mg

**Analysis:** Transfer the *Sample* to a 100-mL beaker, dissolve by stirring in 40 mL of water, and titrate with 0.1 M sodium hydroxide. Use a suitable electrode system (see *Titrimetry* (541)). Each mL of 0.1 M sodium hydroxide is equivalent to 15.81 mg of  $C_4H_6N_4O_3$ .

**Acceptance criteria:** 98.5%–101.0%

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

##### • ORGANIC IMPURITIES

**Adsorbent:** Cellulose

**Diluent:** Methanol and water (1:1)

**Urea stock solution:** 1 mg/mL of USP Urea RS in water

**Standard solution A:** 1 mg/mL of USP Allantoin RS in *Diluent*

**Standard solution B:** 0.1 mg/mL of USP Urea RS in methanol, from *Urea stock solution*

**Standard solution C:** *Standard solution A* and *Standard solution B* (1:1)

**Sample solution A:** Transfer 0.10 g of Allantoin to a 10-mL volumetric flask, add 5 mL of water, dissolve by heating, and allow to cool. Dilute with methanol to volume. [NOTE—Use immediately after preparation.]

**Sample solution B:** Transfer 1 mL of *Sample solution A* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.

**Spray reagent:** 5 mg/mL of *p*-dimethylaminobenzaldehyde in a mixture of methanol and hydrochloric acid (3:1)

##### Application volume

**Standard solution A:** 5  $\mu$ L

**Standard solution B:** 5  $\mu$ L

**Standard solution C:** 5  $\mu$ L

**Sample solution A:** 10  $\mu$ L

**Sample solution B:** 5  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:15:25)

**Analysis:** Proceed as directed for *Chromatography* (621), *Thin-Layer Chromatography*. Develop the chromatogram until the solvent front has moved about 10 cm. Spray the plate with *Spray reagent*, dry in a current of hot air, and after 30 min examine under visible light.

**Acceptance criteria:** Any spot from *Sample solution A*, except for the principal spot, is not more intense than

the spot from *Standard solution B* (NMT 0.5%). The test is not valid unless the principal spots from *Standard solution C* are clearly separated.

### SPECIFIC TESTS

#### • ACIDITY OR ALKALINITY

**Sample solution:** 5 mg/mL in carbon dioxide-free water  
**Analysis:** To 5 mL of the *Sample solution* add 5 mL of water, 0.1 mL of methyl red TS, and 0.2 mL of 0.01 M sodium hydroxide.

**Acceptance criteria:** A yellow color is observed. The solution turns red upon the addition of 0.4 mL of 0.01 M hydrochloric acid.

#### • LOSS ON DRYING (731):

Dry a sample at 105° to constant weight: it loses NMT 0.1% of its weight.

#### • REDUCING SUBSTANCES

**Sample solution:** 1.0 g of Allantoin in 10 mL of water. Shake for 2 min, and filter.

**Analysis:** To the *Sample solution* add 1.5 mL of 0.02 M potassium permanganate.

**Acceptance criteria:** The solution remains violet for at least 10 min.

### ADDITIONAL REQUIREMENTS

#### • USP REFERENCE STANDARDS (11)

USP Allantoin RS

USP Urea RS

## Allopurinol



C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O 136.11

4*H*-Pyrazolo[3,4-*d*]pyrimidin-4-one, 1,5-dihydro-

1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one.

1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol [315-30-0].

» Allopurinol contains not less than 98.0 percent and not more than 102.0 percent of C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at room temperature.

#### USP Reference standards (11)—

USP Allopurinol RS

USP Allopurinol Related Compound A RS

3-Amino-4-carboxamidopyrazole hemisulfate.

(C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> 350.32

USP Allopurinol Related Compound B RS

5-(Formylamino)-1*H*-pyrazole-4-carboxamide.

C<sub>5</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub> 154.13

USP Allopurinol Related Compound C RS

*N*-(4*H*-1,2,4-Triazol-4-yl)-1*H*-pyrazole-4-carboxamide.

C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O 178.15

USP Allopurinol Related Compound D RS

Ethyl 5-amino-1*H*-pyrazole-4-carboxylate.

C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> 155.15

USP Allopurinol Related Compound E RS

Ethyl 5-(formylamino)-1*H*-pyrazole-4-carboxylate.

USP Allopurinol Related Compound F RS

Ethyl 3-(2-carbethoxy-2-cyanoethyl)amino-1*H*-pyrazole-4-carboxylate.

**Identification, Infrared Absorption** (197K).

**Loss on drying** (731)—Dry it in vacuum at 105° for 5 hours: it loses not more than 0.5% of its weight.

**Related compounds**—[NOTE—Store and inject the *Standard solution* and the *Test solution* at 8°, using a cooled autosampler.]

**Solution A**—Dissolve 1.25 g of monobasic potassium phosphate in 1000 mL of water, filter, and degas.

**Solution B**—Use methanol.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of *Solution A* and *Solution B* (90:10).

**Allopurinol related compound F solution**—Accurately weigh about 5 mg of USP Allopurinol Related Compound F RS into a suitable flask. Add 2.0 mL of 0.1 N sodium hydroxide, promptly sonicate with swirling for 1 to 3 minutes to dissolve, add 80 mL of *Diluent*, and sonicate for an additional 5 minutes.

**Standard stock solution**—Accurately weigh about 5 mg each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS, and transfer to a 100-mL volumetric flask. Add 2.0 mL of 0.1 N sodium hydroxide, promptly sonicate with swirling for not more than 1 minute to dissolve, add the entire volume of *Allopurinol related compound F solution* to the flask, rinse the flask in which *Allopurinol related compound F solution* was prepared with a small amount of *Diluent*, and add the rinsings to the solution. Sonicate for an additional 5 minutes. Dilute with *Diluent* to volume. [NOTE—This solution is stable for 48 hours when stored at 8°.]

**Standard solution**—Quantitatively dilute an accurately measured volume of the *Standard stock solution* with *Diluent* to obtain a solution having known concentrations of about 0.5 µg of allopurinol and about 0.5 µg each of allopurinol related compounds A, B, C, D, E, and F per mL.

**Test solution**—Transfer about 25 mg of Allopurinol, accurately weighed, to a 100-mL volumetric flask, add 5.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for not more than 1 minute, add 80 mL of *Diluent*, and sonicate for an additional 5 minutes. Dilute with *Diluent* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at 30°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–30	90→70	10→30	linear gradient
30–35	70	30	isocratic
35–36	70→90	30→10	linear gradient
36–46	90	10	re-equilibration

Chromatograph the *Standard solution*, identify the peaks (see *Table 1*), and record the peak responses as directed for *Procedure*: the resolution, *R*, between allopurinol related compounds C and B is not less than 0.8; and the tailing factor for the allopurinol peak is not more than 1.5.

**Procedure**—Separately inject equal volumes (about 40 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and identify the allopurinol peak and the peaks due to impurities listed in *Table 1*.

Table 1

Name	Relative Retention Time	Limit (%)
Allopurinol related compound A <sup>1</sup>	0.62	0.2
Allopurinol related compound C <sup>2</sup>	0.79	0.2
Allopurinol related compound B <sup>3</sup>	0.81	0.2
Allopurinol	1.0	—
Allopurinol related compound D <sup>4</sup>	4.4	0.2
Allopurinol related compound E <sup>5</sup>	4.8	0.2
Allopurinol related compound F <sup>6</sup>	6.5	0.2

<sup>1</sup>3-Amino-1H-pyrazole-4-carboxamide.

<sup>2</sup>5-(4H-1,2,4-Triazol-4-yl)-1H-pyrazole-4-carboxamide.

<sup>3</sup>5-(Formylamino)-1H-pyrazole-4-carboxamide.

<sup>4</sup>Ethyl-5-amino-1H-pyrazole-4-carboxylate.

<sup>5</sup>Ethyl-5-(formylamino)-1H-pyrazole-4-carboxylate.

<sup>6</sup>Ethyl-(E/Z)-3-(2-carboxy-2-cyanoethenyl)amino-1H-pyrazole-4-carboxylate.

Calculate the percentage of each impurity in the portion of Allopurinol taken by the formula:

$$10(C/W)(r_i / r_s)$$

in which C is the concentration, in µg per mL, of each individual impurity in the *Standard solution*; W is the weight, in mg, of Allopurinol taken to prepare the *Test solution*; and  $r_i$  and  $r_s$  are the peak responses for each individual impurity obtained from the *Test solution* and the *Standard solution*, respectively. [NOTE—For unspecified impurities,  $r_s$  is the peak response for the allopurinol peak obtained from the *Standard solution*.] In addition to not exceeding the limits for each impurity in Table 1, not more than 0.1% of any individual unspecified impurity is found; and not more than 1.0% of total impurities is found.

**Limit of hydrazine**—[NOTE—Under the following conditions, any hydrazine present in the sample will react with benzaldehyde to form benzalazine.]

**Sodium hydroxide 2N solution**—Dissolve 8.5 g of sodium hydroxide in water, and dilute with the same solvent to 100 mL. Alternatively, a commercially available sodium hydroxide 2N solution can be used.

**Diluent**—Prepare a mixture of *Sodium hydroxide 2N solution* and methanol (1:1).

**Benzaldehyde solution**—Prepare a solution in *Diluent* containing 40 mg of benzaldehyde per mL. Prepare immediately before use.

**Hydrazine solution**—Transfer 10.0 mg of hydrazine sulfate to a 50-mL volumetric flask, dissolve in *Diluent* by sonicating for about 2 minutes, and dilute with *Diluent* to volume. Dilute this solution quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 2.0 µg of hydrazine sulfate per mL.

**Standard solution**—Transfer 5.0 mL of the *Hydrazine solution* to a suitable flask, add 4 mL of *Benzaldehyde solution*, mix, and allow to stand for 2.5 hours at room temperature. Add 5.0 mL of hexane, and shake for 1 minute. Allow the layers to separate, and use the upper (hexane) layer.

**Test solution**—Transfer about 250.0 mg of Allopurinol, accurately weighed, to a suitable flask, and dissolve in 5.0 mL of *Diluent*. Add 4 mL of *Benzaldehyde solution*, mix, and allow to stand for 2.5 hours at room temperature. Add 5.0 mL of hexane, and shake for 1 minute. Allow the layers to separate, and use the upper (hexane) layer.

**Blank solution**—Mix 5.0 mL of *Diluent* and 4 mL of *Benzaldehyde solution*, and allow to stand for 2.5 hours at room

temperature. Add 5.0 mL of hexane, and shake for 1 minute. Allow the layers to separate, and use the upper (hexane) layer.

**Mobile phase**—Prepare a mixture of hexane and isopropyl alcohol (95:5).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 310-nm detector and a 4.0-mm × 25-cm column that contains 5-µm packing L10. The column temperature is maintained at 30°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, identify the components on the basis of their relative retention time (1.0 for benzaldehyde and about 0.8 for benzalazine), and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between benzaldehyde and benzalazine is not less than 2.0; and the relative standard deviation for replicate injections is not greater than 15.0% for the benzalazine peak.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Blank solution*, the *Standard solution*, and the *Test solution* into a chromatograph, record the chromatogram for at least two times the retention time of the benzaldehyde peak, identify the components, and measure the responses of peaks due to benzalazine. Calculate the amount, in ppm, of hydrazine in the portion of Allopurinol taken by the formula:

$$1000(32.05 / 130.12) (C_s / C_t)(r_u / r_s)$$

in which 32.05 and 130.12 are the molecular weights of hydrazine and hydrazine sulfate, respectively; 1000 is the conversion coefficient from µg per mL to ppm;  $C_s$  is the concentration, in µg per mL, of hydrazine sulfate in the *Hydrazine solution*;  $C_t$  is the concentration, in mg per mL, of allopurinol in the *Allopurinol solution*; and  $r_u$  and  $r_s$  are the peak responses for the benzalazine peak obtained from the *Test solution* and the *Standard solution*, respectively: not more than 10 ppm of hydrazine is found.

**Assay**—[NOTE—Store and inject the *System suitability solution*, the *Standard preparation*, and the *Assay preparation* at 8°, using a cooled autosampler.]

**Mobile phase**—Dissolve 1.25 g of monobasic potassium phosphate in 1000 mL of water, filter, and degas.

**System suitability solution**—Transfer accurately weighed quantities of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS, each to a suitable volumetric flask, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately and quantitatively dilute with *Mobile phase* to obtain solutions having known concentrations of about 0.05 mg per mL. Transfer 1.0 mL of each of these three solutions to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Allopurinol RS in a small volume of 0.1 N sodium hydroxide, and immediately and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL. Quantitatively dilute an accurately measured volume of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg of allopurinol per mL.

**Assay preparation**—Transfer about 50 mg of Allopurinol, accurately weighed, to a 100-mL volumetric flask, dissolve in 5.0 mL of 0.1 N sodium hydroxide, immediately dilute with *Mobile phase* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg of allopurinol per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.8 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between allopuri-

not related compound B and allopurinol related compound C is not less than 1.1, and that between allopurinol related compound C and allopurinol is not less than 6.0. [NOTE—For the purpose of identification, the relative retention times are about 0.7 for allopurinol related compound B, 0.8 for allopurinol related compound C, and 1.0 for allopurinol.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_5H_4N_4O$  in the portion of Allopurinol taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_U$  and  $C_S$  are the concentrations, in mg per mL, of allopurinol in the *Assay preparation* and the *Standard preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Allopurinol Oral Suspension

### DEFINITION

Allopurinol Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of allopurinol ( $C_5H_4N_4O$ ). Prepare Allopurinol Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Allopurinol	2 g
Glycerin	5 mL
Vehicle for Oral Suspension, NF	45 mL
Vehicle for Oral Solution, NF, a sufficient quantity to make	100 mL

Select the number of tablets that contain the specified amount of allopurinol, and calculate the quantity of each ingredient required for the total amount to be prepared. Count, weigh, or measure each ingredient. Thoroughly pulverize the tablets. Mix the powdered *Allopurinol* tablets and *Glycerin* to form a smooth paste. Incorporate the *Vehicle for Oral Suspension*. Add sufficient *Vehicle for Oral Solution* to volume, and mix well. Adjust the pH, if necessary. Package and label.

### SPECIFIC TESTS

- **pH** (791): 6.5–7.5

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a tight container, and store at controlled room temperature.
- **LABELING:** Label it to state that it is to be shaken well before use, and that it is to be discarded after 60 days. Label it to state that it is to be kept out of the reach of children. Label it to indicate the nominal content of allopurinol in the Oral Suspension.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded

## Allopurinol Tablets

» Allopurinol Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of allopurinol ( $C_5H_4N_4O$ ).

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Allopurinol RS

**Identification**—Extract a quantity of finely powdered Tablets, equivalent to about 50 mg of allopurinol, by trituration with 10 mL of 0.1 N sodium hydroxide. Filter, acidify the filtrate with 1 N acetic acid, collect the precipitated allopurinol (allow 10 to 15 minutes for sufficient precipitation to occur), wash the precipitate with 3 mL of dehydrated alcohol, in portions, and finally wash with 4 mL of anhydrous ethyl ether. Allow to dry in air for 15 minutes, then dry at 105° for 3 hours: the residue so obtained meets the requirements for the *Identification* test under *Allopurinol*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

**Standard stock solution**—Prepare a stock solution by transferring about 40 mg of USP Allopurinol RS, accurately weighed, to a 200-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, sonicate for about 2 minutes, shake by mechanical means for about 10 minutes, dilute with *Dissolution Medium* to volume, and mix.

**Standard solution**—Dilute the *Standard stock solution* with *Dissolution Medium* to obtain a solution having a concentration similar to that expected in the solution under test.

**Procedure**—Determine the amount of  $C_5H_4N_4O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with the *Standard solution*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_5H_4N_4O$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—[NOTE—Do not allow the *Mobile phase* to remain in the column overnight. After performing the procedure, flush the system with water for not less than 20 minutes, and then flush with methanol for 20 minutes.]

**Mobile phase**—Prepare a filtered and degassed 0.05 M solution of monobasic ammonium phosphate.

**Internal standard solution**—On the day of use, dissolve about 50 mg of hypoxanthine in 10 mL of 0.1 N sodium hydroxide, shake by mechanical means until dissolved (about 10 minutes), dilute with water to 50 mL, and mix.

**Standard preparation**—On the day of use, transfer about 50 mg of USP Allopurinol RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, dilute with water to volume, and mix. Transfer 4.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of allopurinol, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, add water to volume, and mix. [NOTE—From this point, conduct the remainder of the Assay without delay.] Filter, rejecting the first 10 mL of the filtrate. Transfer 4.0 mL of the filtrate and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for hypoxanthine and 1.0 for allopurinol; the resolution, *R*, between the analyte and internal standard is not less than 5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of allopurinol (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O) in the portion of Tablets taken by the formula:

$$2.5C(R_U / R_S)$$

in which *C* is the concentration, in µg per mL, of USP Allopurinol RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of allopurinol to hypoxanthine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Allyl Isothiocyanate



C<sub>4</sub>H<sub>5</sub>NS 99.15  
3-Isothiocyanato-1-propene.  
Isothiocyanic acid allyl ester [57-06-7].

» Allyl Isothiocyanate contains not less than 93.0 percent and not more than 105.0 percent of C<sub>4</sub>H<sub>5</sub>NS.

**Caution**—Allyl Isothiocyanate is a potent lachrymator, with a pungent irritating odor. Care should be taken to protect the eyes, to prevent inhalation of fumes, and to avoid tasting.

**Packaging and storage**—Preserve in tight containers.

**Identification, Infrared Absorption** <197F>—The spectrum exhibits pronounced peaks at about 700-cm<sup>-1</sup>, 950-cm<sup>-1</sup>, 980-cm<sup>-1</sup>, 1300-cm<sup>-1</sup>, 1340-cm<sup>-1</sup>, 1350-cm<sup>-1</sup>, 1410-cm<sup>-1</sup>, 1420-cm<sup>-1</sup>, 1650-cm<sup>-1</sup>, 2100-cm<sup>-1</sup>, and 2200-cm<sup>-1</sup>.

**Specific gravity** <841>: between 1.013 and 1.020.

**Refractive index** <831>: between 1.527 and 1.531, determined at 20°.

**Distilling range, Method I** <721>: between 148° and 154°.

**Limit of phenols**—Dilute 1 mL of it with 5 mL of alcohol, and add 1 drop of ferric chloride TS: a blue color is not produced immediately.

**Assay**—Transfer about 4 mL of Allyl Isothiocyanate, accurately weighed, to a 100-mL volumetric flask, add alcohol to volume, and mix. Transfer 5.0 mL of this stock solution to a 100-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 5 mL of ammonia TS. Connect the flask to a reflux condenser, heat on a water bath for 1 hour, and allow to cool to room temperature. Disconnect the flask from the condenser, transfer the contents of the conical flask to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Pass through a dry filter, discarding the first 10 mL of the filtrate. To 50.0 mL of the subse-

quent filtrate add 5 mL of nitric acid and 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Perform a blank determination, using 5 mL of alcohol in place of the stock solution, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 4.958 mg of C<sub>4</sub>H<sub>5</sub>NS.

## Aloe

### DEFINITION

Aloe is the dried latex of the leaves of *Aloe barbadensis* Mill. (*Aloe vera* L.), known in commerce as Curaçao Aloe, or of *Aloe ferox* Mill. and hybrids of this species with *Aloe africana* Mill. and *Aloe spicata* Baker, known in commerce as Cape Aloe (Fam. Liliaceae). Aloe yields NLT 50.0% of water-soluble extractive.

### IDENTIFICATION

- **A.** Powdered Aloe dissolves in nitric acid with effervescence, forming a reddish-brown to brown or green solution.
- **B. PROCEDURE**  
**Sample:** 1 g, finely powdered  
**Analysis:** Mix the *Sample* with 25 mL of cold water. Shake the mixture occasionally during 2 h, filter, and wash the filter and residue with sufficient cold water to make the filtrate measure 100 mL.  
**Acceptance criteria:** The color of the filtrate, viewed in the bulb of a 100-mL volumetric flask, is dark orange with Curaçao Aloe, and greenish yellow with Cape Aloe. The filtrate darkens on standing. [NOTE—Reserve the filtrate for *Identification* tests C and D.]
- **C. PROCEDURE**  
**Sample:** 5 mL of the filtrate obtained in *Identification* test B  
**Analysis:** Add 2 mL of nitric acid to the *Sample*.  
**Acceptance criteria:** The mixture exhibits a reddish-orange color with Curaçao Aloe, and a reddish-brown color that changes rapidly to green with Cape Aloe.
- **D. PROCEDURE**  
**Sample:** 10 mL of the filtrate obtained in *Identification* test B  
**Analysis:** Mix the *Sample* with 2 mL of ammonium hydroxide.  
**Acceptance criteria:** The mixture exhibits an amber color with Cape Aloe, and a dark amber color with Curaçao Aloe.

### ASSAY

#### • WATER-SOLUBLE EXTRACTIVE

**Sample:** 2 g of powdered Aloe  
**Analysis:** Macerate the *Sample* in 70 mL of water in a suitable flask. Shake the mixture during 8 h at 30-min intervals, and allow it to stand for 16 h without shaking. Filter, and wash the flask and residue with small portions of water, passing the washings through the filter, until the filtrate measures 100.0 mL. Evaporate a 50-mL aliquot of the filtrate in a tared dish on a steam bath to dryness, and dry at 110° to constant weight.  
**Acceptance criteria:** The weight of water-soluble extractive so obtained is NLT 50% of the weight of Aloe taken.

### SPECIFIC TESTS

#### • WATER DETERMINATION, Method III <921>

**Sample:** Use a powdered sample. If the Aloe is not powdered, crush it in a mortar until it passes through a no. 40 sieve, and mix the ground material before weighing the sample.

**Analysis:** Dry at 105° for 5 h

**Acceptance criteria:** NMT 12.0%

• **ARTICLES OF BOTANICAL ORIGIN, Total Ash** <561>

**Acceptance criteria:** NMT 4.0%

• **ALCOHOL-INSOLUBLE SUBSTANCES**

**Sample:** 1 g of powdered Aloe

**Analysis:** Add the *Sample* to 50 mL of alcohol in a flask. Heat the mixture to boiling, and maintain at incipient boiling for 15 min, replacing any loss due to evaporation. Remove from the heat, and shake the mixture at intervals during 1 h. Pass through a small dried and tared filter paper or a dried and tared filtering crucible, and wash the residue on the filter with alcohol until the last washing is colorless. Dry the residue at 105° to constant weight.

**Acceptance criteria:** The weight of the residue does not exceed 10.0% of the weight of Aloe taken.

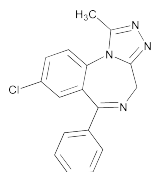
• **BOTANIC CHARACTERISTICS**

**Curaçao aloe:** Brownish black, opaque masses. Its fractured surface is uneven, waxy, and somewhat resinous.

**Cape aloe:** Dusky to dark brown irregular masses, the surfaces of which are often covered with a yellowish powder. Its fracture is smooth and glassy.

**Powdered aloe:** Yellow, yellowish brown to olive-brown in color. When mounted in a bland expressed oil, it appears as greenish-yellow to reddish-brown angular or irregular fragments, the hues of which depend to some extent upon the thickness of the fragments.

## Alprazolam



$C_{17}H_{13}ClN_4$  308.76  
4*H*-[1,2,4]Triazolo[4,3- $\alpha$ ][1,4]benzodiazepine, 8-chloro-1-methyl-6-phenyl-;  
8-Chloro-1-methyl-6-phenyl-4*H*-s-triazolo[4,3- $\alpha$ ][1,4]benzodiazepine [28981-97-7].

### DEFINITION

Alprazolam contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{13}ClN_4$ .

[**CAUTION**—Care should be taken to prevent inhaling particles of Alprazolam and exposing the skin to it.]

### IDENTIFICATION

• **A. INFRARED ABSORPTION** <197M>

- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

### ASSAY

• **PROCEDURE**

**Diluent:** Acetonitrile and water (1:1)

**Buffer:** 1.4 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 25  $\mu$ g/mL of USP Alprazolam RS in *Diluent*. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]

**Sample solution:** 25  $\mu$ g/mL of Alprazolam in *Diluent*. Sonicate for about 1 min. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 231 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

### IMPURITIES

• **RESIDUE ON IGNITION** <281>: NMT 0.5%

• **HEAVY METALS, Method II** <231>: 20 ppm

• **ORGANIC IMPURITIES**

**Diluent, Buffer, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 20  $\mu$ g/mL each of USP Alprazolam RS, USP Alprazolam Related Compound A RS, and USP 2-Amino-5-chlorobenzophenone RS in *Diluent*.  
**Standard solution:** 0.25  $\mu$ g/mL of USP Alprazolam RS in *Diluent*. [NOTE—When stored in closed containers, the solution is stable for 48 h at room temperature.]

**Sample solution:** 250  $\mu$ g/mL of Alprazolam in *Diluent*. Sonicate for about 1 min. [NOTE—When stored in closed containers, the *Sample solution* is stable for 24 h at room temperature.]

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—For relative retention times, see *Table 1*.]

### Suitability requirements

**Resolution:** NLT 2.0 between alprazolam related compound A and alprazolam, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity in the *Sample solution*

$r_S$  = peak response for alprazolam from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of Alprazolam in the *Sample solution* ( $\mu$ g/mL)

$F$  = relative response factor (see *Table 1*)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A	0.8	0.76	0.15
Alprazolam	1.0	1.0	—
2-Amino-5-chlorobenzophenone	4.0	1.0	0.15
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 1 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Alprazolam RS
  - USP Alprazolam Related Compound A RS
  - 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.
  - USP 2-Amino-5-chlorobenzophenone RS
  - 2-Amino-5-chlorobenzophenone.
  - C<sub>13</sub>H<sub>10</sub>ClNO 231.68

## Alprazolam Oral Suspension

#### DEFINITION

Alprazolam Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>).

Prepare Alprazolam Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Solutions* (795)).

Alprazolam	100 mg
Vehicle: a mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF (1:1), a sufficient quantity to make	100 mL

Commminute tablets in a suitable mortar to a fine powder, or add *Alprazolam* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

#### ASSAY

##### PROCEDURE

**Buffer:** 0.04 M sodium acetate solution. Adjust with glacial acetic acid to a pH of 2.4.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (45:8:47)

**Standard solution:** 20 µg/mL of USP Alprazolam RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable vol-

ume of the Oral Suspension in *Mobile phase* to obtain a nominal concentration of 20 µg/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 0.6 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of alprazolam is about 10 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 1.4% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of alprazolam in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **pH** (791): 4.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or under refrigeration.
- **LABELING:** Label it to state that it is to be well-shaken before use, and to state the *Beyond-Use Date*.  
**Beyond-Use Date:** NMT 60 days after the day on which it was compounded
- **USP REFERENCE STANDARDS** (11)
  - USP Alprazolam RS

## Alprazolam Tablets

#### DEFINITION

Alprazolam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>).

#### IDENTIFICATION

##### A. INFRARED ABSORPTION

**Sample:** An amount of finely powdered Tablets, equivalent to 15 mg of alprazolam, prepared as follows. Dissolve the *Sample* in 10 mL of 10 mg/mL of sodium carbonate solution. Add 15 mL of chloroform, and shake vigorously for 30 min. Centrifuge, withdraw the aqueous layer, and transfer the chloroform to a clean container. Add 200 mg of potassium bromide. Evaporate the chloroform from this mixture to dryness, and dry the dispersion in vacuum at 60° for 24 h. Grind this dispersion into a fine powder. Prepare a suitable pellet for testing by placing 100 mg of dried potassium bromide into a die. Sprinkle 20 mg of the finely ground alprazolam–potassium bromide dispersion onto the dried potassium bromide layer, and cover with another specimen of 100 mg of dried potassium bromide.

**Acceptance criteria:** The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima characteristic of alprazolam, as compared to

that of a similar preparation of USP Alprazolam RS, at the following wavenumbers: at 1609, 1578, 1566, 1539, 1487, and 1379 wavenumbers in the region of 1650–1300  $\text{cm}^{-1}$ ; at 932, 891, 826, 779, 746, 696, and 658 wavenumbers in the region of 975–600  $\text{cm}^{-1}$ .

## ASSAY

### PROCEDURE

**Mobile phase:** Acetonitrile, chloroform, butyl alcohol, glacial acetic acid, and water (850: 80: 50: 0.5: 20)

**Internal standard solution:** 0.25 mg/mL of triazolam in acetonitrile

**Standard stock solution:** 0.25 mg/mL of USP Alprazolam RS in *Internal standard solution*

**Standard solution:** 25  $\mu\text{g/mL}$  of USP Alprazolam RS from *Standard stock solution* in acetonitrile

**Sample solution:** Nominally 25  $\mu\text{g/mL}$  of alprazolam from finely powdered Tablets (NLT 20) prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 1% of the flask volume of water. Transfer 10% of the flask volume of *Internal standard solution*, shake vigorously for 10 min, and dilute with acetonitrile to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  30-cm; packing L3

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu\text{L}$

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 2.0 between triazolam and alprazolam

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Sample solution*

$R_S$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of alprazolam in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION, Procedure for a Pooled Sample <711>

**Buffer stock solution:** Dissolve 80 g of monobasic potassium phosphate and 20 g of dibasic potassium phosphate in 1 L of water. Add, with mixing, phosphoric acid or potassium hydroxide solution (45 in 100), as necessary to adjust the solution, such that the resulting solution has a pH of  $6.0 \pm 0.1$ .

**Buffer:** Prepare a 1-in-10 dilution of the *Buffer stock solution* to obtain a solution that has a pH of  $6.0 \pm 0.1$ .

**Medium:** *Buffer*; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (35:5:60)

**Standard stock solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** Add 50 mL of *Buffer stock solution* and 250 mL of water to a 500-mL flask. Add to the flask 5.0 mL of *Standard stock solution* for every 0.25 mg of alprazolam contained in the Tablet being assayed. Dilute with water to volume.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L7

**Flow rate:** 1 mL/min

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 500 theoretical plates

**Relative standard deviation:** NMT 3.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) is dissolved.

### UNIFORMITY OF DOSAGE UNITS <905>

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Internal standard solution** 0.032 mg/mL of triazolam in acetonitrile

**Standard solution:** 0.025 mg/mL of USP Alprazolam RS in *Internal standard solution*

**Sample solution:** Transfer 1 Tablet to a container. Add 0.4 mL of water directly onto the Tablet, allow the Tablet to stand for 2 min, and then swirl the container to disperse the Tablet. For every 0.25 mg of alprazolam contained in the Tablet, add 10.0 mL of *Internal standard solution* to the container. Shake, and centrifuge if necessary.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times C \times V \times (100/L)$$

$R_U$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Sample solution*

$R_S$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Standard solution*

$C$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$V$  = volume of *Internal standard solution* used to prepare the *Sample solution* (mL)

$L$  = label claim (mg/Tablet)

**Acceptance criteria:** Meet the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS <11>**

USP Alprazolam RS



## Alprazolam Extended-Release Tablets

### DEFINITION

Alprazolam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ).

### IDENTIFICATION

**A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, water, and phosphoric acid (350:650:1)

**Standard solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Sample solution:** Transfer an appropriate number of Tablets into a suitable volumetric flask to obtain a nominal concentration of about 0.05 mg/mL of alprazolam. Sonicate in 80% of the flask volume of methanol for 15 min, shake mechanically for 30 min, dilute with methanol to final volume, filter a portion of the solution, and discard the first 3 mL of filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for alprazolam

**Efficiency:** NLT 3000 theoretical plates for alprazolam

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ( $C_{17}H_{13}ClN_4$ ), based on the label claim, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0  $\pm$  0.1); 500 mL

**Apparatus 1:** 100 rpm

**Time:** 1, 4, 8, and 12 h

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Medium* (7:1:12)

**Standard stock solution:** 0.5 mg/mL of USP Alprazolam RS in acetonitrile

**Standard solution:** (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L7

**Flow rate:** 1 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 3000 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved		
	0.5-mg Tablet	2-mg Tablet	3-mg Tablet
1	NMT 25%	NMT 20%	NMT 20%
4	40%–60%	30%–55%	30%–55%
8	70%–90%	65%–90%	65%–90%
12	NLT 85%	NLT 85%	NLT 85%

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of 6.0  $\pm$  0.1); 500 mL

**Apparatus 1:** 100 rpm

**Time:** 1, 4, 8, and 16 h

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Medium* (35:5:60)

**Standard stock solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  7.5-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.3 mL/min

**Injection size:** 80  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point  $i$ , ( $Q_i$ ):

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100] + [Q_1 \times (V_S/V)]$$

$$Q_8 = [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100] + [Q_1 \times (V_S/V)] + [Q_4 \times V_S/(V - V_S)]$$

$$Q_{16} = [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100] + [Q_1 \times V_S/V] + [Q_4 \times V_S/(V - V_S)] + [Q_8 \times V_S/(V - 2V_S)]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL) $L$  = label claim (mg/Tablet) $V$  = initial volume of *Medium*, 500 mL $V_S$  = volume of the sample withdrawn at each time point (mL)**Tolerances:** See *Table 2*.**Table 2**

Time (h)	Amount Dissolved			
	0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	NMT 25%	NMT 25%	NMT 20%	NMT 20%
4	45%–60%	40%–55%	30%–50%	25%–45%
8	70%–90%	65%–85%	55%–75%	50%–70%
16	NLT 85%	NLT 85%	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water; adjust with phosphoric acid or potassium hydroxide to a pH of  $6.0 \pm 0.1$ ); 500 mL, deaerated.**Apparatus 1:** 100 rpm**Times:** 1, 4, and 8 h for Tablets labeled to contain 0.5 mg or 1 mg; 1, 4, 8, and 16 h for Tablets labeled to contain 2 mg or 3 mg**Mobile phase:** Acetonitrile and *Medium* (40:60)**Standard stock solution:** 0.5 mg/mL of USP Alprazolam RS in methanol**Standard solution:** ( $L/500$ ) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where  $L$  is the label claim in mg/Tablet**Sample solution:** Pass a portion of the solution under test through a suitable filter of 1- $\mu$ m pore size.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm  $\times$  10-cm; 3- $\mu$ m or 5- $\mu$ m packing L7**Flow rate:** 1 mL/min**Injection size:** 100  $\mu$ L**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point  $i$ , ( $Q_i$ ):

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100] + [Q_1 \times (V_S/V)]$$

$$Q_8 = [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100] + [Q_1 \times (V_S/V)] + [Q_4 \times V_S/(V - V_S)]$$

$$Q_{16} = [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100] + [Q_1 \times V_S/V] + [Q_4 \times V_S/(V - V_S)] + [Q_8 \times V_S/(V - 2V_S)]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL) $L$  = label claim (mg/Tablet) $V$  = initial volume of *Medium*, 500 mL $V_S$  = volume of the sample withdrawn at each time point (mL)**Tolerances:** See *Table 3*.**Table 3**

Time (h)	Amount Dissolved			
	0.5-mg Tablet	1-mg Tablet	2-mg Tablet	3-mg Tablet
1	15%–35%	10%–30%	10%–30%	5%–25%
4	50%–75%	45%–65%	30%–55%	25%–50%
8	NLT 75%	NLT 70%	60%–80%	50%–75%
16	—	—	NLT 85%	NLT 80%

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

- UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

**IMPURITIES****ORGANIC IMPURITIES****Buffer:** 5.4 g/L of monobasic potassium phosphate ( $KH_2PO_4$ ) in water. Adjust with phosphoric acid to a pH of 3.4.**Solution A:** Acetonitrile, methanol, and *Buffer* (27:10:63)**Solution B:** Acetonitrile, methanol, and *Buffer* (7:3:10)**System suitability solution:** 1  $\mu$ g/mL each of USP Chlordiazepoxide Related Compound A RS, USP Alprazolam Related Compound A RS, and USP Nordazepam RS; and 0.4  $\mu$ g/mL of USP Alprazolam RS in methanol**Standard solution:** 0.4  $\mu$ g/mL of USP Alprazolam RS in methanol**Sample solution:** From NLT 20 Tablets ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.2 mg/mL of alprazolam in methanol. [NOTE—Sonicate for 15 min to dissolve the contents.] Filter a portion, and discard the first 1 mL of filtrate.**Mobile phase:** See *Table 4*.**Table 4**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5

Table 4 (Continued)

Time (min)	Solution A (%)	Solution B (%)
25	15	85
60	15	85
60.1	95	5
70	95	5

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Flow rate:** 1.5 mL/min**Injection size:** 10 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*[NOTE—The relative retention times are listed in *Table 5*.]**Suitability requirements****Resolution:** NLT 1.5 between nordazepam and alprazolam; NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution***Tailing factor:** NMT 2.0 for the alprazolam peak, *System suitability solution***Relative standard deviation:** NMT 5%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of the impurity from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of alprazolam in the *Sample solution* (mg/mL) $F$  = relative response factor (see *Table 5*)**Acceptance criteria** See *Table 5*.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A <sup>a,e</sup>	0.36	1.0	—
USP Alprazolam Related Compound A RS <sup>b</sup>	0.45	0.7	0.5
Nordazepam <sup>c,e</sup>	0.8	1.0	—
Alprazolam	1.0	—	—
2-Amino-5-chloro-benzophenone	1.8	0.9	0.5
Amino-derivative <sup>d</sup>	2.2	1.2	0.5

<sup>a</sup> α-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide.<sup>b</sup> 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.<sup>c</sup> 7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (reported as unspecified impurity).<sup>d</sup> 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-a]quinolin-4-amine.<sup>e</sup> If present meets the requirement for any other individual degradation product.

Table 5 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

<sup>a</sup> α-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide.<sup>b</sup> 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.<sup>c</sup> 7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (reported as unspecified impurity).<sup>d</sup> 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-a]quinolin-4-amine.<sup>e</sup> If present meets the requirement for any other individual degradation product.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.**• LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.**• USP REFERENCE STANDARDS <11>**

USP Alprazolam RS

USP Alprazolam Related Compound A RS

2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

C<sub>15</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>2</sub> 286.72

USP Nordazepam RS

**Alprazolam Orally Disintegrating Tablets****DEFINITION**Alprazolam Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>).**IDENTIFICATION****•** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****• PROCEDURE****Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.**Diluent:** Acetonitrile and water (60:40)**Mobile phase:** Acetonitrile, methanol, and *Buffer* (35:10:55)**Standard solution:** 10 μg/mL of USP Alprazolam RS in *Diluent***Sample solution:** 10 μg/mL of alprazolam in *Diluent*.

Prepare using 10 Tablets, and pass through a suitable filter. [NOTE—Sonicate with intermittent shaking to help dissolve, if necessary.]

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 221 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L7**Column temperature:** 30°**Flow rate:** 1.5 mL/min**Injection volume:** 30 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) in the portion of Tablets taken:

Result = (r<sub>U</sub>/r<sub>S</sub>) × (C<sub>S</sub>/C<sub>U</sub>) × 100

r<sub>U</sub> = peak response from the Sample solution

r<sub>S</sub> = peak response from the Standard solution

C<sub>S</sub> = concentration of USP Alprazolam RS in the Standard solution (μg/mL)

C<sub>U</sub> = nominal concentration of alprazolam in the Sample solution (μg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• DISINTEGRATION <701>

• Test 1 • (RB 1-May-2012)

Time: NMT 60 s

• Test 2: If the product complies with this test, the labeling indicates that it meets USP Disintegration Test 2.

Time: NMT 30 s • (RB 1-May-2012)

Change to read:

• DISSOLUTION <711>

• Test 1 • (RB 1-May-2012)

Medium: pH 6.0 phosphate buffer (•8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or diluted potassium hydroxide to a pH of 6.0 ± 0.1 • (RB 1-May-2012)); 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the Assay, except use an injection size of 100 μL.

Standard stock solution: 50 μg/mL of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]

Standard solution: (L/1000) μg/mL of USP Alprazolam RS in Medium from the Standard stock solution, where L is the label claim in μg/Tablet

Sample solution: Pass a portion of the solution under test through a nylon membrane filter of 0.45-μm pore size, discarding the first few mL.

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) dissolved:

Result = (r<sub>U</sub>/r<sub>S</sub>) × C<sub>S</sub> × V × (1/L) × 100

r<sub>U</sub> = peak response from the Sample solution

r<sub>S</sub> = peak response from the Standard solution

C<sub>S</sub> = concentration of USP Alprazolam RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) is dissolved.

• Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium

phosphate in water, adjusted with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 2: 50 rpm

Time: 10 min

Buffer: 1.36 g/L of monobasic potassium phosphate adjusted with dilute sodium hydroxide to a pH of 6.0

Mobile phase: Acetonitrile and Buffer (35:65)

Standard stock solution: 50 μg/mL of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]

Standard solution: (L/500) μg/mL of USP Alprazolam RS in Medium from the Standard stock solution, where L is the label claim in μg/Tablet

Sample solution: Pass a 5-mL aliquot of the solution under test through a suitable filter of 0.45-μm pore size, discarding the first 3 mL.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 7.5-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 40 μL

Run time: 3 times the retention time of alprazolam

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) dissolved:

Result = (r<sub>U</sub>/r<sub>S</sub>) × C<sub>S</sub> × V × (1/L) × 100

r<sub>U</sub> = peak response from the Sample solution

r<sub>S</sub> = peak response from the Standard solution

C<sub>S</sub> = concentration of USP Alprazolam RS in the Standard solution (mg/mL)

V = volume of Medium, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) is dissolved. • (RB 1-May-2012)

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer and Diluent: Prepare as directed in the Assay.

Solution A: Acetonitrile, methanol, and Buffer (25:20:55)

Solution B: Acetonitrile, methanol, and Buffer (40:5:55)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
15	0	100
60	0	100
65	100	0
70	100	0

Standard solution: 0.6 μg/mL of USP Alprazolam RS in Diluent

Sample solution: 200 μg/mL of alprazolam in Diluent. Prepare using 10 Tablets, and pass through a suitable filter.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L7**Column temperature:** 30°**Flow rate:** 1.2 mL/min**Injection volume:** 25 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Theoretical plates:** NLT 2000**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 6.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of alprostadil from the *Standard solution* $C_S$  = concentration of USP Alprostadil RS in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of alprostadil in the *Sample solution* (μg/mL) $F$  = relative response factor (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard any peaks less than 0.05%.**Table 2**

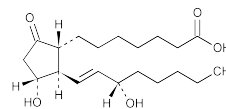
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprostadil related compound A <sup>a,b</sup>	0.8	—	—
Alprostadil	1.0	—	—
2-Amino-5-chlorobenzophenone	2.9	1.9	0.5
Any other unknown impurity	—	1.0	0.5
Total impurities	—	—	2.0

<sup>a</sup> 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.<sup>b</sup> Disregard the peak due to alprostadil related compound A, because it is a process impurity in alprostadil.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

**Add the following:**

- LABELING:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. • (RB 1-May-2012)
- USP REFERENCE STANDARDS** <11>  
USP Alprostadil RS

**Alprostadil** $C_{20}H_{34}O_5$  354.48

Prost-13-en-1-oic acid, 11,15-dihydroxy-9-oxo-, (11α,13E,15S)-

(1R,2R,3R)-3-Hydroxy-2-[(E)-(3S)-3-hydroxy-1-octenyl]-5-oxocyclopentane heptanoic acid [745-65-3].

» Alprostadil contains not less than 95.0 percent and not more than 105.0 percent of  $C_{20}H_{34}O_5$ , calculated on the anhydrous basis.**Caution**—Great care should be taken to prevent inhaling particles of Alprostadil and exposing the skin to it.**Packaging and storage**—Preserve in tight containers, and store in a refrigerator.**USP Reference standards** <11>—

USP Alprostadil RS

USP Prostaglandin A<sub>1</sub> RSUSP Prostaglandin B<sub>1</sub> RS $C_{20}H_{32}O_4$  336.47**Identification, Infrared Absorption** <197M>.**Water, Method I** <921>: not more than 0.5%, using 0.5 g.**Residue on ignition** <281>: not more than 0.5%, using 0.3 g.**Limit of chromium—****Standard solution**—Dissolve an accurately weighed quantity of chromium trichloride in 0.05 M nitric acid, and dilute, stepwise if necessary, with 0.05 M nitric acid to obtain a solution having a known concentration of about 3.04 μg per mL. Transfer 2 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. This solution contains 20 ng of chromium per mL.**Test solution**—Transfer about 10 mg of Alprostadil, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix.**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>), equipped with a chromium hollow-cathode lamp, with a drying temperature of 100°, an ashing temperature of 1000°, and an atomization temperature of 2700°. Use alcohol as the blank. Concomitantly determine the absorbances at the chromium emission line at 357.9 nm, and calculate the percentage of chromium (Cr) in the portion of Alprostadil taken by the formula:

$$100(C_S/C_A)(A_U/A_S)$$

in which  $C_S$  is the concentration, in mg per mL, of chromium in the *Standard solution*;  $C_A$  is the concentration, in mg per mL, of Alprostadil in the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and *Standard solution*, respectively: not more than 0.002% is found.**Limit of rhodium—****Standard solution**—Dissolve an accurately weighed quantity of rhodium chloride hydrate in 1.2 M hydrochloric acid, and dilute, stepwise if necessary, with 1.2 M hydrochloric acid to obtain a solution having a known concentration of 100 μg of rhodium per mL. Transfer 5 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume,

and mix. Transfer 2 mL of this solution to a 200-mL volumetric flask, dilute with alcohol to volume, and mix. This solution contains 50 ng of rhodium per mL.

**Test solution**—Transfer about 20 mg of Alprostadil, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into a suitable graphite furnace atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)), equipped with a rhodium hollow-cathode lamp, with a drying temperature of 100°, an ashing temperature of 1000°, and an atomization temperature of 2800°. Use alcohol as the blank. Simultaneously determine the absorbances at the rhodium emission line at 343.5 nm, and calculate the percentage of rhodium (Rh) in the portion of Alprostadil taken by the formula:

$$100(C_S / C_A)(A_U / A_S)$$

in which  $C_S$  is the concentration, in mg per mL, of rhodium in the *Standard solution*;  $C_A$  is the concentration, in mg per mL, of Alprostadil in the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.002% is found.

#### Limit of foreign prostaglandins—

##### TEST 1—

**NOTE**—Use freshly prepared solutions. Measure the peak responses at the following wavelengths: prostaglandin  $A_1$  at 224 nm; prostaglandin  $B_1$  at 280 nm; and all other foreign prostaglandin impurities at 200 nm.

**Mobile phase**—Proceed as directed in the Assay.

**Standard solution**—Dissolve accurately weighed quantities of USP Alprostadil RS, USP Prostaglandin  $A_1$  RS, and USP Prostaglandin  $B_1$  RS in a mixture of methanol and water (9:1), and dilute quantitatively, and stepwise if necessary, with a mixture of methanol and water (9:1) to obtain a solution having known concentrations of about 6  $\mu$ g per mL, 15  $\mu$ g per mL, and 6  $\mu$ g per mL, respectively.

**Test solution**—Dissolve about 15 mg of Alprostadil, accurately weighed, in 5 mL of a mixture of methanol and water (9:1), and mix.

**Chromatographic system**—Proceed as directed in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution between prostaglandin  $A_1$  and alprostadil is not less than 7.5, and the relative standard deviation from the peaks at their respective wavelength for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses at 200 nm, 224 nm, and 280 nm. Calculate the percentage of prostaglandin  $A_1$  and prostaglandin  $B_1$  in the portion of Alprostadil taken by the formula:

$$500(C_S / W)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Prostaglandin  $A_1$  RS or USP Prostaglandin  $B_1$  RS in the *Standard solution*;  $W$  is the weight, in mg, of Alprostadil taken for the *Test solution*;  $r_i$  is the peak response for prostaglandin  $A_1$  or prostaglandin  $B_1$  obtained from the *Test solution*; and  $r_s$  is the peak response of prostaglandin  $A_1$  or prostaglandin  $B_1$  obtained from the *Standard solution*: not more than 1.5% of prostaglandin  $A_1$  is found; and not more than 0.1% of prostaglandin  $B_1$  is found. Calculate the percentage of each impurity occurring at 200 nm and eluting before pros-

taglandin  $A_1$  in the portion of Alprostadil taken by the formula:

$$500(C_S / W)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Alprostadil RS in the *Standard solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*;  $r_s$  is the peak response for alprostadil obtained from the *Standard solution*; and the other terms are as defined herein: not more than 0.9% of any foreign prostaglandin impurity is found. Calculate the percentage of any impurity having a relative retention time of 0.6, relative to the prostaglandin  $A_1$  peak detected at 224 nm, in the portion of Alprostadil taken by the formula:

$$500(C_S / W)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Prostaglandin  $A_1$  RS in the *Standard solution*;  $r_i$  is the peak response for any impurity having a relative retention time of 0.6, relative to the prostaglandin  $A_1$  peak, obtained from the *Test solution*;  $r_s$  is the peak response of prostaglandin  $A_1$  obtained from the *Standard solution*; and the other terms are as defined herein: not more than 0.9% of any impurity having a relative retention time of 0.6, relative to the prostaglandin  $A_1$  peak, is found.

##### TEST 2—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, acetonitrile, and 0.02 M monobasic potassium phosphate (2:1:1), and adjust with phosphoric acid to a pH of 3. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Alprostadil RS in a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

**Test solution**—Dissolve about 25 mg of Alprostadil, accurately weighed, in 5 mL of a mixture of acetonitrile and water (1:1), using ultrasound if necessary.

**Identification solution**—Use the *Standard solution* under Test 1.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a photodiode array detector or equivalent capable of detecting UV wavelengths between 200 nm and 300 nm and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Identification solution* as directed for *Procedure*: the relative retention times for prostaglandin  $A_1$  and alprostadil are about 1.2 and 1.0, respectively; the resolution between prostaglandin  $A_1$  and alprostadil is not less than 4.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation determined from the main peak for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses at 200 nm, 224 nm, and 280 nm. Calculate the percentage of each impurity occurring at 200 nm and eluting after prostaglandin  $A_1$ , excluding prostaglandin  $B_1$ , in the portion of Alprostadil taken by the formula:

$$500(C_S / W)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Alprostadil RS in the *Standard solution*;  $W$  is the weight, in mg, of Alprostadil taken for the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for alprostadil obtained from the *Standard solution*: the sum of the peaks having relative retention times of 2.0 and 2.3 is not more than 0.6%; not more than 0.9% of any other foreign prostaglandin impurity

is found; and not more than 2.0% of total impurities is found, the results for *Test 1* and *Test 2* being added.

#### Assay—

NOTE—Use freshly prepared solutions.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, 0.1 M monobasic potassium phosphate and acetonitrile (2:2:1), and adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Dissolve an accurately weighed quantity of ethylparaben in a mixture of methanol and water (9:1), and dilute quantitatively, and stepwise if necessary, with a mixture of methanol and water (9:1) to obtain a solution having a concentration of about 0.05 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Alprostadil RS in a mixture of methanol and water (9:1), and dilute quantitatively, and stepwise if necessary, with a mixture of methanol and water (9:1) to obtain a solution having a known concentration of about 0.3 mg per mL. To 2.0 mL of this solution, add 1.0 mL of *Internal standard solution*, and mix.

**System suitability solution**—Dilute an accurately measured amount of USP Prostaglandin A<sub>1</sub> RS with *Standard preparation* to obtain a solution having a concentration of 4.5 µg of prostaglandin A<sub>1</sub> per mL. To 2.0 mL of this solution, add 1.0 mL of *Internal standard solution*, and mix.

**Assay preparation**—Transfer about 7.5 mg of Alprostadil, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with a mixture of methanol and water (9:1) to volume, and mix. To 2.0 mL of this solution, add 1.0 mL of *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a photodiode array detector or equivalent capable of detecting UV wavelengths between 200 nm and 300 nm and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between prostaglandin A<sub>1</sub> and alprostadil is not less than 7.5, and between prostaglandin A<sub>1</sub> and ethylparaben is not less than 2.0; and the relative standard deviation determined from the peak area ratio of alprostadil to ethylparaben for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms at 200 nm, and measure the areas for the major peaks. Calculate the quantity, in mg, of C<sub>20</sub>H<sub>34</sub>O<sub>5</sub> in the portion of Alprostadil taken by the formula:

$$25C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Alprostadil RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak area ratios of alprostadil to ethylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Alprostadil Injection

» Alprostadil Injection is a sterile solution of Alprostadil in Dehydrated Alcohol. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>).

**Packaging and storage**—Preserve in tight, single-dose containers, preferably of Type I glass. Store in a refrigerator.

#### USP Reference standards <11>—

USP Alprostadil RS

USP Endotoxin RS

#### Identification, Infrared Absorption <197K>—

**Test specimen**—Dry an amount of Injection, equivalent to about 2 mg of alprostadil, on about 500 mg of spectroscopic grade potassium bromide at about 40° to 50° under vacuum. Prepare a pellet from this mixture.

**Standard specimen**: a similar preparation of USP Alprostadil RS, in dehydrated alcohol.

**Bacterial endotoxins** <85>—It contains not more than 5 USP Endotoxin units per 100 µg of alprostadil.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Water, Method I** <921>: not more than 0.4%.

**Other requirements**—It meets the requirements under *Injections* <1>.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methylene chloride, 1,3-butanediol, and water (1000:6:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Dissolve ethylparaben in methylene chloride to obtain a solution containing about 0.05 mg per mL.

**Standard stock preparation**—Dissolve an accurately weighed quantity of USP Alprostadil RS in dehydrated alcohol, and quantitatively dilute with dehydrated alcohol to obtain a solution having a known concentration of about 0.5 mg per mL.

**Standard preparation**—Gently evaporate a 0.5-mL portion of the *Standard stock preparation* to dryness with a stream of nitrogen. Proceed as directed for the *Assay preparation* beginning with "Add 150 µL of a 1 in 25 freshly prepared solution."

**Assay preparation**—Pool the contents of several containers of the Injection, and gently evaporate an accurately measured volume, equivalent to about 0.25 mg of alprostadil, to dryness using a stream of nitrogen. Add 150 µL of a 1 in 25 freshly prepared solution of α-bromo-2'-acetonephthone in acetonitrile, rinse the inside of the container with this solution, and swirl. Add 150 µL of a freshly prepared 1 in 200 solution of diisopropylethylamine in acetonitrile to the container, rinse the inside of the container with this solution, and swirl. Cap and sonicate to dissolve. Heat the container at 45° for 45 minutes, swirling occasionally. Sonicate again after heating is complete. [NOTE—If the entire sample does not dissolve, the specimen should be discarded.] Evaporate the solution using a stream of nitrogen, add 2.0 mL of *Internal standard solution*, and mix. Sonicate to dissolve. [NOTE—If incomplete dissolution is still observed, discard the specimen.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.4-mm × 25-cm column that contains packing L18. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 and 1.0, for ethylparaben and alprostadil, respectively; the resolution, *R*, between alprostadil and the internal standard is not less than 9.0; and the relative standard deviation for replicate injections is not more than 2.5%.

**Procedure**—Separately inject equal volumes of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg,

of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>) in the volume of Injection taken by the formula:

$$C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Alprostadil RS in the *Standard stock preparation*; and R<sub>U</sub> and R<sub>S</sub> are the peak response ratios of alprostadil to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Alteplase

SYQVICRDEK	TQMIYQQHQSS	WLRPVLRSMR	VEYCWNSGR
AQCHSVPVKS	CSEPRCFNGG	TCQALYFSD	FVQCQPEGFA
GKCCSIDTRA	TCYEDQGTSY	RGTWSTAESG	AECTNWNSSA
LAQKPYSGRR	PDAIRLGLGN	HNYCRNPDRD	SKPWICYVKA
GKYSSEFCST	PACSEGNSDQ	YFGNSAYRG	THSLTESGAS
CLPWNSMILI	GKYYTAQNPS	AQALGLGKHN	YCRNPDGDAK
PWCHVLKNRR	LTWEYCDVPS	CSTGLRQYS	QPQFR
IKGGLFADIA	SHPWQAAIFA	KHRRSPGERF	LCGGILISSC
WILSAAHCFQ	ERFPPHLLTV	ILGRTRYRVP	GEEEQKFEVE
KYIVHKEFDD	DTYNDIALLL	QLKSDSSRCA	QESSVVRTVC
LPPADLQLPD	WTECELSGGY	KHEALSPFYS	ERLKEAHVRL
YPSRRCSTSQH	LLNRTVTDNM	LCAGDTRSGG	PQANLHDACQ
GDSGGPLYCL	NDGRMTLVGI	ISWGLGCGQK	DVPGVYTKVT
NYLDWIRDNM	RP		

C<sub>2569</sub>H<sub>3894</sub>N<sub>746</sub>O<sub>781</sub>S<sub>40</sub> 59,007.61 [105857-23-6].

» Alteplase is a highly purified glycosylated serine protease with fibrin-binding properties and plasminogen-specific proteolytic activities. It is produced by recombinant DNA synthesis in mammalian cell culture. It has a biological potency of not less than 90.0 percent and not more than 115.0 percent of the potency stated on the label, the potency being 580,000 USP Alteplase Units per mg of protein.

The presence of host cell DNA and host cell protein impurities in Alteplase is process-specific; the limits of these impurities are determined by validated methods.

**Packaging and storage**—Preserve in tight containers, and store in the frozen state at a temperature of –20° or below.

**USP Reference standards** (11)—  
USP Alteplase RS  
USP Endotoxin RS

**Identification**—To each of three test tubes transfer 1 mL of a solution of H-D-isoleucyl-prolyl-arginyl-*p*-nitroaniline dihydrochloride containing 0.5 mg per mL. Prepare a test solution of Alteplase in water containing 1.0 to 2.5 mg per mL. Separately transfer 200 µL of this solution and 200 µL of a Standard solution of USP Alteplase RS, similarly prepared, to two of the test tubes, and to the third test tube, add 200 µL of 0.2 M arginine solution that has been adjusted with phosphoric acid (negative control) to a pH of 7.3. Mix the solutions in the test tubes, and allow to stand for 1 minute: a yellow color is produced in the solutions from the test specimen and the USP Reference Standard, while no yellow color is produced in the negative control.

**Peptide mapping**—

*Solution A*—Dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid

to a pH of 2.85. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Solution B*—Use acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Dialysis solution*—Prepare an aqueous solution containing, in each mL, 480 mg of urea, 44 mg of tris(hydroxymethyl)aminomethane, and 0.88 mg of edetic acid. Adjust with hydrochloric acid to a pH of 8.6.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Alteplase RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 1.0 mg per mL. Dialyze about 2.0 mL of this solution into the *Dialysis solution* at room temperature for not less than 12 hours. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10 µL of 1 M dithiothreitol. Incubate at room temperature for 4 hours, then add 25 µL of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 minutes. Quench the reaction by the addition of 50 µL of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 hours, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20 µg of trypsin, and incubate for 6 to 8 hours at room temperature. Again add 20 µg of trypsin, and incubate for 16 to 18 hours for a total of 24 hours of incubation of the trypsin-treated solution. [NOTE—Store this *Standard preparation* in a freezer.]

*Test preparation*—Using an accurately weighed quantity of Alteplase, proceed as directed for *Standard preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1 mL per minute. The system is programmed to provide a *Mobile phase* consisting of variable mixtures of *Solution A* and *Solution B*. The system is equilibrated with 100% *Solution A*. After injection of the solution under test, the proportion of *Solution B* is increased linearly from 0% to 30% at a rate of 0.33% per minute. The proportion of *Solution B* is then increased linearly at a rate of 1.0% per minute until the proportion of *Solution B* is 60%, and is held at that composition for 10 minutes. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R<sub>s</sub> between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet is not less than 1.5, and the times of their baseline widths are not more than 0.5 minutes.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard preparation*, the *Test preparation*, and a mixture of the *Standard preparation* and the *Test preparation* (1:1) into the chromatograph, record the chromatograms, and measure the responses for not less than 20 major peaks as defined in the USP Alteplase RS Data Sheet: the retention times of corresponding peaks from the *Standard preparation* and the *Test preparation* do not differ by more than 0.4 minutes, and the peak area ratios relative to peak 19 (as shown on the USP Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak area response of not less than 5% of peak 19.

**Bacterial endotoxins** (85)—It contains not more than 1 USP Endotoxin Unit per mg of alteplase.

**Chromatographic purity** (see *Electrophoresis* (726))—  
*SDS buffer*—Prepare a solution in sodium dodecyl sulfate solution (8 in 100) containing, in each mL, 400 mg of glycerol, 5.52 mg of tris(hydroxymethyl)aminomethane hydrochloride, 3.28 mg of tris(hydroxymethyl)aminomethane,



0.20 mg of bromophenol blue, and 0.20 mg of xylene cyanole FF.

**Diluted SDS buffer**—Dilute 1 volume of *SDS buffer* with 4 volumes of water.

**Ammoniacal silver nitrate solution**—Transfer 105 mL of sodium hydroxide solution (0.36 in 100) and 7.0 mL of ammonium hydroxide to a 500-mL volumetric flask, and add slowly, with stirring, 20.0 mL of silver nitrate solution (20 in 100). Dilute with water to volume, and mix. [NOTE—Prepare this solution immediately before use and protect it from light. This amount of solution is sufficient for two slab gels.]

**Citric acid-formaldehyde solution**—To 500 mL of water add 25 mg of citric acid, 0.25 mL of formaldehyde, and 0.025 mL of methanol, omitting the methanol if the formaldehyde is preserved with methanol. [NOTE—Prepare this solution fresh at the time of use. This amount of solution is sufficient for two slab gels.]

**Running buffer**—Prepare a buffer solution in sodium dodecyl sulfate (1 in 1000) containing 3.03 mg of tris(hydroxymethyl)aminomethane and 14.26 mg of glycine per mL.

**Carboxymethylation buffer**—Prepare an aqueous solution containing, in each mL, 480 mg of urea, 44 mg of tris(hydroxymethyl)aminomethane, and 1.2 mg of edetic acid. Adjust with hydrochloric acid if necessary to a pH of 8.6.

**Gel**—Prepare a 10% T (total acrylamide)-0.25% C (cross-linked bisacrylamide) resolving gel containing 0.1% sodium dodecyl sulfate, 0.375 M tris(hydroxymethyl)aminomethane hydrochloride, and 0.05 M tris(hydroxymethyl)aminomethane.

**0.2 M Arginine solution**—Prepare a solution of arginine in water containing 34.8 mg per mL. Adjust with phosphoric acid to a pH of 7.3.

**Stock standard solution**—Dissolve an accurately weighed quantity of USP Alteplase RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 1 mg per mL.

**Standard solution**—Dilute an accurately measured volume of *Stock standard solution* with 0.02 M Arginine solution to obtain a solution having a concentration of 0.25 mg per mL. Heat 0.5 mL of this solution with 116  $\mu$ L of *SDS buffer* and 10  $\mu$ L of 1 M dithiothreitol at 80° for 2 minutes.

**Carboxymethylated standard solution**—Dilute 1.0 mL of *Stock standard solution* with 1 mL of *Carboxymethylation buffer*, and adjust with 1 M sodium hydroxide to a pH of 8.5. Add 20  $\mu$ L of 1 M dithiothreitol, and incubate at 37° for 60 minutes. Add 100  $\mu$ L of 1 M iodoacetic acid, and incubate in the dark for 20 minutes. Desalt by passing the solution through a chromatographic column containing fine gel chromatographic packing equilibrated with a buffer solution containing, in each mL, 20 mg of sodium dodecyl sulfate, 100 mg of glycerol, 1.42 mg of tris(hydroxymethyl)aminomethane hydrochloride, and 0.85 mg of tris(hydroxymethyl)aminomethane. Collect the protein fraction of the preparation by elution with the same buffer, and add 20  $\mu$ L of 1 M dithiothreitol. Adjust the protein concentration to about 0.2 mg per mL with a buffer solution containing, in each mL, 20 mg of sodium dodecyl sulfate, 100 mg of glycerol, 1.42 mg of tris(hydroxymethyl)aminomethane hydrochloride, 0.85 mg of tris(hydroxymethyl)aminomethane, 1.06 mg of dithiothreitol, 0.05 mg of bromophenol blue, and 0.05 mg of xylene cyanole FF.

**Stock test solution, Test solution, and Carboxymethylated test solution**—Using an accurately weighed quantity of Alteplase, proceed as directed for *Stock standard solution*, *Standard solution*, and *Carboxymethylated standard solution*, respectively.

**Molecular weight standard preparation**—Use a commercially available preparation of low molecular weight protein standards (10,000 to 100,000 Da) at about 2 mg per mL. Mix 990  $\mu$ L of *Diluted SDS buffer* and 10  $\mu$ L of the molecular weight standard mixture.

**Control solutions**—Prepare a control solution of bovine serum albumin containing 10  $\mu$ g per mL. For a 10 ng per 25  $\mu$ L load, mix 600  $\mu$ L of *Diluted SDS buffer* and 25  $\mu$ L of the control solution, and heat at 90° for 2 minutes. For a 2.5 ng per 25  $\mu$ L load, mix 594  $\mu$ L of *Diluted SDS buffer* and 6  $\mu$ L of the control solution, and heat at 90° for 2 minutes.

**Blank**—Mix 500  $\mu$ L of water, 126  $\mu$ L of *SDS buffer*, and 10  $\mu$ L of 1 M dithiothreitol.

**Procedure**—Separately apply equal volumes (about 25  $\mu$ L) of the *Test solution*, *Standard solution*, *Carboxymethylated test solution*, and *Carboxymethylated standard solution* at the 5  $\mu$ g load; apply equal volumes (about 38  $\mu$ L) of the *Standard solution* and the *Carboxymethylated standard solution* at the 7.5  $\mu$ g load; and apply the *Control solutions* at the 10 ng and 2.5 ng load onto separate lanes of the gel. Apply about 25  $\mu$ L of the *Molecular weight standard preparation* to each side of the gel, and apply about 25  $\mu$ L of the *Blank* onto a separate lane. Apply the *Test solution* and the *Standard solution* on one half and the *Carboxymethylated test solution* and *Carboxymethylated standard solution* on the other half. Perform the electrophoresis using a constant current of 1.3 to 1.5 mA per cm of gel length and the *Running buffer*. Remove the gel from the apparatus 10 to 20 minutes after the tracking dye starts to move. Place the gel in 250 mL of a solution of 20% alcohol and 6% glacial acetic acid for not less than 1 hour, and change the solution every 20 minutes, leaving the gel to soak overnight following the last change. Perform silver staining of the gel by placing the gel in 250 mL of a solution (1 in 10) in a shallow dish, and shake for about 30 minutes. Replace the glutaraldehyde solution with distilled water, allow gel to soak for about 20 minutes, and then change the water. Repeat for a total of three washings. Transfer the gel to a dish, and cover with 250 mL of *Ammoniacal silver nitrate solution*. Place the dish on a shaker for about 15 minutes. Rinse 4 times with 250 mL of water, rocking the dish for 1 minute between rinses. Continue rocking to prevent the gel from sticking and to facilitate washing. Transfer the gel to a clear dish containing 250 mL of *Citric acid-formaldehyde solution*, and rock the dish. Protein bands become visible. When the gel is visibly stained, wash immediately with water, and rinse it repeatedly with water to remove the *Citric acid-formaldehyde solution*. Rinse the gel for not less than 1 hour, and dry. Soak cellophane membranes in glycerol solution (2 in 100). Roll a membrane onto a rigid sheet of plastic. Roll the gel onto the membrane, and cover with another membrane. Lay a frame on the edges of the membranes, and clamp it to the rigid plastic sheet. Dismantle the dryer, and cut off excess cellophane when dry (about 24 hours). Visually examine the gel under light. The *Test solution* exhibits 3 major bands in the region between 66,000 Da and 31,000 Da, corresponding to the major bands from the *Standard solution*. The *Carboxymethylated test solution* exhibits 6 major bands in the region between 92,500 Da and 45,000 Da, corresponding to the major bands from the *Carboxymethylated standard solution*.

**System suitability**—The 2.5 ng and 10 ng controls must be visible. The nonreduced controls solutions migrate with an apparent molecular weight of slightly less than 66,000 Da, as compared with the molecular weight standards.

#### Single-chain content—

**Mobile phase**—Dissolve 27.6 g of monobasic sodium phosphate in 1000 mL of sodium dodecyl sulfate solution (1 in 1000), and adjust with sodium hydroxide to a pH of 6.8. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**0.02 M Dithiothreitol solution**—Prepare a solution of dithiothreitol in *Mobile phase* containing 3.12 mg per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Alteplase RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1 mL of this solution into a glass tube, add 3 mL of 0.02 M

*Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3 to 5 minutes at about 80°.

*Test solution*—Using an accurately weighed quantity of Alteplase, proceed as directed for *Standard solution*.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 7.5-mm × 60-cm column that contains packing L25. The flow rate is about 0.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the single-chain and two-chain alteplase peaks is not less than 1.1.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.] No peaks or shoulders in the chromatogram of the *Test solution* that are not present in the chromatogram of the *Standard solution* are found. Calculate the percentage of single-chain alteplase in the portion of Alteplase taken by the formula:

$$100(r_a / r_s)$$

in which  $r_a$  is the peak response for single-chain alteplase, and  $r_s$  is the sum of the responses of all of the alteplase peaks: not less than 60% is found.

**Protein content** (see *Spectrophotometry and Light-Scattering* <851>)—

*0.2 M Arginine solution*—Prepare a solution of arginine in water containing 34.8 mg per mL. Adjust with phosphoric acid to a pH of 7.3.

*Test solution*—Dissolve an accurately weighed quantity of Alteplase in water to obtain a solution containing about 1 mg per mL. Dilute an accurately measured volume of this solution with a volume of *0.2 M Arginine solution* to obtain a solution having an absorbance value of 0.5 to 1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume,  $V$ .

*Procedure*—Obtain an absorption spectrum of the *Test solution* in a 1-cm cell from 240 nm to 500 nm, and determine the absorbance at 320 nm and at the wavelength of maximum absorbance at about 280 nm, using *0.2 M Arginine solution* as the blank. Calculate the protein content in the portion of Alteplase taken by the formula:

$$V(A_{\max} - A_{320})/1.9$$

in which  $V$  is the volume of *0.2 M Arginine solution* required to prepare the *Test solution*,  $A_{\max}$  is the absorbance value at the wavelength of maximum absorbance, and  $A_{320}$  is the absorbance of the *Test solution* at 320 nm.

#### Assay for biological potency—

*Buffer*—Prepare an aqueous solution containing, in each mL, 1.38 mg of monobasic sodium phosphate, 7.10 mg of anhydrous dibasic sodium phosphate, 0.20 mg of sodium azide, and 0.10 mg of polysorbate 80.

*Human thrombin solution*—Prepare a solution of human thrombin in *Buffer* containing 33 U.S. Units in terms of the U.S. Standard Thrombin per mL.

*Human fibrinogen solution*—Prepare a solution of human fibrinogen in *Buffer* containing 2 mg per mL.

*Human plasminogen solution*—Prepare a solution of human plasminogen in *Buffer* containing 1 mg per mL.

*Standard preparations*—Dissolve an accurately weighed quantity of USP Alteplase RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 1.0 mg (580,000 USP Alteplase Units) per mL. Dilute accurately measured volumes of this solution quantitatively and stepwise with water to obtain a series of five *Standard preparations* having known concentrations ranging from 145 to 9.3 USP Alteplase Units per mL.

*Assay preparations*—Dissolve an accurately weighed quantity of Alteplase in water, and dilute with water to obtain a solution having a concentration of about 1 mg per mL. Dilute an accurately measured volume of this solution quantitatively and stepwise with *Buffer* to obtain a series of dilutions of about 1:20,000, 1:10,000, and 1:5,000.

*Procedure*—To a set of labeled glass test tubes, add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard* and *Assay preparation*, mix, and store on ice. To a second set of labeled glass tubes, add 20  $\mu$ L of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, mix, and store on ice. Beginning with the *Standard/thrombin mixture* containing the lowest number of USP Units per mL, record the time, and separately add 200  $\mu$ L of each of the thrombin mixtures to the test tubes containing the plasminogen-fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 seconds, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 seconds, followed by the formation of bubbles within the clot. Record the clot lysis time,  $t_{cl}$ , from the first addition of the Alteplase solution to the last bubble to rise to the surface. Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units per mL, versus the log values of their clot lysis times in seconds taken by the formula:

$$\log t = m(\log U_s) + b$$

in which  $t$  is the time, in seconds, to bubble release;  $U_s$  is the activity, in USP Alteplase Units per mL, of the *Standard preparation*;  $m$  is the slope of the line; and  $b$  is the y-intercept of the line. The correlation coefficient is not less than -0.9900. From the line equation and using the log of the clot lysis time for the *Assay preparation*, calculate the log of the activity,  $U_a$ , by the equation:

$$\log U_a = [(\log t) - b]/m.$$

Calculate the alteplase activity, in USP Alteplase Units per mL, taken by the formula:

$$D(10^{\log U})$$

in which  $D$  is the dilution factor for the *Assay preparation*. Calculate the specific activity in the portion of Alteplase taken by the formula:

$$U_a / P$$

in which  $P$  is the concentration of protein obtained in the test for *Protein content*.

## Alteplase for Injection

» Alteplase for Injection is a sterile lyophilized preparation of Alteplase. Its biological activity is not less than 90 percent and not more than 115 percent of that stated on the label in USP Alteplase Units. It contains not less than 95 percent and not more than 111 percent of the total protein content stated on the label.

**Packaging and storage**—Preserve in hermetic, light-resistant containers, and store in a refrigerator.

**Labeling**—Label it to state the biological activity in USP Alteplase Units per vial and the amount of protein per vial.

**USP Reference standards** <11>—

USP Alteplase RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

**Identification**—It responds to the tests for *Identification* and *Peptide mapping* under *Alteplase*.

**Bacterial endotoxins** ⟨85⟩—It contains less than 1 USP Endotoxin Unit per mg.

**Safety**—It meets the requirements for biologics as set forth for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* ⟨88⟩.

**Sterility** ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** ⟨905⟩: meets the requirements for *Content Uniformity*.

**pH** ⟨791⟩: between 7.1 and 7.5, in the solution constituted as directed in the labeling.

**Water, Method I** ⟨921⟩: not more than 4.0%.

**Single-chain content**—When constituted with water, it meets the requirements for *Single-chain content* under *Alteplase*.

**Percent monomer**—

**Mobile phase**—Dissolve 34.84 g of arginine, 158.56 g of ammonium sulfate, and 100 mL of isopropyl alcohol in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 7.3, degas, and pass through a 0.45-μm porosity filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

**Standard solution**—Dissolve an accurately weighed quantity of USP Alteplase RS in water to obtain a solution having a known concentration of about 1 mg per mL.

**Test solution**—Dissolve an accurately weighed quantity of Alteplase for Injection in water to obtain a solution having a concentration of about 1 mg per mL.

**Resolution solution**—Prepare a solution containing 1 mg per mL of each of chicken ovalbumin and bovine gamma globulin.

**Chromatographic system** (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 7.5-mm × 30-cm column that contains packing L25. The flow rate is between 0.5 and 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between gamma globulin and ovalbumin is not less than 1.6. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the alteplase peak is not less than 1200 theoretical plates.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percent monomer in the portion of Alteplase for Injection taken by the formula:

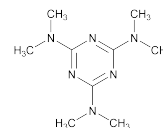
$$100(r_m / r_s)$$

in which  $r_m$  is the peak response for the alteplase monomer, and  $r_s$  is the sum of the responses of all of the alteplase related peaks: not less than 95.0% is found.

**Protein content**—Proceed as directed for *Protein content* under *Alteplase*.

**Assay for biological potency**—When constituted with water, Alteplase for Injection meets the requirements for *Assay for biological potency* under *Alteplase*.

## Altretamine



$C_9H_{18}N_6$  210.28  
1,3,5-Triazine-2,4,6-triamine, *N,N,N',N',N'',N''*-hexamethyl-; Hexamethylmelamine [645-05-6].

### DEFINITION

Altretamine contains NLT 98.0% and NMT 102.0% of altretamine ( $C_9H_{18}N_6$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of  $8.0 \pm 0.05$ .

**Diluent:** Methanol and water (65:35)

**Mobile phase:** Methanol and *Buffer* (65:35)

**Standard stock solution:** 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.

**Standard solution:** 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*

**Sample stock solution:** Transfer 25 mg of Altretamine to a 50-mL volumetric flask. Dissolve in 35 mL of methanol, and dilute with water to volume.

**Sample solution:** 0.05 mg/mL of Altretamine in *Diluent*, from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

**Mode:** LC

**Detector:** UV 227 nm

**Column:** 4.6-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of altretamine ( $C_9H_{18}N_6$ ) in the portion of Altretamine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, Method II (231): NMT 40 ppm

**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method I (921): NMT 1%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Altretamine RS

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**Altretamine Capsules**


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**DEFINITION**

Altretamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of altretamine ( $C_9H_{18}N_6$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)  
**Sample:** Remove as completely as possible the contents of 5 Capsules, and dissolve, with shaking, in 10 mL of chloroform. Filter, and evaporate the chloroform solution to dryness.  
**Acceptance criteria:** Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- **PROCEDURE**  
**Buffer:** 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of  $8.0 \pm 0.05$ .  
**Diluent:** Methanol and water (65:35)  
**Mobile phase:** Methanol and *Buffer* (65:35)  
**Standard stock solution:** 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.  
**Standard solution:** 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*  
**Sample stock solution:** Remove as completely as possible the contents of NLT 20 Capsules, and weigh. Mix the combined contents, and transfer as completely as possible to a 500-mL volumetric flask. Add 325 mL of methanol, and sonicate. Dilute with water to volume.  
**Sample solution:** Transfer a volume of the *Sample stock solution*, equivalent to 10 mg of altretamine, to a 200-mL volumetric flask, and dilute with *Diluent* to volume.  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 227 nm  
**Column:** 4.6-mm  $\times$  30-cm; packing L1  
**Flow rate:** 2 mL/min  
**Injection volume:** 10  $\mu$ L  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Tailing factor:** NMT 1.5  
**Relative standard deviation:** NMT 2.0%  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of altretamine ( $C_9H_{18}N_6$ ) in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION** (711)  
**Medium:** 0.1 N hydrochloric acid; 900 mL  
**Apparatus 1:** 100 rpm  
**Time:** 30 min  
**Detector:** UV 242 nm  
**Standard solution:** USP Altretamine RS in *Medium*  
**Analysis:** Determine the amount of  $C_9H_{18}N_6$  dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted if necessary with *Medium*, compared with the *Standard solution*.  
**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_9H_{18}N_6$  is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Altretamine RS

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**Ammonium Alum**


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$AlNH_4(SO_4)_2 \cdot 12H_2O$	453.33
$AlNH_4(SO_4)_2$	237.15
Sulfuric acid, aluminum ammonium salt (2:1:1), dodecahydrate;	
Aluminum ammonium sulfate (1:1:2), dodecahydrate [7784-26-1].	
Anhydrous [7784-25-0].	

**DEFINITION**

Ammonium Alum contains NLT 99.0% and NMT 100.5% of ammonium alum [ $AlNH_4(SO_4)_2$ ], calculated on the dried basis.

**IDENTIFICATION****Change to read:**

- **A.**  
**Sample solution:** 50 mg/mL  
**Analysis:** Add 1 N sodium hydroxide dropwise to the *Sample solution*.  
**Acceptance criteria:** A precipitate is formed, and it dissolves in an excess of the reagent with the evolution of ammonia, recognizable by  $\blacktriangle_{USP36}$  its alkaline effect upon moistened red litmus paper exposed to the vapor.
- **B. IDENTIFICATION TESTS—GENERAL, Aluminum** (191)  
**Sample solution:** 50 mg/mL  
**Acceptance criteria:** Meets the requirements

**Change to read:**

- **C. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)  
**Sample solution:** 50 mg/mL  
**Analysis:** Proceed as directed in *Identification Tests—General, Sulfate* (191), except centrifuge the neutral solutions of sulfates and use the supernatants for the lead acetate test.  $\blacktriangle_{USP36}$

**Acceptance criteria:** Meets the requirements

## ASSAY

### Change to read:

#### • PROCEDURE

▲**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.▲<sup>USP36</sup>

**Sample:** 800 mg of Ammonium Alum

**Analysis:** Transfer the *Sample* to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until the solution is complete, and boil gently for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate ▲the excess edetate disodium▲<sup>USP36</sup> with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 11.86 mg of  $\text{AlNH}_4(\text{SO}_4)_2$ .

**Acceptance criteria:** 99.0%–100.5% on the dried basis

## IMPURITIES

#### • HEAVY METALS, Method I (231)

**Test preparation:** Dissolve 1 g in 20 mL of water, and add 5 mL of 0.1 N hydrochloric acid. Evaporate the solution in a porcelain evaporating dish to dryness. Treat the residue with 20 mL of water, and add 50 mg of hydroxylamine hydrochloride. Heat the solution on a steam bath for 10 min, cool, and dilute with water to 25 mL.

**Analysis:** Proceed as directed in the chapter, except add 50 mg of hydroxylamine hydrochloride to the *Standard Preparation*.

**Acceptance criteria:** 20 ppm

#### • IRON

**Sample solution:** 6.7 mg/mL

**Analysis:** Add 5 drops of potassium ferrocyanide TS to 20 mL of the *Sample solution*.

**Acceptance criteria:** No blue color is produced immediately.

## SPECIFIC TESTS

#### • LOSS ON DRYING (731)

**Sample:** 2.0 g

**Analysis:** Transfer the *Sample*, in a tared porcelain crucible, to a muffle furnace at 200°. Increase the temperature to 300°, and dry at 300° to a constant weight. Cool in a desiccator, and weigh.

**Acceptance criteria:** 45.0%–48.0%

#### • LIMIT OF ALKALIES AND ALKALINE EARTHS

**Sample:** 1 g

**Analysis:** Completely precipitate the aluminum from a boiling solution of the *Sample* in 100 mL of water by the addition of sufficient 6 N ammonium hydroxide to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite.

**Acceptance criteria:** The weight of the residue is NMT 5 mg (0.5%).

## Potassium Alum

$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  474.39

Sulfuric acid, aluminum potassium salt (2:1:1), dodecahydrate.

Aluminum potassium sulfate (1:1:2) dodecahydrate [7784-24-9].

Anhydrous 258.21 [10043-67-1].

» Potassium Alum contains not less than 99.0 percent and not more than 100.5 percent of  $\text{AlK}(\text{SO}_4)_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store at room temperature.

### Identification—

**A:** Add 1 N sodium hydroxide dropwise to a solution of it (1 in 20): a precipitate is formed that dissolves in an excess of the reagent. Ammonia is not evolved (*distinction from Ammonium Alum*).

**B:** Hold it in a nonluminous flame: a violet color is imparted to the flame.

**C:** Add 10 mL of sodium bitartrate TS to 5 mL of a saturated solution of it: a white, crystalline precipitate is formed within 30 minutes.

**D:** A solution (1 in 20) responds to the tests for *Aluminum* (191) and for *Sulfate* (191).

**Loss on drying** (731)—Transfer 2.0 g, in a tared porcelain crucible, to a muffle furnace at 200°. Increase the temperature to 400°, and dry at 400° to constant weight. Cool in a desiccator, and weigh: it loses between 43.0% and 46.0% of its weight.

**Heavy metals, Method I** (231)—Dissolve 1 g in water to make 20 mL, and add 5 mL of 0.1 N hydrochloric acid. Evaporate the solution in a porcelain evaporating dish to dryness. Treat the residue with 20 mL of water, and add 50 mg of hydroxylamine hydrochloride. Heat the solution on a steam bath for 10 minutes, cool, dilute with water to 25 mL, and proceed as directed, except to add 50 mg of hydroxylamine hydrochloride to the *Standard Preparation*: the limit is 0.002%.

**Iron**—Add 5 drops of potassium ferrocyanide TS to 20 mL of a solution (1 in 150): no blue color is produced immediately.

### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

*Procedure*—Transfer about 800 mg of Potassium Alum, accurately weighed, to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of *Edetate disodium titrant*, and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until solution is complete, and boil gently for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 12.91 mg of  $\text{AlK}(\text{SO}_4)_2$ .

## Alumina and Magnesia Oral Suspension

» Alumina and Magnesia Oral Suspension is a mixture containing aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and Magnesium Hydroxide  $[\text{Mg}(\text{OH})_2]$ . It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ . It may contain a flavoring agent, and may contain suitable antimicrobial agents.

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**Labeling**—Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent

amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

#### Identification—

**A:** To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**B:** Wash the precipitate obtained in *Identification* test A with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

**Acid-neutralizing capacity (301)**—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343 M)$$

in which 0.0385 and 0.0343 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$ , respectively, and A and M are the quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$  in the specimen tested, based on the labeled quantities.

**pH (791):** between 7.3 and 8.5.

**Chloride (221)**—Dissolve 5.0 g in the minimum volume of nitric acid required to achieve complete solution, add 1 mL of acid in excess, then add water to make 100 mL, and filter: a 10-mL portion of the filtrate shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid (0.14%).

**Sulfate (221)**—Dissolve 5.0 g in 5 mL of 3 N hydrochloric acid, with gentle heating. Cool, add water to make 250 mL, mix, and filter: a 20-mL portion of the filtrate shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid (0.1%).

**Other requirements**—It meets the requirements of the tests for *Arsenic* and *Heavy metals* under *Aluminum Hydroxide Gel*.

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Assay preparation**—Transfer an accurately measured quantity of Oral Suspension, previously well shaken in its original container, equivalent to about 1200 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of Assay preparation into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Eдетate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the Assay preparation, and make any necessary correction. Each mL of 0.05 M *Eдетate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium hydroxide—

**Assay preparation**—Prepare as directed in the Assay for aluminum hydroxide.

**Procedure**—Pipet a volume of Assay preparation, equivalent to about 40 mg of magnesium hydroxide, into a

400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the Assay preparation, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

## Alumina and Magnesia Tablets

» Alumina and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Tablets prepared with the use of Dried Aluminum Hydroxide Gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

#### Identification—

**A:** To a 0.7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**B:** Wash the precipitate obtained in *Identification* test A with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Disintegration (701):** 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units (905):** meet the requirements for *Weight Variation* with respect to alumina and to magnesia.

**Acid-neutralizing capacity (301)**—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343 M)$$

in which 0.0385 and 0.0343 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$ , respectively, and A and M are the quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$  in the specimen tested, based on the labeled quantities.

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1200 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Proceed as directed for Assay preparation in the Assay for aluminum hydroxide under *Alumina and Magnesia Oral Suspension*, beginning with "Heat gently, if necessary."

**Procedure**—Proceed as directed for *Procedure* in the *Assay for aluminum hydroxide* under *Alumina and Magnesia Oral Suspension*. Each mL of 0.05 M *Edetate disodium* titrant is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### **Assay for magnesium hydroxide—**

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for magnesium hydroxide* under *Alumina and Magnesia Oral Suspension*.

## **Alumina, Magnesia, and Calcium Carbonate Oral Suspension**

» Alumina, Magnesia, and Calcium Carbonate Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate  $(\text{CaCO}_3)$ .

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**Labeling**—Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

#### **Identification—**

**A:** To 5 g of Oral Suspension add 25 mL of 2 N sulfuric acid, stir, and allow to stand for 5 minutes. Add 25 mL of alcohol, stir, and place in an ice bath for 30 minutes. Filter while cold, retaining the filtrate for *Identification* test **B**. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings: the precipitate so obtained, dissolved in 3 N hydrochloric acid and filtered, responds to the tests for *Calcium* (191).

**B:** To the filtrate obtained in *Identification* test **A** add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 minutes, and filter through hardened filter paper. (Retain the filtrate for *Identification* test **C**.) Wash the precipitate with 350 mL of a hot ammonium chloride solution (1 in 50), discarding the washings: the precipitate so obtained, dissolved in 3 N hydrochloric acid, responds to the tests for *Aluminum* (191).

**C:** The filtrate obtained in *Identification* test **B** responds to the tests for *Magnesium* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

**Acid-neutralizing capacity (301)**—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343 M) + 0.9(0.02C)$$

in which 0.0385, 0.0343, and 0.02 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$ , respectively, and A, M, and C are the respective quantities, in mg, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantities.

**pH (791):** between 7.5 and 8.5.

**Chloride (221)**—Dissolve 5.0 g in 3 mL of nitric acid, add water to make 100 mL, and filter: a 10.0-mL portion of the

filtrate shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid (0.14%).

**Sulfate (221)**—Dissolve 5.0 g in 7 mL of 3 N hydrochloric acid, with gentle heating. Cool, add water to make 250 mL, mix, and filter: a 20.0-mL portion of the filtrate shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid (0.1%).

**Other requirements**—It meets the requirements of the tests for *Arsenic* and *Heavy metals* under *Aluminum Hydroxide Gel*.

#### **Assay for aluminum hydroxide—**

**Edetate disodium titrant**—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Assay preparation**—Transfer an amount of Oral Suspension, previously shaken in its original container, equivalent to about 600 mg of aluminum hydroxide, to a tared beaker, and weigh accurately. Add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Edetate disodium* titrant and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium* titrant consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### **Assay for magnesium hydroxide—**

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Pipet a volume of *Assay preparation*, equivalent to about 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethylamine, and mix. Add 50 mL of ammonia–ammonium chloride buffer TS and 2 drops of eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethylamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersing the beaker in an ice bath, and titrate with 0.05 M *edetate disodium* VS until the color changes to pure blue. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. From the volume of 0.05 M *edetate disodium* consumed, subtract the volume of 0.05 M *edetate disodium* consumed in the *Assay for calcium carbonate*. Each mL of 0.05 M *edetate disodium* is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

#### **Assay for calcium carbonate—**

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Pipet a volume of the *Assay preparation*, equivalent to about 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, 5 mL of sodium hydroxide solution (1 in 2), and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M *edetate disodium* VS until the solution is distinctly blue. Each mL of 0.05 M *edetate disodium* is equivalent to 5.004 mg of calcium carbonate  $(\text{CaCO}_3)$ .

## Alumina, Magnesia, and Calcium Carbonate Chewable Tablets

*Former Title: Alumina, Magnesia, and Calcium Carbonate Tablets*

» Alumina, Magnesia, and Calcium Carbonate Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate ( $\text{CaCO}_3$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Chewable Tablets prepared using dried aluminum hydroxide gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

**Identification**—To a 3-g portion of finely powdered Chewable Tablets add 25 mL of water and 25 mL of 2 N sulfuric acid, stir, and heat on a steam bath for 10 minutes. Cool, add 50 mL of alcohol, and stir: the mixture so obtained meets the requirements of *Identification* tests A, B, and C under *Alumina, Magnesia, and Calcium Carbonate Oral Suspension*, beginning under *Identification* test A with "place in an ice bath for 30 minutes."

**Disintegration** (701): 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to alumina, to magnesia, and to calcium carbonate.

**Acid-neutralizing capacity** (301)—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343M) + 0.9(0.02C)$$

in which 0.0385, 0.0343, and 0.02 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$ , respectively; and A, M, and C are the respective quantities, in mg, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantities.

### Assay for aluminum hydroxide—

*Edetate disodium titrant*—Prepare as directed in the *Assay* under *Ammonium Alum*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* for aluminum hydroxide under *Alumina, Magnesia, and Calcium Carbonate Oral Suspension*. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

### Assay for magnesium hydroxide—

*Edetate disodium titrant* and *Assay preparation*—Prepare as directed in the *Assay* for aluminum hydroxide.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* for magnesium hydroxide under *Alumina, Magnesia, and Calcium Carbonate Oral Suspension*. Each mL of 0.05 M *edetate disodium* is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

### Assay for calcium carbonate—

*Edetate disodium titrant* and *Assay preparation*—Prepare as directed in the *Assay* for aluminum hydroxide.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* for calcium carbonate under *Alumina, Magnesia, and Calcium Carbonate Oral Suspension*. Each mL of 0.05 M *edetate disodium* is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

## Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets

*Former Title: Alumina, Magnesia, Calcium Carbonate, and Simethicone Tablets*

» Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate ( $\text{CaCO}_3$ ), and an amount of polydimethylsiloxane  $([-(\text{CH}_3)_2\text{SiO}-]_n)$  that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the Chewable Tablets to indicate that they are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, if it is greater than 5 mg per Chewable Tablet.

**USP Reference standards** (11)—

USP Polydimethylsiloxane RS

### Identification—

**A:** Cut a Chewable Tablet into pieces, add 50 mL of 1 N sulfuric acid, stir until the pieces disintegrate, and heat on a steam bath for 10 minutes. Cool, add 50 mL of alcohol, and stir. The mixture so obtained responds to *Identification* tests A, B, and C under *Alumina, Magnesia, and Calcium Carbonate Oral Suspension*, beginning under *Identification* test A with "place in an ice bath for 30 minutes."

**B: Infrared Absorption** (197S)—

Cell: 0.5 mm.

*Solution:* prepared as directed in the *Assay* for polydimethylsiloxane.

### Microbial enumeration tests (61) and Tests for specified microorganisms (62)—

The total aerobic microbial count does not exceed 200 cfu per g, the total combined molds and yeasts count does not exceed 200 cfu per g, and the Chewable Tablets meet the requirements of the test for the absence of *Salmonella* species and *Escherichia coli*.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide, to magnesium hydroxide, and to calcium carbonate.

**Acid-neutralizing capacity** (301)—Dissolve an accurately counted number of Chewable Tablets, equivalent to about 120 mEq of acid-neutralizing capacity, in about 400 mL of water. Transfer the mixture to a 500-mL volumetric flask, dilute with water to volume, and mix. Use 75.0 mL of this solution as the *Test preparation*. Proceed as directed in the section *Procedure for Powders, Effervescent Solids, Suspensions and Other Liquids, Nonchewable Tablets, Chewable Tablets, and Capsules*. The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq.



and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343 M) + 0.9(0.02C)$$

in which 0.0385, 0.0343, and 0.02 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$ , respectively; and  $A$ ,  $M$ , and  $C$  are the quantities, in mg, of  $\text{Al}(\text{OH})_3$ ,  $\text{Mg}(\text{OH})_2$ , and  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantities.

#### Sodium content—

**Potassium chloride solution**—Dissolve 3 g of potassium chloride in water in a 100-mL volumetric flask, dilute with water to volume, and mix.

**Dilute hydrochloric acid**—Prepare by mixing 226 mL of hydrochloric acid with sufficient water to make 1000 mL.

**Standard solution**—Transfer 2.5420 g of sodium chloride, previously dried at  $105^\circ$  for 2 hours, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. To three separate 100-mL volumetric flasks, each containing 10.0 mL of *Potassium chloride solution* and 3.0 mL of *Dilute hydrochloric acid*, add, respectively, 10.0, 20.0, and 30.0 mL of this solution. These solutions contain 1.0, 2.0, and 3.0  $\mu\text{g}$  of sodium (Na) per mL, respectively.

**Test solution**—Accurately weigh 10 Chewable Tablets, and determine the average weight,  $A$ , in mg. Cut 4 Chewable Tablets into pieces, combine the pieces, and weigh them. Transfer the combined pieces to a 500-mL volumetric flask, add 150 mL of *Dilute hydrochloric acid*, and swirl gently to disintegrate the pieces. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Blank solution**—Transfer 3.0 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in  $\mu\text{g}$  per mL, of sodium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu\text{g}$  per mL, of sodium in the *Test solution*. Calculate the mg of sodium (Na) in each Tablet taken by the formula:

$$5C(A/W)$$

in which  $A$  is the average weight, in mg, of each Chewable Tablet; and  $W$  is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Test solution*. Chewable Tablets contain not more than 5 mg of sodium per Chewable Tablet, except when labeled to contain more than 5 mg of sodium per Chewable Tablet, they contain not more than 110% of the labeled amount.

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Assay preparation**—Transfer an accurately counted number of Chewable Tablets, equivalent to about 665 mg of aluminum hydroxide, to a suitable beaker. Add 15 mL of hydrochloric acid, and swirl to dissolve the Chewable Tablets. Add 80 mL of water, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 20 mL of *Assay preparation* into a 250-mL beaker, then add, in the order named and with continuous stirring, 25.0 mL of *Eдетate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and making any necessary correction. Each mL of 0.05 M *Eдетate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium hydroxide—

**Lanthanum chloride solution**—Transfer 17.6 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

**Dilute hydrochloric acid**—Prepare by mixing 226 mL of hydrochloric acid with sufficient water to make 1000 mL.

**Potassium chloride solution**—Dissolve 3 g of potassium chloride in water in a 100-mL volumetric flask, dilute with water to volume, and mix.

**Magnesium stock solution**—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. This solution contains 20  $\mu\text{g}$  of magnesium (Mg) per mL.

**Standard preparations**—To three separate 100-mL volumetric flasks each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 2.0, and 3.0 mL, respectively, of the *Magnesium stock solution*. Dilute each with water to volume, and mix. These solutions contain 0.1, 0.2, and 0.3  $\mu\text{g}$  of magnesium (Mg) per mL, respectively.

**Assay preparation**—Transfer an accurately counted number of Chewable Tablets, equivalent to about 250 mg of magnesium hydroxide (100 mg of magnesium), to a 1000-mL volumetric flask. Add 500 mL of *Dilute hydrochloric acid*, and swirl to disintegrate the Chewable Tablets. Add 100.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

**Blank**—Add 50 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a third 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a magnesium hollow-cathode lamp and an air-acetylene flame, using the *Blank* to set the instrument. Plot the absorbances of the *Standard preparations* versus concentration, in  $\mu\text{g}$  per mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu\text{g}$  per mL, of magnesium in the *Assay preparation*. Calculate the quantity, in mg, of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in each Chewable Tablet taken by the formula:

$$(58.34/24.305)(500C/N)$$

in which 58.34 is the molecular weight of magnesium hydroxide; 24.305 is the atomic weight of magnesium; and  $N$

is the number of Chewable Tablets taken to prepare the Assay preparation.

#### Assay for calcium carbonate—

*Assay preparation*—Prepare as directed in the Assay for aluminum hydroxide.

*Procedure*—Pipet a volume of the Assay preparation, equivalent to about 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, a volume of sodium hydroxide solution (1 in 2) equivalent to the volume of the Assay preparation taken, and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Perform a blank determination, substituting a volume of water equivalent to the volume of the Assay preparation taken, and make any necessary correction. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

#### Assay for polydimethylsiloxane—

*Dilute hydrochloric acid*—Prepare by mixing 400 mL of hydrochloric acid with sufficient water to make 1000 mL.

*Standard preparation*—Transfer about 60 mg of USP Polydimethylsiloxane RS, accurately weighed, to a separator, add 30.0 mL of chloroform and 60 mL of Dilute hydrochloric acid, shake for 30 seconds, and allow the phases to separate. Remove about 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 60 mg of simethicone, to a suitable screw-capped bottle, add 30.0 mL of chloroform and 60 mL of Dilute hydrochloric acid, and allow to stand, with frequent shaking, until the Chewable Tablets are dissolved. Transfer the contents of the bottle to a separator, shake, and allow the phases to separate. Remove about 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

*Blank*—Place 30.0 mL of chloroform and 60 mL of Dilute hydrochloric acid in a separator, shake for 30 seconds, and allow the phases to separate. Remove about 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

*Procedure*—Concomitantly determine the absorbances of the Standard preparation and the Assay preparation in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9  $\mu\text{m}$ , with a suitable IR spectrophotometer, using the Blank to set the instrument. Calculate the quantity, in mg, of  $[-(\text{CH}_3)_2\text{SiO}-]_n$  in each Chewable Tablet taken by the formula:

$$(W/N)(A_U / A_S)$$

in which *W* is the weight, in mg, of USP Polydimethylsiloxane RS used to prepare the Standard preparation; *N* is the number of Chewable Tablets taken to prepare the Assay preparation; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the Assay preparation and the Standard preparation, respectively.

## Alumina, Magnesia, and Simethicone Oral Suspension

» Alumina, Magnesia, and Simethicone Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and an amount of polydimethylsiloxane  $[-(\text{CH}_3)_2\text{SiO}-]_n$  that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**Labeling**—Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ . Label it to state the sodium content if it is greater than 1 mg per mL.

#### USP Reference standards (11)—

USP Polydimethylsiloxane RS

#### Identification—

**A:** Infrared Absorption (197S)—

Cell: 0.5 mm.

**Solution:** prepared as directed in the Assay for polydimethylsiloxane.

**B:** To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution just changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate so obtained responds to the tests for Magnesium (191).

**C:** Wash the precipitate obtained in Identification test B with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions: the dropwise addition of 6 N ammonium hydroxide to one portion yields a gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide. The dropwise addition of 1 N sodium hydroxide to the other portion yields a gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

**Acid-neutralizing capacity (301)**—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343M)$$

in which 0.0385 and 0.0343 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$ , respectively, and *A* and *M* are the quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$  in the specimen tested, based on the labeled quantities.

**pH (791):** between 7.0 and 8.6.

#### Sodium content—

**Potassium chloride solution**—Prepare a solution of potassium chloride in water containing 38 mg per mL.

**Sodium chloride stock solution**—Dissolve a suitable quantity of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, in water, and dilute quantitatively

and stepwise with water to obtain a solution containing 25.42 µg per mL (10 µg of sodium per mL).

**Standard preparations**—On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 mL and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume, and mix. These solutions contain about 0.5 µg and 1.0 µg of sodium per mL, respectively.

**Test preparation**—Transfer 5.0 mL of Oral Suspension, previously well-shaken in its original container, to a 100-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, boil for 15 minutes, cool to room temperature, dilute with water to volume, and mix. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using as a blank a solution prepared by pipeting 4 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* into a 100-mL volumetric flask, diluting with water to volume, and mixing. Plot the absorbances of the *Standard preparations* versus concentrations, in µg per mL, of sodium and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of sodium in the *Test preparation*. Calculate the quantity, in mg, of sodium in each mL of Oral Suspension taken by the formula:

$$0.4C.$$

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Assay preparation**—Transfer an accurately measured volume of Oral Suspension, previously well-shaken in its original container, equivalent to about 800 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Eдетate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and making any necessary correction. Each mL of 0.05 M *Eдетate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium hydroxide—

**Assay preparation**—Prepare as directed in the Assay for aluminum hydroxide.

**Procedure**—Pipet a volume of the *Assay preparation*, equivalent to about 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath,

then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

**Assay for polydimethylsiloxane**—Transfer an accurately measured volume of Oral Suspension, equivalent to about 50 mg of simethicone, to a suitable round, narrow-mouth, screw-capped, 120-mL bottle, add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 25.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 15 minutes, accurately timed, on a reciprocating shaker (e.g., about 200 oscillations per minute and a stroke of  $38 \pm 2$  mm). Transfer the mixture to a 125-mL separator. Remove about 5 mL of the upper, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant (*Assay preparation*) is obtained. Prepare a *Standard preparation* similarly, except to dissolve about 50 mg of USP Polydimethylsiloxane RS, accurately weighed, in 25.0 mL of toluene, add 40 mL of 0.1 N sodium hydroxide, and add a volume of water equal to that of the specimen of Oral Suspension taken. Prepare a blank by mixing 10 mL of toluene with 0.5 g of anhydrous sodium sulfate and centrifuging to obtain a clear supernatant. Concomitantly determine the absorbances of the solutions in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $[-(\text{CH}_3)_2\text{SiO}-]_n$  in each mL of the Oral Suspension taken by the formula:

$$(W / V)(A_U / A_S)$$

in which *W* is the weight, in mg, of USP Polydimethylsiloxane RS used in preparing the *Standard preparation*; *V* is the volume, in mL, of Oral Suspension taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Alumina, Magnesia, and Simethicone Chewable Tablets

*Former Title: Alumina, Magnesia, and Simethicone Tablets*

» Alumina, Magnesia, and Simethicone Chewable Tablets contain the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and an amount of polydimethylsiloxane  $[-(\text{CH}_3)_2\text{SiO}-]_n$  that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Label the Chewable Tablets to state the sodium content if it is greater than 5 mg per Tablet. The Chewable Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

**USP Reference standards** (11)—  
USP Polydimethylsiloxane RS

**Identification—**

**A:** *Infrared Absorption* (197S)—

**Cell:** 0.5 mm.

**Solution:** prepared as directed in the *Assay for polydimethylsiloxane*.

**B:** To a portion of finely powdered Chewable Tablets, equivalent to about 600 mg of magnesium hydroxide, add 25 mL of 3 N hydrochloric acid and 25 mL of water, and mix. Boil gently for 2 minutes. Allow to cool, and filter. Add 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution just turns to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate so obtained meets the requirements of the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification* test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution so obtained meets the requirements for *Identification* test C under *Alumina, Magnesia, and Simethicone Oral Suspension*.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium hydroxide.

**Acid-neutralizing capacity** (301)—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.0343M)$$

in which 0.0385 and 0.0343 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$ , respectively, and *A* and *M* are the quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{Mg}(\text{OH})_2$  in the specimen tested, based on the labeled quantities.

**Sodium content—**

**Potassium chloride solution, Sodium chloride stock solution, and Standard preparations—**Prepare as directed in the test for *Sodium content* under *Alumina, Magnesia, and Simethicone Oral Suspension*.

**Test preparation—**Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to the average weight of 1 Chewable Tablet, to a 100-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, boil for 15 minutes, cool to room temperature, dilute with water to volume, and mix. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Procedure—**Proceed as directed in the test for *Sodium content* under *Alumina, Magnesia, and Simethicone Oral Suspension*. Calculate the quantity, in mg, of sodium per Chewable Tablet taken by the formula:

$$2C$$

**Assay for aluminum hydroxide—**

**Edetate disodium titrant—**Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Assay preparation—**Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 800 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool to room temperature, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure—**Proceed as directed for *Procedure* in the *Assay for aluminum hydroxide* under *Alumina, Magnesia, and Simethicone Oral Suspension*.

**Assay for magnesium hydroxide—**

**Assay preparation—**Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure—**Proceed as directed for *Procedure* in the *Assay for magnesium hydroxide* under *Alumina, Magnesia, and Simethicone Oral Suspension*.

**Assay for polydimethylsiloxane—**Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 33 mg of simethicone, to a suitable round, narrow-mouth, screw-capped, 120-mL bottle, add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 20.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 30 minutes, accurately timed, on a reciprocating shaker (e.g., about 200 oscillations per minute and a stroke of  $38 \pm 2$  mm). Transfer the mixture to a 125-mL separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing about 2 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant (*Assay preparation*) is obtained. Similarly prepare a *Standard preparation*, using about 33 mg of USP Polydimethylsiloxane RS, accurately weighed. Prepare a blank by mixing 10 mL of toluene with about 1 g of anhydrous sodium sulfate and centrifuging to obtain a clear supernatant. Concomitantly determine the absorbances of the solutions in 0.5-mm cells at the wavelength of maximum absorbance at about  $7.9 \mu\text{m}$  ( $1265.8 \text{ cm}^{-1}$ ), with a suitable IR spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $[-(\text{CH}_3)_2\text{SiO}-]_n$  in the portion of Chewable Tablets taken by the formula:

$$(W)(A_U / A_S)$$

in which *W* is the weight, in mg, of USP Polydimethylsiloxane RS used to prepare the *Standard preparation*, and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

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**Alumina and Magnesium Carbonate Oral Suspension**


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» Alumina and Magnesium Carbonate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium carbonate ( $\text{MgCO}_3$ ).

**Packaging and storage—**Preserve in tight containers, and avoid freezing.

**Identification—**

**A:** Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification* test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.024M)$$

in which 0.0385 and 0.024 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{MgCO}_3$ , respectively; and *A* and *M* are the respective quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantities.

**pH** (791): between 7.5 and 9.5.

#### **Assay for aluminum hydroxide—**

**Potassium chloride solution**—Prepare a solution containing 4.5 g of potassium chloride in each 100 mL.

**Aluminum stock solution**—Transfer 1.000 g of aluminum wire to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks, each containing 10 mL of *Potassium chloride solution*, transfer 9.0, 10.0, and 11.0 mL, respectively, of *Aluminum stock solution*, dilute with water to volume, and mix. These *Standard preparations* contain 90.0, 100.0, and 110.0 µg of aluminum per mL, respectively.

**Assay preparation**—Transfer an accurately measured quantity of Oral Suspension, previously shaken in its original container, equivalent to about 75 mg of aluminum hydroxide, to a suitable beaker. Add 25 mL of 6 N hydrochloric acid, and heat on a steam bath for 30 minutes, with occasional swirling. Cool, and transfer with the aid of water to a 250-mL volumetric flask containing 25 mL of *Potassium chloride solution*. Dilute with water to volume, mix, and filter.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with an aluminum hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Calculate the quantity, in mg, of  $\text{Al}(\text{OH})_3$  in the portion of Oral Suspension taken by the formula:

$$(78.00/26.98)(0.25)(A_U / R_S)$$

in which 78.00 is the molecular weight of aluminum hydroxide; 26.98 is the atomic weight of aluminum; *A<sub>U</sub>* is the absorbance of the *Assay preparation*; and *R<sub>S</sub>* is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in µg of aluminum per mL.

#### **Assay for magnesium carbonate—**

**Lanthanum chloride solution**—Prepare a solution of lanthanum chloride in water containing 5 mg per mL.

**Magnesium stock solution**—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks, each containing 10 mL of *Lanthanum chloride solution*, transfer 1.70 mL and 1.80 mL, respectively, of *Magnesium stock solution*, dilute with water to volume, and mix. These

*Standard preparations* contain 1.7 µg of magnesium per mL and 1.8 µg of magnesium per mL, respectively.

**Assay preparation**—Quantitatively dilute an accurately measured volume of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide* with water to obtain a solution having a concentration of about 6 µg of magnesium carbonate per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a magnesium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Calculate the quantity, in mg, of magnesium carbonate ( $\text{MgCO}_3$ ) in the portion of Oral Suspension taken by the formula:

$$(84.31/24.31)(L/D)(A_U / R_S)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; *L* is the labeled quantity, in mg, of magnesium carbonate in the portion of Oral Suspension taken; *D* is the concentration, in µg of magnesium carbonate per mL, of the *Assay preparation*, based on the labeled amount of magnesium carbonate in the portion of Oral Suspension taken and the extent of dilution; *A<sub>U</sub>* is the absorbance of the *Assay preparation*; and *R<sub>S</sub>* is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in µg of magnesium per mL.

## **Alumina and Magnesium Carbonate Tablets**

» Alumina and Magnesium Carbonate Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [ $\text{Al}(\text{OH})_3$ ] and magnesium carbonate ( $\text{MgCO}_3$ ).

**Packaging and storage**—Preserve in tight containers.

#### **Identification—**

**A:** Place about 1 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To a 7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification* test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium carbonate.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**Assay for aluminum hydroxide—**

*Potassium chloride solution*—Prepare a solution containing 38.1 g of potassium chloride in each 1000 mL.

*Digestion fluid*—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

*Aluminum stock solution*—Transfer 1.000 g of aluminum metal to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

*Standard preparations*—To separate 100-mL volumetric flasks transfer 3.0 mL, 4.0 mL, and 5.0 mL of *Aluminum stock solution*, respectively. To each flask add 10 mL of *Potassium chloride solution* and 7.5 mL of *Digestion fluid*, dilute with water to volume, and mix. These *Standard preparations* contain 30, 40, and 50 µg of aluminum per mL, respectively.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of aluminum hydroxide, to a 100-mL volumetric flask, add 25-mL of *Digestion fluid*, and heat on a steam bath for 30 minutes or on a hot plate until the volume is reduced by about one-half. Cool, dilute with water to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer 15.0 mL of the filtrate to a 50-mL volumetric flask, add 5.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

[NOTE—Reserve a portion of the filtrate for use in the *Assay for magnesium carbonate*.]

*Procedure*—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with an aluminum hollow-cathode lamp and a nitrous oxide-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of aluminum, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of aluminum in each mL of the *Assay preparation*. Calculate the quantity, in mg, of aluminum hydroxide [Al(OH)<sub>3</sub>] in the portion of Tablets taken by the formula:

$$(78.00 / 26.98)(C / 3)$$

in which 78.00 is the molecular weight of aluminum hydroxide, and 26.98 is the atomic weight of aluminum.

**Assay for magnesium carbonate—**

*Lanthanum chloride solution*—Transfer 17.6 g of lanthanum chloride to a 1000-mL volumetric flask, add 500 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

*Digestion fluid*—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

*Magnesium stock solution*—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

*Standard preparations*—To separate 100-mL volumetric flasks transfer 4.0, 6.0, and 8.0 mL of *Magnesium stock solution*, respectively. To each flask add 0.5 mL of *Digestion fluid* and 10 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. These *Standard preparations* contain 0.40, 0.60, and 0.80 µg of magnesium per mL, respectively.

*Assay preparation*—Transfer an accurately measured volume of the filtrate used to prepare the *Assay preparation* in the *Assay for aluminum hydroxide*, equivalent to about 0.4 mg of magnesium carbonate, to a 200-mL volumetric

flask, add 20 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a magnesium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of magnesium in each mL of the *Assay preparation*. Calculate the quantity, in mg, of magnesium carbonate (MgCO<sub>3</sub>) in the portion of Tablets taken by the formula:

$$(84.31 / 24.31)(20C / V)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; and V is the volume taken, in mL, of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide*.

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**Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets**


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» Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [Al(OH)<sub>3</sub>] and magnesium carbonate (MgCO<sub>3</sub>), and not less than 85.0 percent and not more than 115.0 percent of the labeled amount of magnesium oxide (MgO).

**Packaging and storage**—Preserve in tight containers.

**Identification—**

**A:** Place about 3 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To the solution in the flask obtained in *Identification* test A add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 minutes, and filter through hardened filter paper. (Retain the filtrate for *Identification* test C.) Wash the precipitate with 350 mL of a hot ammonium chloride solution (1 in 50), discarding the washings: the precipitate so obtained, dissolved in 3 N hydrochloric acid, responds to the tests for *Aluminum* (191).

**C:** The filtrate obtained in *Identification* test B responds to the tests for *Magnesium* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to alumina, to magnesium carbonate, and to magnesium oxide.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**Assay for aluminum hydroxide—**

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1200 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

**Assay for magnesium carbonate**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 750 mg of magnesium carbonate, to a 250-mL conical flask fitted with a two-hole stopper. Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and accurately weigh the tube and the contents. Into the other arm of the tube place 9.5 g to 10.5 g of soda lime, and again weigh accurately. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with soda lime to a calcium chloride drying tube, which in turn is connected to one of the holes in the stopper of the 250-mL conical flask. Attach a dropping funnel to the other hole in the stopper of the 250-mL conical flask. Add 100 mL of water and 10 mL of a mixture of hydrochloric acid and nitric acid (4:1) to the 250-mL conical flask through the dropping funnel, and close the dropping funnel. Heat the 250-mL conical flask at 95° for 1 hour, and allow the evolved carbon dioxide to pass through the U-tube. Replace the dropping funnel with a source of carbon dioxide-free air, and pass the carbon dioxide-free air through the apparatus at a rate of about 75 mL per minute for 30 minutes. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh. The increase in weight corresponds to the quantity of carbon dioxide evolved. Calculate the quantity, in mg, of magnesium carbonate in each Tablet taken by the formula:

$$(84.31 / 44.01)(I)(W_A / W_P)$$

in which 84.31 and 44.01 are the molecular weights of magnesium carbonate and carbon dioxide, respectively; *I* is the quantity, in mg, of carbon dioxide evolved from the portion of Tablets taken;  $W_A$  is the average weight, in g, of 1 Tablet; and  $W_P$  is the weight, in g, of the portion of Tablets taken.

**Assay for magnesium oxide**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of magnesium carbonate and magnesium oxide combined, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix. Transfer 20.0 mL of this solution to a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice

bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 1.216 mg of Mg. Calculate the quantity, in mg, of magnesium equivalent in each Tablet taken by the formula:

$$10T(W_A / W_P)$$

in which *T* is the magnesium equivalent obtained in the titration;  $W_A$  is the average weight, in g, of 1 Tablet; and  $W_P$  is the weight, in g, of the portion of Tablets taken. Calculate the quantity, in mg, of magnesium oxide in each Tablet taken by the formula:

$$(40.30 / 24.31)(A - 0.2883B)$$

in which 40.30 and 24.31 are the molecular weight of magnesium oxide and the atomic weight of magnesium, respectively; *A* is the quantity, in mg, of magnesium equivalent in each Tablet; and *B* is the quantity, in mg, of magnesium carbonate in each Tablet, as determined in the *Assay for magnesium carbonate*.

## Alumina and Magnesium Trisilicate Oral Suspension

» Alumina and Magnesium Trisilicate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [ $\text{Al}(\text{OH})_3$ ] and magnesium trisilicate ( $\text{Mg}_2\text{Si}_3\text{O}_8$ ).

**Packaging and storage**—Preserve in tight containers.

### Identification—

**A:** To a mixture of 5 mL in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**B:** Wash the solids on the filter obtained in *Identification* test A with hot ammonium chloride solution (1 in 50), add 10 mL of 3 N hydrochloric acid, and filter: the filtrate responds to the tests for *Aluminum* (191).

**C:** Transfer the filter paper and contents from *Identification* test B to a small platinum dish, ignite, cool in a desiccator, and weigh. Moisten the residue with water and add 6 mL of hydrofluoric acid. Evaporate to dryness, ignite for 5 minutes, cool in a desiccator, and weigh: a loss of more than 10% in relation to the weight of the residue from the initial ignition indicates  $\text{SiO}_2$ .

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**pH** (791): between 7.5 and 8.5.

### Assay for aluminum hydroxide—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Assay preparation**—Transfer about 10 g of well-shaken Oral Suspension to a tared beaker, and weigh accurately. Add 50 mL of water and 10 mL of hydrochloric acid, and digest on a steam bath for 1 hour. Cool, and filter into a 200-mL volumetric flask, washing the filter with water into the flask. Dilute with water to volume, and mix.

**Procedure**—Pipet 20 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order

named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium trisilicate—

*Assay preparation*—Prepare as directed in the *Assay for aluminum hydroxide*.

*Procedure*—Pipet 20 mL of *Assay preparation* into a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 6.521 mg of  $\text{Mg}_2\text{Si}_3\text{O}_8$ .

## Alumina and Magnesium Trisilicate Tablets

» Alumina and Magnesium Trisilicate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium trisilicate ( $\text{Mg}_2\text{Si}_3\text{O}_8$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Tablets prepared with the use of *Dried Aluminum Hydroxide Gel* may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ . Tablets intended for the temporary relief of heartburn (acid indigestion) due to acid reflux are so labeled. Tablets that must be chewed before swallowing are so labeled.

**Identification**—One powdered Tablet responds to the *Identification* tests under *Alumina and Magnesium Trisilicate Oral Suspension*.

**Disintegration** <701>: 10 minutes, simulated gastric fluid TS being substituted for water in the test. [NOTE—Tablets that must be chewed before swallowing are exempt from this requirement.]

**Uniformity of dosage units** <905>: meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium trisilicate.

**Acid-neutralizing capacity** <301>—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling. [NOTE—Tablets labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux are exempt from this requirement.]

**Foam** [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]—Finely powder a number of Tablets, accurately counted, equivalent to the minimum single dose recommended in the labeling, and transfer the powder to a 100-mL beaker having an inside diameter of 45 mm. Add 5 mL of alcohol and sufficient water to make 40 mL. Mix at 300 rpm for 60 seconds, using

a magnetic stirrer and a 9.5- × 38-mm polytetrafluoroethylene-coated stirring bar. Stop the stirrer, and carefully add 10 mL of 0.5 N hydrochloric acid down the side of the beaker. Stir for 30 seconds at 300 rpm. Allow to stand for 10 minutes, and measure the thickness of the foam layer above the liquid in the beaker: the thickness of the foam is not less than 10 mm.

**pH** <791> [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]: not less than 4.5, determined on the foam layer obtained in the *Foam* test. [NOTE—Take care that the electrodes do not touch the liquid beneath the foam.]

#### Assay for aluminum hydroxide—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay under Ammonium Alum*.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

*Procedure*—Pipet 10 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium trisilicate—

*Potassium chloride solution*—Prepare a solution in water containing 5 g of potassium chloride per 100 mL.

*Magnesium standard solution*—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

*Standard preparations*—Transfer 16.0 mL, 18.0 mL, and 20.0 mL of *Magnesium standard solution* to separate 100-mL volumetric flasks, add 2.0 mL of *Potassium chloride solution* to each flask, dilute with water to volume, and mix. These *Standard preparations* contain 1.6, 1.8, and 2.0 µg of magnesium per mL, respectively. [NOTE—Prepare these solutions on the day of use.]

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of magnesium trisilicate, to a 100-mL volumetric flask, and add 10 mL of 18 N sulfuric acid. Heat on a steam bath for 30 minutes with occasional swirling. Allow to cool, dilute with water to volume, and mix. Filter this solution, discarding the first 20 mL of the filtrate. Transfer 20.0 mL of the filtrate to a second 100-mL volumetric flask, add 2.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

*Procedure*—Concomitantly determine the absorbance of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)), equipped with a magnesium hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations*, in µg per mL, of magnesium, and draw the line best fitting the three plotted points. From the graph so obtained determine the concentration, C, in µg per mL, of magnesium in the *Assay preparation*. Calculate the quantity,

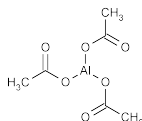


in mg, of magnesium trisilicate ( $\text{Mg}_2\text{Si}_3\text{O}_8$ ) in the portion of Tablets taken by the formula:

$$0.5C(260.86 / 48.62)$$

in which 260.86 is the molecular weight of anhydrous magnesium trisilicate and 48.62 is twice the atomic weight of magnesium.

## Aluminum Acetate Topical Solution



$\text{C}_6\text{H}_9\text{AlO}_6$  204.11

Acetic acid, aluminum salt.

Aluminum acetate [139-12-8].

» Aluminum Acetate Topical Solution yields, from each 100 mL, not less than 1.20 g and not more than 1.45 g of aluminum oxide ( $\text{Al}_2\text{O}_3$ ), and not less than 4.24 g and not more than 5.12 g of acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ), corresponding to not less than 4.8 g and not more than 5.8 g of aluminum acetate ( $\text{C}_6\text{H}_9\text{AlO}_6$ ). Aluminum Acetate Topical Solution may be stabilized by the addition of not more than 0.6 percent of Boric Acid.

Aluminum Subacetate Topical Solution . . . . .	545 mL
Glacial Acetic Acid . . . . .	15 mL
Purified Water, a sufficient quantity, to make . . . . .	1000 mL

Add the Glacial Acetic Acid to the Aluminum Subacetate Topical Solution and sufficient water to make 1000 mL. Mix, and filter, if necessary.

NOTE—Dispense only clear Aluminum Acetate Topical Solution.

**Packaging and storage**—Preserve in tight containers.

**Identification**—It responds to the tests for *Aluminum* (191) and for the ferric chloride test for *Acetate* (191) with a deep red color upon the addition of ferric chloride TS. This color is destroyed by the addition of a mineral acid.

**pH** (791): between 3.6 and 4.4.

**Limit of boric acid**—Pipet 25 mL into 75 mL of water in a conical flask. Add 3 mL of phenolphthalein TS, then add 0.5 N sodium hydroxide VS from a buret until a faint pink color is obtained. Heat to boiling, and again neutralize. Add 150 mL of glycerin to the neutralized solution, and titrate with 0.5 N sodium hydroxide VS. Perform a blank determination in a similar manner. From the volume of 0.5 N sodium hydroxide VS used after the addition of the glycerin, subtract the volume used in the blank. Each mL of 0.5 N sodium hydroxide is equivalent to 30.92 mg of  $\text{H}_3\text{BO}_3$ .

**Heavy metals** (231)—Dilute 2 mL of it with water to 25 mL: the limit is 0.001%.

**Assay for aluminum oxide**—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Pipet 25 mL of Topical Solution into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, dilute with water to volume, and mix. Pipet 25 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting water for the sample, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 2.549 mg of  $\text{Al}_2\text{O}_3$ .

**Assay for acetic acid**—Pipet 20 mL of Topical Solution into a Kjeldahl flask containing a mixture of 20 mL of phosphoric acid and 150 mL of water. Connect the flask to a condenser, the delivery tube from which dips beneath the surface of 50.0 mL of 0.5 N sodium hydroxide VS contained in a receiving flask. Distill about 160 mL, then remove the delivery tube from below the surface of the liquid, allow the distilling flask to cool, add 50 mL of water, and distill an additional 40 to 45 mL into the receiving flask. Add phenolphthalein TS to the distillate, and titrate the excess 0.5 N sodium hydroxide VS with 0.5 N sulfuric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 30.03 mg of  $\text{C}_2\text{H}_4\text{O}_2$ .

## Aluminum Chloride

$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  241.43

Aluminum chloride, hexahydrate.

Aluminum chloride hexahydrate [7784-13-6].

Anhydrous 133.34 [7446-70-0].

» Aluminum Chloride contains not less than 95.0 percent and not more than 102.0 percent of  $\text{AlCl}_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**Water**, *Method I* (921): between 42.0% and 48.0%.

**Sulfate**—The addition of 0.2 mL of barium chloride TS to 10 mL of a solution (1 in 100) produces no turbidity within 1 minute.

**Limit of alkalies and alkaline earths**—To a boiling solution of 1.0 g in 150 mL of water add a few drops of methyl red TS, then add 6 N ammonium hydroxide until the color of the solution just changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to constant weight: the weight of the residue does not exceed 2.5 mg (0.5%).

**Heavy metals**, *Method I* (231)—Dissolve 1 g in 1 mL of 1 N acetic acid and sufficient water to make 25 mL: the limit is 0.002%.

**Iron** (241)—Dissolve 1.0 g in 45 mL of water, and add 2 mL of hydrochloric acid: the limit is 0.001%.

**Assay**—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Transfer to a 250-mL volumetric flask about 5 g of Aluminum Chloride, accurately weighed, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant*, and 20 mL of acetic acid–ammonium acetate buffer TS, and boil gently for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a

blank determination, substituting 10 mL of water for the assay preparation, and make any necessary correction. Each mL of 0.05 M *Eдетate disodium titrant* is equivalent to 6.667 mg of  $\text{AlCl}_3$ .

## Aluminum Chlorohydrate

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z \cdot \text{H}_2\text{O}$

Aluminum chlorohydroxide.

Aluminum hydroxychloride.

Dihydrate [12042-91-0].

Anhydrous [1327-41-9].

Aluminum chlorohydroxide, dihydrate.

Aluminum hydroxychloride, dihydrate.

Dihydrate 210.48 [12042-91-0].

Anhydrous 174.45 [1327-41-9].

» Aluminum Chlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum chlorohydrate.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic**, *Method I* (211): 2 µg per g.

**Heavy metals**, *Method I* (231): 0.002%.

**Limit of iron**—

*Standard preparation*—Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* (241), to a 50-mL beaker.

*Test preparation*—Transfer 2.7 g of Aluminum Chlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

*Procedure*—To each of the beakers containing the *Standard preparation* and the *Test preparation* add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3 to 5 minutes. Allow to cool, add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed under *Iron* (241), transfer to separate 50-mL color comparison tubes, dilute with water to volume, and mix: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (150 µg per g).

**Content of aluminum**—

*Eдетate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*, except to use 37.2 g of edetate disodium instead of 18.6 g.

*Test solution*—Transfer about 200 mg of Aluminum Chlorohydrate, accurately weighed, to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for not less than 5 minutes, and allow to cool.

*Procedure*—To the *Test solution* add 25.0 mL of *Eдетate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid-ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate with 0.1 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank ti-

tration, and make any necessary correction. Each mL of 0.1 M *Eдетate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Transfer about 700 mg of Aluminum Chlorohydrate, accurately weighed, to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with 0.1 N silver nitrate VS using a glass silver-silver chloride electrode and a silver billet electrode system, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.10:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrate by the formula:

$$\text{Al} \{ 26.98x + [17.01(3x - 1)] + 35.453 \} / 26.98x$$

in which *Al* is the percentage of aluminum as obtained in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Chlorohydrate Solution

» Aluminum Chlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride ratios between 1.91:1 and 2.10:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum chlorohydrate contained therein.

**Identification**—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum chlorohydrate per mL responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D: Identification of alcohol** (where stated on the label)—In a small beaker mix 5 drops of Solution with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid. Immediately cover the beaker with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferrocyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color fading after a few minutes.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 ppm.

**Heavy metals, Method I** (231)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 0.001%.

#### Limit of iron—

**Standard preparation**—Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* (241), to a 50-mL beaker.

**Test preparation**—Transfer 5.3 g of the Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

**Procedure**—To each of the beakers containing the *Standard preparation* and the *Test preparation* add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3 to 5 minutes. Allow to cool, add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed under *Iron* (241), transfer to separate 50-mL color comparison tubes, dilute with water to volume, and mix: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (75 µg per g).

#### Content of aluminum—

**Edetate disodium titrant**—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*, except to use 37.2 g of edetate disodium, instead of 18.6 g.

**Test solution**—Transfer about 400 mg of the Solution, accurately weighed, to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, and boil on a hot plate for not less than 5 minutes, and allow to cool.

**Procedure**—To the *Test solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid-ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate with 0.1 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Calculate the percentage of aluminum (Al) found, and use this value to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Transfer about 1.4 g of the Solution, accurately weighed, to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with 0.1 N silver nitrate VS using a silver-silver chloride glass electrode and a silver billet electrode system, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Calculate the percentage of chloride (Cl) found, and use this value to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride* and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.10:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Solution by the formula:

$$Al\{[26.98x + (17.01)(3x-1) + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide ion (OH), and 35.453 is the atomic weight of chloride (Cl).

## Aluminum Chlorohydrate Polyethylene Glycol

$Al_x(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$   
Aluminum chlorohydroxide polyethylene glycol complex.  
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Chlorohydrate Polyethylene Glycol consists of aluminum chlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum chlorohydrate.

#### Identification—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Infrared Absorption* (197F)—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen:** a similar preparation of polyethylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231): 20 µg per g.

**Limit of iron**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453

and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.10:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrate Polyethylene Glycol by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Chlorohydrate Propylene Glycol

$Al_x(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$

$Al_2(H_2O)_{y-z}(OH)_{6-n}(Cl)_n(C_3H_8O_2)_z$

Aluminum chlorohydroxide, hydrate: propylene glycol complex (1:1).

Aluminum hydroxychloride, hydrate: propylene glycol complex (1:1) [53026-85-0].

» Aluminum Chlorohydrate Propylene Glycol is a complex of aluminum chlorohydrate and propylene glycol in which some of the waters of hydration of the aluminum chlorohydrate have been replaced by propylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum chlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Infrared Absorption* (197F)—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen:** a similar preparation of propylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231): 20 µg per g.

**Limit of iron**—Using Aluminum Chlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

### Content of aluminum—

**EDETATE DISODIUM TITRANT**—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*, except to use 37.2 g of edetate disodium instead of 18.6 g.

**Test solution**—Transfer about 1.6 g of Aluminum Chlorohydrate Propylene Glycol, accurately weighed, to a 100-mL beaker, add 15 to 20 mL of water and 5 to 6 mL of hydrochloric acid, and boil on a hot plate for 15 to 20 minutes. Cool the solution, and with the aid of water transfer to

a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—Transfer 5.0 mL of the *Test solution* to a 250-mL beaker, add 10 to 15 mL of water, and adjust with 1 N sodium hydroxide to a pH of  $1.5 \pm 0.5$ . Add 10.0 mL of *EDETATE DISODIUM TITRANT*, and heat to boiling. Cool the solution and carefully introduce a magnetic stirring bar into the beaker. Add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, 40 to 50 mL of alcohol, and while stirring adjust with glacial acetic acid to a pH of  $4.6 \pm 0.1$ . Add 1 to 2 mL of dithizone TS and 40 to 50 mL of alcohol, and titrate with 0.1 M zinc sulfate VS until the color changes from a green-violet to a rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *EDETATE DISODIUM TITRANT* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Chlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the chloride content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the *Assay* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.1:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrate Propylene Glycol by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide ion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate

$Al_x(OH)_{3y-z}Cl_z \cdot nH_2O$

Aluminum chlorohydroxide.

Aluminum hydroxychloride.

» Aluminum Dichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum dichlorohydrate.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231): 20 µg per g.

**Limit of iron**—Using Aluminum Dichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride* and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrate by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate Solution

» Aluminum Dichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum dichlorohydrate contained therein.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum dichlorohydrate per mL responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Infrared Absorption* (197F) (where propylene glycol is indicated on the label)—

*Test specimen*—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath. Deposit this solution on a silver chloride disk.

*Standard specimen:* a similar preparation of propylene glycol.

**C:** *Infrared Absorption* (197F) (where dipropylene glycol is indicated on the label)—

*Test specimen*—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath. Deposit this solution on a silver chloride disk.

*Standard specimen:* a similar preparation of dipropylene glycol.

**D:** *Identification of alcohol* (where stated on the label)—Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferrocyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 10 µg per g.

**Limit of iron**—Using Aluminum Dichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of aluminum* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of chloride* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum respectively: the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Solution by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate Polyethylene Glycol

$Al_x(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$   
Aluminum chlorohydroxide polyethylene glycol complex.  
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Dichlorohydrate Polyethylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum dichlorohydrate.

**Identification**—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Infrared Absorption* (197F)—

*Test specimen*—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

*Standard specimen:* a similar preparation of polyethylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic**, *Method I* (211): 2 µg per g.

**Heavy metals**, *Method I* (231): 20 µg per g.

**Limit of iron**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrate Propylene Glycol by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate Propylene Glycol

$Al_3(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$

Aluminum chlorohydroxide propylene glycol complex.

Aluminum hydroxychloride propylene glycol complex.

» Aluminum Dichlorohydrate Propylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum dichlorohydrate.

**Identification**—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic**, *Method I* (211): 2 µg per g.

**Heavy metals**, *Method I* (231): 20 µg per g.

**Limit of iron**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrate Propylene Glycol by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Hydroxide Gel

$Al(OH)_3$  78.00

Aluminum hydroxide.

Aluminum hydroxide [21645-51-2].

» Aluminum Hydroxide Gel is a suspension of amorphous aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide  $[Al(OH)_3]$ . It may contain Peppermint Oil, Glycerin, Sorbitol, Sucrose, Saccharin, or other suitable flavors, and it may contain suitable antimicrobial agents.

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**Identification**—

**A:** Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

**Acid-neutralizing capacity (301)**—Not less than 65.0% of the expected mEq value, calculated from the results of the *Assay*, is obtained. Each mg of  $\text{Al}(\text{OH})_3$  has an expected acid-neutralizing capacity value of 0.0385 mEq.

**pH (791):** between 5.5 and 8.0, determined potentiometrically.

**Chloride**—Transfer an accurately measured quantity of the Gel, equivalent to 0.6 g of  $\text{Al}(\text{OH})_3$ , to a porcelain dish. Add 0.1 mL of potassium chromate TS and 25 mL of water. Stir, and add 0.10 N silver nitrate until a faint, persistent pink color is obtained: not more than 8.0 mL of 0.10 N silver nitrate is required [4.7%, based on the  $\text{Al}(\text{OH})_3$  content].

**Sulfate (221)**—Add 5.0 mL of 3 N hydrochloric acid to an accurately measured quantity of the Gel, equivalent to 0.3 g of  $\text{Al}(\text{OH})_3$ , and heat to dissolve the specimen under test. Cool, dilute with water to 250 mL, and filter if necessary: a 20-mL portion of the filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid [0.8%, based on the  $\text{Al}(\text{OH})_3$  content].

**Arsenic, Method I (211)**—Prepare a *Standard Preparation* as directed in the test for *Arsenic* (211), except to prepare it to contain 5  $\mu\text{g}$  of arsenic instead of 3  $\mu\text{g}$ . Prepare the *Test Preparation* as follows. Dissolve an accurately measured quantity of the Gel, equivalent to 0.5 g of  $\text{Al}(\text{OH})_3$ , in 20 mL of 7 N sulfuric acid. The limit is 0.001%, based on the  $\text{Al}(\text{OH})_3$  content.

**Heavy metals (231)**—Dissolve an accurately measured quantity of the Gel, equivalent to 0.24 g of  $\text{Al}(\text{OH})_3$ , in 10 mL of 3 N hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 mL: the limit is 0.0083%, based on the  $\text{Al}(\text{OH})_3$  content.

**Assay**—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Procedure**—Transfer an accurately measured quantity of Gel, equivalent to about 1.5 g of  $\text{Al}(\text{OH})_3$ , to a beaker, add 15 mL of hydrochloric acid, and heat gently until solution is complete. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the sample, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

## Dried Aluminum Hydroxide Gel

$\text{Al}(\text{OH})_3$  78.00

Aluminum hydroxide [21645-51-2].

» Dried Aluminum Hydroxide Gel is an amorphous form of aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 76.5 percent of  $\text{Al}(\text{OH})_3$ , and it may contain varying quantities of basic aluminum carbonate and bicarbonate.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where the quantity of dried aluminum hydroxide gel equivalent is stated in the labeling of any preparation, this shall be understood to be on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

**USP Reference standards (11)**—

USP Dried Aluminum Hydroxide Gel RS

**Identification**—

**A: Infrared Absorption (197K).**

**B:** Dissolve 500 mg in 10 mL of 3 N hydrochloric acid, with gentle warming: the solution responds to the tests for *Aluminum* (191).

**Acid-neutralizing capacity (301):** not less than 25.0 mEq per g, 400 mg being tested as directed for *Powders* under *Test Preparation*.

**pH (791):** not higher than 10.0, in an aqueous dispersion (1 in 25).

**Chloride (221)**—Dissolve 1.0 g in 30 mL of 2 N nitric acid, heat to boiling, add water to make 100 mL, and filter: a 5.0-mL portion of the filtrate, diluted with an equal volume of water, shows no more chloride than corresponds to 0.60 mL of 0.020 N hydrochloric acid (0.85%).

**Sulfate (221)**—Dissolve 330 mg in 15 mL of 3 N hydrochloric acid, heat to boiling, add water to make 250 mL, and filter: a 25-mL portion of the filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.6%).

**Arsenic, Method I (211)**—Dissolve 1.5 g in 80 mL of 7 N sulfuric acid, and dilute with water to 220 mL: 55 mL of the resulting solution meets the requirements of the test, the addition of 20 mL of 7 N sulfuric acid specified under *Procedure* being omitted. The limit is 8 ppm.

**Heavy metals (231)**—Dissolve 330 mg in 10 mL of 3 N hydrochloric acid with the aid of heat, filter if necessary, and dilute with water to 25 mL: the limit is 0.006%.

**Assay**—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Procedure**—Weigh accurately about 2 g of Gel, and dissolve in 15 mL of hydrochloric acid, with the aid of heat. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting 20 mL of water for the sample solution, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

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## Dried Aluminum Hydroxide Gel Capsules

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» Dried Aluminum Hydroxide Gel Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The Capsules may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

### Identification—

**A:** Place a portion of Capsule contents, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 10 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of  $\text{Al}(\text{OH})_3$ , is obtained. Each mg of  $\text{Al}(\text{OH})_3$  has an expected acid-neutralizing capacity value of 0.0385 mEq.

### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Weigh accurately the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, to a beaker, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the Assay under *Dried Aluminum Hydroxide Gel*, beginning with “dilute with water to volume.” Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

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## Dried Aluminum Hydroxide Gel Tablets

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» Dried Aluminum Hydroxide Gel Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

### Identification—

**A:** Place a quantity of finely ground Tablets, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped

with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation*.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of  $\text{Al}(\text{OH})_3$ , is obtained. Each mg of  $\text{Al}(\text{OH})_3$  has an expected acid-neutralizing capacity value of 0.0385 mEq.

### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the Assay under *Dried Aluminum Hydroxide Gel*, beginning with “dilute with water to volume.” Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

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## Aluminum Phosphate Gel

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Phosphoric acid, aluminum salt (1:1).  
Aluminum phosphate (1:1) [7784-30-7].

» Aluminum Phosphate Gel is a water suspension containing not less than 4.0 percent and not more than 5.0 percent (w/w) of aluminum phosphate ( $\text{AlPO}_4$ ). It may contain sodium benzoate, benzoic acid, or other suitable agent, in an amount not exceeding 0.5 percent, as a preservative.

**Packaging and storage**—Preserve in tight containers.

### Identification—

**A:** A solution of it in hydrochloric acid meets the requirements of the tests for *Aluminum* (191).

**B:** A solution of it in 2 N nitric acid meets the requirements of the tests for *Phosphate* (191).

**pH** (791): between 6.0 and 7.2.

**Soluble phosphate**—Filter 20 g, and wash the residue with 30 mL of water. Add to the filtrate 2 mL of nitric acid, heat to 60°, and add 20 mL of ammonium molybdate TS. Heat at 50° for 30 minutes, filter, wash the precipitate with dilute nitric acid (1 in 36), then wash with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 2.065 mg of  $\text{PO}_4$ . The soluble phosphate, calculated as  $\text{PO}_4$ , does not exceed 0.30%.

**Sulfate** (221)—Add 10 mL of 3 N hydrochloric acid to 10 g of Gel, and heat to boiling. Cool, dilute with water to 250 mL, and filter, if necessary. A 10-mL portion of the solution shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid: not more than 0.05% is found.



**Arsenic, Method I** (211)—Prepare the *Test Preparation* by dissolving 5.0 g of Gel in the smallest necessary volume of 3 N hydrochloric acid. The limit is 0.6 ppm.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to make the following modifications.

*Standard Preparation*—Into a 50-mL color-comparison tube pipet 4.0 mL of *Standard Lead Solution*, dilute with water to 25 mL, adjust with 6 N ammonium hydroxide to a pH between 1.9 and 2.1, dilute with water to 40 mL, and mix.

*Test Preparation*—Dissolve 8.0 g in 5 mL of 3 N hydrochloric acid, warming if necessary, dilute with water to 25 mL, and adjust with 6 N ammonium hydroxide to a pH between 1.9 and 2.1. Transfer to a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

*Monitor Preparation*—Into a 50-mL color-comparison tube place 25 mL of the *Test Preparation*, add 4.0 mL of *Standard Lead Solution*, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 1.9 and 2.1, dilute with water to 40 mL, and mix.

*Procedure*—Proceed as directed in the chapter, except to omit the addition of 2 mL of pH 3.5 Acetate Buffer. Not more than 5 µg per g is found.

**Chloride**—Transfer 25 g to a beaker with the aid of about 50 mL of water, add 5 mL of nitric acid, mix, then add, with stirring, 30.0 mL of 0.1 N silver nitrate VS. Warm on a steam bath for 30 minutes, filter, and wash the precipitate with water acidified with nitric acid. To the filtrate add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not more than 0.16% of chloride is found.

**Assay**—To about 20 g of Gel, accurately weighed, in a 100-mL volumetric flask, add nitric acid to effect solution, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 400-mL beaker, dilute with water to 100 mL, heat to 60°, add an excess of ammonium molybdate TS, and maintain at 50° for 30 minutes. Filter, and wash the precipitate with dilute nitric acid (1 in 36), then with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 2.651 mg of  $\text{AlPO}_4$ .

## Aluminum Sesquichlorohydrate

$\text{Al}_2(\text{OH})_3\text{Cl}_2 \cdot n\text{H}_2\text{O}$

Aluminum chlorohydroxide.

Aluminum hydroxychloride [11097-68-0].

» Aluminum Sesquichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231): 20 µg per g.

**Limit of iron**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Sesquichlorohydrate Solution

» Aluminum Sesquichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum sesquichlorohydrate contained therein.

**Identification**—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum sesquichlorohydrate per mL responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the

same wavelengths as that of a similar preparation of a film of propylene glycol.

**C: Identification of dipropylene glycol** (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D: Identification of alcohol** (where stated on the label)—Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferrocyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

**pH** {791}: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** {211}—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** {231}—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 10 µg per g.

**Limit of iron**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of aluminum* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of chloride* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Solution by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Sesquichlorohydrate Polyethylene Glycol

$Al_2(OH)_3 \cdot xCl_2 \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$   
Aluminum chlorohydroxide polyethylene glycol complex.  
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Sesquichlorohydrate Polyethylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic

ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* {191} and for *Chloride* {191}.

**B: Infrared Absorption** {197F}—

*Test specimen*—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

*Standard specimen:* a similar preparation of polyethylene glycol.

**pH** {791}: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** {211}: 2 µg per g.

**Heavy metals, Method I** {231}: 20 µg per g.

**Limit of iron**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate Polyethylene Glycol by the formula:

$$Al\{26.98x + [17.01(3x - 1)] + 35.453\} / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Sesquichlorohydrate Propylene Glycol

$Al_2(OH)_3 \cdot xCl_2 \cdot nH_2O \cdot mC_3H_8O_2$   
Aluminum chlorohydroxide propylene glycol complex.  
Aluminum hydroxychloride propylene glycol complex.

» Aluminum Sesquichlorohydrate Propylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have

been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

**Identification**—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B: Infrared Absorption** (197F)—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen:** a similar preparation of propylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211):  $2 \mu\text{g}$  per g.

**Heavy metals, Method I** (231):  $20 \mu\text{g}$  per g.

**Limit of iron**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is  $150 \mu\text{g}$  per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate Propylene Glycol by the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Subacetate Topical Solution

» Aluminum Subacetate Topical Solution yields, from each 100 mL, not less than 2.30 g and not more than 2.60 g of aluminum oxide ( $\text{Al}_2\text{O}_3$ ), and not less than 5.43 g and not more than

6.13 g of acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ). It may be stabilized by the addition of not more than 0.9 percent of boric acid.

Aluminum Subacetate Topical Solution may be prepared as follows.

Aluminum Sulfate . . . . .	145 g
Acetic Acid . . . . .	160 mL
Calcium Carbonate . . . . .	70 g
Purified Water, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the Aluminum Sulfate in 600 mL of cold water, filter the solution, and add the Calcium Carbonate gradually, in several portions, with constant stirring. Then slowly add the Acetic Acid, mix, and set the mixture aside for 24 hours. Filter the product with the aid of vacuum if necessary, returning the first portion of the filtrate to the funnel. Wash the magma on the filter with small portions of cold water, until the total filtrate measures 1000 mL.

**Packaging and storage**—Preserve in tight containers.

**Identification**—It responds to the tests for *Aluminum* (191) and for the ferric chloride test for *Acetate* (191) with a deep red color upon the addition of ferric chloride TS. This color is destroyed by the addition of a mineral acid.

**pH** (791): between 3.8 and 4.6.

**Limit of boric acid**—Proceed as directed in the test for *Limit of boric acid* under *Aluminum Acetate Topical Solution*.

**Assay for aluminum oxide**—

**Edetate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Pipet 20 mL of Topical Solution into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, dilute with water to volume, and mix. Pipet 25 mL of this dilution into a 250-mL beaker, and proceed as directed for *Procedure in the Assay for aluminum oxide* under *Aluminum Acetate Topical Solution*, beginning with “add, in the order named.” Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 2.549 mg of  $\text{Al}_2\text{O}_3$ .

**Assay for acetic acid**—Proceed as directed in the Assay for *acetic acid* under *Aluminum Acetate Topical Solution*.

## Aluminum Sulfate

$\text{Al}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$ (anhydrous)	342.15
Sulfuric acid, aluminum salt (3:2), hydrate.	
Aluminum sulfate (2:3) hydrate	[17927-65-0].
Anhydrous	342.16 [10043-01-3].

» Aluminum Sulfate contains not less than 54.0 percent and not more than 59.0 percent of  $\text{Al}_2(\text{SO}_4)_3$ . It contains a varying amount of water of crystallization.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* and for *Sulfate* (191).

**pH** <791>: not less than 2.9, in a solution (1 in 20).

**Water**, Method I <921>: not less than 41.0% and not more than 46.0%.

**Heavy metals** <231>—Dissolve 1.0 g in 2 mL of 1 N acetic acid, and dilute with water to 25 mL. The limit is 20 µg per g.

**Limit of alkalies and alkaline earths**—To a boiling solution of 1.0 g in 150 mL of water add a few drops of methyl red TS and then add 6 N ammonium hydroxide just until the color of the solution changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to constant weight: not more than 2 mg of residue remains (0.4%).

**Limit of ammonium salts**—Heat 1 g with 10 mL of 1 N sodium hydroxide on a steam bath for 1 minute: the odor of ammonia is not perceptible.

**Iron**—To 20 mL of a solution (1 in 150) add 0.3 mL of potassium ferrocyanide TS: no blue color is produced immediately.

#### Assay—

*Eдетate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Transfer about 7.5 g of Aluminum Sulfate, accurately weighed, to a 250-mL volumetric flask, and dissolve in water. Dilute with water to volume, mix, and pipet 10 mL of the solution into a 250-mL beaker. Proceed as directed in the Assay for aluminum oxide under *Aluminum Acetate Topical Solution*, beginning with “add, in the order named.” Each mL of 0.05 M *Eдетate disodium titrant* is equivalent to 8.554 mg of  $\text{Al}_2(\text{SO}_4)_3$ .

### Aluminum Sulfate and Calcium Acetate for Topical Solution

» Aluminum Sulfate and Calcium Acetate for Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$  and calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$ .

**Packaging and storage**—Preserve in single-unit containers, and protect from excessive heat.

#### Identification—

**A:** Place approximately 0.25 g of Aluminum Sulfate and Calcium Acetate for Topical Solution in a test tube. Add 10 mL of water and 0.25 g of calcium carbonate. Heat on a steam bath for 10 minutes, and filter. Add 3 to 4 drops of ferric chloride TS to the filtrate. A reddish-brown color or precipitate indicates acetate. [NOTE—After the addition of the ferric chloride TS, the solution may be heated for 1 minute to speed the reaction.]

**B:** Suspend 2 g of sample in 50 mL of water, and filter. The filtrate meets the requirements of the tests for *Sulfate* <191> and for *Calcium* <191>.

**pH** <791>: between 4.0 and 4.8 in a solution (1 in 200).

#### Assay for aluminum sulfate—

*Assay preparation*—Transfer 10 g of Aluminum Sulfate and Calcium Acetate for Topical Solution, accurately weighed, to a 1000-mL volumetric flask. Add 100 mL of 1.2 M hydrochloric acid and approximately 250 mL of water. Heat on a steam bath or hot plate until dissolved. Cool, dilute with water to volume, and mix. [NOTE—Retain a portion of this Assay preparation for the Assay for calcium acetate.]

*Procedure*—Transfer a 5.0-mL aliquot of the Assay preparation to a 250-mL conical flask. Add 40.0 mL of 0.01 M

edetate disodium VS and 20 mL of acetic acid–ammonium acetate buffer TS, and mix well. Add 50 mL of alcohol and 2 mL of dithizone TS. [NOTE—Follow the given order of addition.] Titrate with 0.02 M zinc sulfate VS until the color changes from green-violet to clear rose-pink. Perform a blank titration, substituting 5.0 mL of water for the Assay preparation. Each mL of 0.01 M edetate disodium is equivalent to 2.972 mg of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ . Calculate the percentage of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$  by the formula:

$$[(1000)(100)C_F M(V_B - V_U)] / 5.0 M_T W$$

in which 1000/5.0 is the dilution factor; 100 is the conversion factor to percentage;  $C_F$  is the conversion factor (2.972 mg of sample per mL of 0.01 M edetate disodium);  $M$  is the actual molarity of the titrant;  $V_B$  is the blank titration volume, in mL;  $V_U$  is the sample titration volume, in mL;  $M_T$  is the theoretical molarity of the titrant (0.02); and  $W$  is the weight of the sample, in mg.

#### Assay for calcium acetate—

*Procedure*—Transfer a 5.0-mL aliquot of the Assay preparation retained from the Assay for aluminum sulfate to a 250-mL conical flask. Add 1 to 2 mL of 50% triethanolamine to mask the aluminum. Mix well. Add 100 mL of water, 15 mL of 1 N sodium hydroxide, and approximately 300 mg of hydroxy naphthol blue. [NOTE—Follow the given order of addition.] Titrate the solution with 0.01 M edetate disodium VS. The indicator will change from purple to a clear blue color at the endpoint. Each mL of 0.01 M edetate disodium is equivalent to 1.762 mg of calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$ . Calculate the percentage of calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$  by the formula:

$$[(1000)(100)V_U C_F M] / 5.0 M_T W$$

in which 1000/5.0 is the dilution factor; 100 is the conversion factor to percentage;  $V_U$  is the sample titration volume, in mL;  $C_F$  is the conversion factor (1.762 mg of sample per mL of 0.01 M edetate disodium);  $M$  is the actual molarity of the titrant;  $M_T$  is the theoretical molarity of the titrant (0.01); and  $W$  is the weight of the sample, in mg.

### Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution

» Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$  and calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$ .

**Packaging and storage**—Preserve in tight containers, and avoid excessive heat.

#### Identification—

**A:** Suspend 2 g of ground Tablet powder in 50 mL of water, and filter. Mix 2 mL of the filtrate with 2 mL of water and 2 drops of 3 N hydrochloric acid: the solution responds to the ammonium hydroxide test for *Aluminum* <191>. [NOTE—Retain the remaining filtrate for Identification test B.]

**B:** A portion of the filtrate retained from Identification test A responds to the tests for *Sulfate* <191> and for *Calcium* <191>.

**Disintegration** <701>: 10 minutes.

**Uniformity of dosage units** <905>: meet the requirements for *Weight Variation*.

**pH** (791): between 4.0 and 4.8, in a solution (2 g of ground Tablet powder in 500 mL of water).

**Loss on drying** (731)—Dry ground Tablet powder at 150° for 15 minutes: it loses not more than 18% of its weight.

**Assay for aluminum sulfate—**

**Assay preparation**—Finely powder and mix not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 2.8 g of aluminum sulfate, and transfer to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 100 mL of water, and heat on a steam bath, with occasional swirling, to dissolve the powder. Allow the solution to cool, dilute with water to volume, and mix. [NOTE—Retain a portion of this Assay preparation for the Assay for calcium acetate.]

**Procedure**—Transfer 25.0 mL of the Assay preparation to a 250-mL conical flask. Add 40.0 mL of 0.01 M edetate disodium VS and 20 mL of acetic acid–ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate with 0.02 M zinc sulfate VS until the color changes from green-violet to a clear rose-pink. Perform a blank determination, substituting 25 mL of water for the Assay preparation, and make any necessary correction. Each mL of 0.01 M edetate disodium is equivalent to 2.972 mg of  $\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$ .

**Assay for calcium acetate**—Transfer 20.0 mL of the Assay preparation retained from the Assay for aluminum sulfate to a 125-mL conical flask. With constant stirring, add in the order named, about 0.5 mL of triethylamine, 10 mL of ammonia–ammonium chloride buffer TS, and 3 drops of a solution prepared by dissolving 500 mg of eriochrome black T in 10 mL of methanol, and titrate with 0.01 M edetate disodium VS to a violet endpoint. Each mL of 0.01 M edetate disodium is equivalent to 1.762 mg of  $\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O}$ .

## Aluminum Zirconium Octachlorohydrate

$\text{Al}_x\text{Zr}(\text{OH})_{3y+4-x}\text{Cl}_x \cdot n\text{H}_2\text{O}$

» Aluminum Zirconium Octachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium octachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for Chloride (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hy-

drochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for Test Preparation if necessary.

**Procedure**—To each of the three tubes containing the Standard Preparation, the Test Preparation, and the Monitor Preparation, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the Standard Preparation add 6 drops of sodium sulfide TS. To the Test Preparation and the Monitor Preparation add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation, and the color of the solution from the Monitor Preparation is the same as or darker than the color of the solution from the Standard Preparation. If the color of the solution from the Monitor Preparation is lighter than the color of the solution from the Standard Preparation, repeat the procedure with the following modification: after the heating step, to the Monitor Preparation and the Test Preparation add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron—**

**Standard preparation**—Transfer 2.0 mL of Standard Iron Solution, prepared as directed under Iron (241), to a 50-mL beaker.

**Test preparation**—Transfer 2.7 g of Aluminum Zirconium Octachlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

**Procedure**—To each of the beakers containing the Standard preparation and the Test preparation add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3 to 5 minutes. Allow to cool, add 5 mL of Ammonium Thiocyanate Solution, prepared as directed under Iron (241), transfer to separate 50-mL color comparison tubes, dilute with water to volume, and mix: the color of the solution from the Test preparation is not darker than that of the solution from the Standard preparation (150 µg per g).

**Content of aluminum**—Transfer about 0.15 g of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 5 minutes. Add 40 mL of water and 15.0 mL of 0.1 M edetate disodium VS. Heat the solution to boiling, and continue boiling for 5 minutes. Allow the solution to cool, add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, and adjust with ammonium hydroxide to a pH of  $4.5 \pm 0.1$ . Add 20 mL of alcohol, and adjust with ammonium hydroxide to a pH of  $4.6 \pm 0.1$ . Add 5 to 10 drops of dithizone TS, and titrate with 0.1 M zinc sulfate VS until the first permanent purple-pink color appears. Perform a blank determination, and make any necessary correction. Calculate the percentage of aluminum (Al) in the Aluminum Zirconium Octachlorohydrate by the formula:

$$2.698[15.0 M_e - (z M_z + Z_e)] / W$$

in which  $M_e$  is the molarity of the edetate disodium VS;  $z$  is the volume, in mL, of zinc sulfate VS consumed;  $M_z$  is the molarity of the zinc sulfate VS;  $W$  is the quantity, in g, of Aluminum Zirconium Octachlorohydrate taken;  $Z_e$  is the equivalent volume, in mL, of edetate disodium VS consumed by the zirconium moiety, calculated as follows:

$$(Zr/M_e)(W/92.97)$$

in which  $Zr$  is the percentage of zirconium as determined in the test for Content of zirconium, 92.97 is the atomic weight

of zirconium corrected for 2% hafnium content, and the other terms are as defined above. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 6 to 8 minutes. Add 30 to 40 mL of water and 5 mL of hydrochloric acid, and heat to boiling. Add 1 drop of xylenol orange TS, and, while still hot, titrate with 0.1 M edetate disodium VS until the color of the solution changes from pink to yellow. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M edetate disodium is equivalent to 9.297 mg of zirconium (Zr). Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 250-mL beaker, add 100 to 120 mL of water and 20 mL of diluted nitric acid, and swirl to dissolve. Titrate with 0.05 N silver nitrate VS using a calomel electrode and a silver billet electrode system, determining the endpoint potentiometrically. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of chloride (Cl). Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)] / (Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrate by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1) / z\} / 26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Solution

» Aluminum Zirconium Octachlorohydrate Solution consists of complex basic aluminum chloride

that is polymeric and encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211): Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Prep-*

aration and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Octachlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Gly

» Aluminum Zirconium Octachlorohydrate Gly is a derivative of Aluminum Zirconium Octachlorohydrate in which some of the water molecules have

been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium*

atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrate Gly by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Gly Solution

» Aluminum Zirconium Octachlorohydrate Gly Solution is a solution of Aluminum Zirconium Octachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of

not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.



**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Octachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate

$Al_3Zr(OH)_{3y+4-x}Cl_x \cdot nH_2O$

» Aluminum Zirconium Pentachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a

range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium pentachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is

the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride, as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrate by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Solution

» Aluminum Zirconium Pentachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solu-

tion, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Pentachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/chloride atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum*

by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al\{[26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Gly

» Aluminum Zirconium Pentachlorohydrate Gly is a derivative of Aluminum Zirconium Pentachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio—**Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay—**Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrate Gly by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Gly Solution

» Aluminum Zirconium Pentachlorohydrate Gly Solution is a solution of Aluminum Zirconium Pentachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

*Test Preparation*—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

*Monitor Preparation*—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

*Procedure*—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron—**Using about 5.4 g of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum—**Using about 0.3 g of Aluminum Zirconium Pentachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium—**Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio—**Divide the percentage of aluminum found in the test for *Content of aluminum*

by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

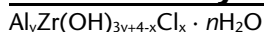
in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al\{[26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate



» Aluminum Zirconium Tetrachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium tetrachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chlo-*

ride, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrate by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Solution

» Aluminum Zirconium Tetrachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respec-

tively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Gly

» Aluminum Zirconium Tetrachlorohydrate Gly is a derivative of Aluminum Zirconium Tetrachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor*

*Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrate Gly by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium



atomic ratio found in the test for *Aluminum/zirconium atomic ratio*,  $z$  is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Gly Solution

» Aluminum Zirconium Tetrachlorohydrate Gly Solution is a solution of Aluminum Zirconium Tetrachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room tempera-

ture. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium



content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

### Aluminum Zirconium Trichlorohydrate

$Al_xZr(OH)_{3y+4-x}Cl_x \cdot nH_2O$

» Aluminum Zirconium Trichlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium trichlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or

darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrate by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate Solution

» Aluminum Zirconium Trichlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solu-

tion from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Trichlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio as determined in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride ratio determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate Gly

» Aluminum Zirconium Trichlorohydrate Gly is a derivative of Aluminum Zirconium Trichlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium trichlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 *Acetate Buffer*, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found.

**Limit of iron**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum*

*Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrate Gly by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate Gly Solution

» Aluminum Zirconium Trichlorohydrate Gly Solution is a solution of Aluminum Zirconium Trichlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It

encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

**Identification**—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic**, *Method I* (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

**Heavy metals**, *Method I* (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or

darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found.

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Trichlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

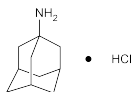
in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio, as determined in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio as determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Amantadine Hydrochloride



$C_{10}H_{17}N \cdot HCl$  187.71  
Tricyclo[3.3.1.1<sup>3,7</sup>]decan-1-amine, hydrochloride;  
1-Adamantanamine hydrochloride [665-66-7].

### DEFINITION

Amantadine Hydrochloride contains NLT 98.5% and NMT 101.5% of  $C_{10}H_{17}N \cdot HCl$ .

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197S)

Cell: 1 mm

**Sample solution:** 50 mg in 10 mL of 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a suitable separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample:** Dissolve 120 mg of Amantadine Hydrochloride in a mixture of 30 mL of glacial acetic acid and 10 mL of mercuric acetate TS.

**Analysis:** Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using suitable electrodes. Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 18.77 mg of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**Acceptance criteria:** 98.5%–101.5%

### IMPURITIES

#### • HEAVY METALS, Method I (231)

**Test preparation:** Use 1 mL of 1 N acetic acid.

**Acceptance criteria:** NMT 10 ppm

#### • ORGANIC IMPURITIES

**Internal standard solution:** 50 mg/mL of adamantane in dichloromethane

**Standard solution:** Transfer 10 mg of USP Amantadine Hydrochloride RS to a separator. Add 20 mL of 5.0 N sodium hydroxide and 18 mL of dichloromethane, and shake for 10 min. Remove the water layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure that all remaining water has been removed. Filter, collect the filtrate in a 20-mL volumetric flask, add 0.2 mL of *Internal standard solution*, and dilute with dichloromethane to volume.

**Sample solution:** Transfer 1.0 g of Amantadine Hydrochloride to a separator. Add 20 mL of 5.0 N sodium hydroxide and 18 mL of dichloromethane, and shake for 10 min. Remove the water layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure that all remaining water has been removed. Filter, collect the filtrate in a 20-mL volumetric flask, add 0.2 mL of *Internal standard solution*, and dilute with dichloromethane to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Detector temperature:** 300°

**Column:** 0.53-mm  $\times$  30-m fused-silica column coated with 1.0- $\mu$ m G27 stationary phase

Column temperature: See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	0	70	5
70	10	250	At least 17

**Carrier gas:** Helium

**Flow rate:** 4 mL/min

**Injection size:** 2  $\mu$ L

**Injector temperature:** 220°

**Injection type:**

**Split flow:** 200 mL/min

**Split flow ratio:** 50:1

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for adamantane and amantadine are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 20 between adamantane and amantadine

**Relative standard deviation:** NMT 5.0% determined from the peak response ratios of amantadine to adamantane

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of each impurity to adamantane from the *Sample solution*

$R_S$  = peak response ratio of amantadine to adamantane from the *Standard solution*

$C_S$  = concentration of USP Amantadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 0.3%

**Total impurities:** NMT 1.0%

### SPECIFIC TESTS

#### • pH (791)

**Sample:** 0.2 g/mL in water

**Acceptance criteria:** 3.0–5.5

#### • CLARITY AND COLOR OF SOLUTION

**Sample:** Dissolve 2 g in 10 mL of water.

**Acceptance criteria:** Solution is clear and nearly colorless.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Amantadine Hydrochloride RS

## Amantadine Hydrochloride Capsules

### DEFINITION

Amantadine Hydrochloride Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**IDENTIFICATION**• **INFRARED ABSORPTION** <197S>

Cell: 1 mm

**Sample solution:** Place the contents of Capsules, equivalent to 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.

**ASSAY**• **PROCEDURE**

**Internal standard solution:** 0.4 mg/mL of naphthalene in hexane

**Standard stock solution:** 2 mg/mL of USP Amantadine Hydrochloride RS in water

**Standard solution:** Pipet 25.0 mL of *Standard stock solution* into a 250-mL separator, and add 25 mL of 2.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 min, and collect the hexane layer (*Standard solution*).

**Sample solution:** Transfer NLT 20 Capsules to a 200-mL volumetric flask. Add 40 mL of 0.1 N hydrochloric acid, and heat gently to achieve complete dissolution. Cool, and dilute with water to volume. Pipet 5.0 mL of the solution into a 250-mL separator, and add 40 mL of 1.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 min, and collect the hexane layer (*Sample solution*).

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 2-mm  $\times$  1.22-m; glass column packed with 10% phase G1 on 100- to 120-mesh support S1A**Temperature****Column:** 115°**Injector:** 250°**Detector block:** 250°**Injection size:** 1  $\mu$ L**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2 between naphthalene and amantadine**Tailing factor:** NMT 2.0 for the analyte peak**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $C_{10}H_{17}N \cdot HCl$  in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 $R_U$  = peak response ratios from the *Sample solution* $R_S$  = peak response ratios from the *Standard solution* $C_S$  = concentration of USP Amantadine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–105.0%**PERFORMANCE TESTS**• **DISSOLUTION** <711>**Test 1:** Procedure for a Pooled Sample**Medium:** Water; 900 mL**Apparatus 1:** 100 rpm**Time:** 45 min**Internal standard solution:** 0.054 mg/mL of naphthalene in hexane**Standard stock solution:** 0.1 mg/mL of USP

Amantadine Hydrochloride RS in water

**Standard solution:** Pipet 15.0 mL of *Standard stock solution* into a 50-mL screw-capped test tube, add 5.0 mL of 5 N sodium hydroxide and 10.0 mL of *Internal standard solution*, and shake for 60 min. Collect the hexane layer.

**Sample solution:** Filter 15.0 mL of the solution under test, and place into a 50-mL screw-capped test tube. Pipet 5.0 mL of 5 N sodium hydroxide and 10.0 mL of the *Internal standard solution* into the test tube, and shake for 60 min. Collect the hexane layer (*Sample solution*).

**Chromatographic system:** Proceed as directed in the *Assay*.

**Injection size:** 2.5  $\mu$ L**Analysis****Samples:** *Standard solution* and *Sample solution***Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{10}H_{17}N \cdot HCl$  is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.qia-llc.com or www.tabletdissolution.com or www.labhut.com.]

**Time:** 45 min**Internal standard solution:** 0.06 mg/mL of naphthalene in hexanes**Standard stock solution:** 0.12 mg/mL of USPAmantadine Hydrochloride RS in *Medium*

**Standard solution:** Transfer 60.0 mL of the *Standard stock solution* to a 200-mL volumetric flask. Add 20 mL of 5 N sodium hydroxide and 40.0 mL of *Internal standard solution*. Shake the flask for approximately 10 min, and allow the layers to separate. Use the top layer for injection. The final concentration is about 0.18 mg/mL.

**Sample solution:** Transfer 3.0 mL of the solution under test to a centrifuge tube. Add 1.0 mL of 5 N sodium hydroxide and 2.0 mL of *Internal standard solution*. Shake the tube for approximately 10 min, and allow the layers to separate. Use the top layer for injection.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 0.32-mm  $\times$  30-cm, 0.25- $\mu$ m film, phase G1**Temperature****Oven:** 100° for 3 min, to 200° at 10°/min, held at 200° for 2 min**Injector:** 250°**Detector:** 300°**Carrier gas:** Helium, 1.4 mL/min**Flow rate:** 20 mL/min**Injection size:** 2  $\mu$ L**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2 between naphthalene and amantadine hydrochloride**Tailing factor:** NMT 2.0 for amantadine hydrochloride**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amantadine hydrochloride released:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

 $R_U$  = ratio of the peak areas from the *Sample solution*

- $R_S$  = average ratio of the peak areas from the *Standard solution*  
 $C_S$  = concentration of amantadine hydrochloride in the *Standard stock solution* (mg/mL)  
 $L$  = label claim (mg/capsule)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of amantadine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Amantadine Hydrochloride RS

### Amantadine Hydrochloride Oral Solution

» Amantadine Hydrochloride Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Amantadine Hydrochloride RS

#### Identification, Infrared Absorption (197S)—

*Cell:* 1 mm.

*Solution*—Place a volume of Oral Solution, equivalent to about 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 10 mL of 0.5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.

#### Assay—

*Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Amantadine Hydrochloride Capsules*.

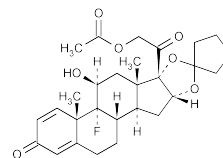
*Assay preparation*—Pipet 5.0 mL of the Oral Solution into a 250-mL conical flask, and add 45 mL of 1.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 minutes, and collect the hexane layer (*Assay preparation*).

*Procedure*—Proceed as directed in the Assay under *Amantadine Hydrochloride Capsules*. Calculate the quantity, in mg, of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amantadine Hydrochloride RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Amcinonide



$C_{28}H_{35}FO_7$  502.57

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-16,17-[cyclopentylidenebis(oxy)]-9-fluoro-11-hydroxy-, (11 $\beta$ , 16 $\alpha$ )-.

9-Fluoro-11 $\beta$ ,16 $\alpha$ ,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclopentanone, 21-acetate [51022-69-6].

» Amcinonide contains not less than 97.0 percent and not more than 102.0 percent of  $C_{28}H_{35}FO_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Amcinonide RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 40  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +89.4° and +94.0°.

*Test solution:* 10 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at 105° for 4 hours; it loses not more than 1.0% of its weight.

**Heavy metals, Method II** (231): 0.002%.

#### Assay—

*Solution A*—Prepare a filtered and degassed mixture of water and acetonitrile (13:7).

*Solution B*—Prepare a filtered and degassed mixture of acetonitrile and water (7:3).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments to either *Solution* as necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve suitable quantities of butylparaben and USP Amcinonide RS in *Solution B* to obtain separate solutions containing 12.5  $\mu$ g per mL and 20  $\mu$ g per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Amcinonide RS in *Solution B*, and dilute quantitatively, and stepwise if necessary, with *Solution B* to obtain a solution having a known concentration of about 0.02 mg per mL.

*Assay preparation*—Transfer about 20 mg of Amcinonide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Solution B* to volume, sonicate for 5 minutes, and mix. Transfer 5 mL of this solution to a 50-mL volumetric flask, dilute with *Solution B* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1, and is programmed to provide variable mixtures of *Solution A* and *Solution B*. The flow rate is about 2 mL per minute. Equilibrate the system with *Solution A*. From 2.5 minutes to 10 minutes after injection, linearly increase the amount of *Solution B* to 100%. Chromatograph the *System suitability*

solution, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.78 for butylparaben and 1.0 for amcinonide, and the resolution,  $R$ , between butylparaben and amcinonide is not less than 8.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation*. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{28}H_{35}FO_7$  in the portion of Amcinonide taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amcinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amcinonide Cream

» Amcinonide Cream is Amcinonide in a suitable cream base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of amcinonide ( $C_{28}H_{35}FO_7$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Amcinonide RS

**Identification**—Place 2 g of the Cream in a 150-mL beaker, add 50 mL of chloroform and 15 g of anhydrous sodium sulfate, and stir with a glass rod to dissolve the specimen. Filter the solution, and clarify the filtrate, if necessary, by the further addition of anhydrous sodium sulfate and a second filtration. Evaporate the filtrate to dryness, and dissolve the residue in chloroform to obtain a solution containing about 100  $\mu$ g of amcinonide per mL. Apply 25  $\mu$ L of this solution, and 25  $\mu$ L of a solution of USP Amcinonide RS in chloroform containing 100  $\mu$ g per mL, on a line parallel to and about 3 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a developing chamber containing and equilibrated with ether. Develop the chromatogram until the solvent front has moved about 12 cm above the line of application. Remove the plate, allow the solvent to evaporate, and locate the spots on the plate by viewing under short-wavelength UV light: the intensity and the  $R_f$  value of the principal spot obtained from the solution under test are similar to those of the spot obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 3.5 and 5.2.

**Assay**—

*Solution A, Solution B, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay under Amcinonide*.

*Assay preparation*—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of Amcinonide, to a 50-mL volumetric flask, add 5 mL of *Solution B* and 15 mL of acetonitrile, and heat over a steam bath until dissolved.

Add 20 mL of *Solution B* while hot, cool to room temperature, dilute with *Solution B* to volume, and refrigerate for 30 minutes. Vigorously shake the solution to disperse the mixture, and filter while cold. Transfer 5 mL of this solution to a 50-mL volumetric flask, dilute with *Solution B* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation*. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amcinonide ( $C_{28}H_{35}FO_7$ ) in the portion of Cream taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amcinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amcinonide Ointment

» Amcinonide Ointment is Amcinonide in a suitable ointment base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of amcinonide ( $C_{28}H_{35}FO_7$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Amcinonide RS

**Identification**—It meets the requirements for the *Identification test under Amcinonide Cream*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Solution A, Solution B, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay under Amcinonide*, except to use a 240-nm detector.

*Assay preparation*—Dissolve an accurately weighed quantity of Ointment in a suitable volume of a mixture of acetonitrile and chloroform (4:1) by heating in a hot water bath, cooling, and adjusting quantitatively with the same solvent mixture to obtain a solution having a concentration of about 0.2 mg of amcinonide per mL. Cool to room temperature, dilute with acetonitrile to volume, and filter. Transfer 5 mL of this solution to a 50-mL volumetric flask, dilute with *Solution B* to volume, and mix.

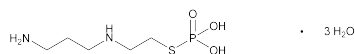
*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amcinonide ( $C_{28}H_{35}FO_7$ ) in the portion of Ointment taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amcinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Amifostine



$C_5H_{15}N_2O_3PS \cdot 3H_2O$  268.27  
 Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate;  
 S-[2-(3-Aminopropyl)amino]ethyl]dihydrogen phosphorothioate, trihydrate [112901-68-5].

### DEFINITION

Amifostine contains NLT 78.0% and NMT 82.0% of  $C_5H_{15}N_2O_3PS$ , calculated on the as-is basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (7:18)

**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]

**Sample solution:** 3 mg/mL of Amifostine in water.

[NOTE—Inject immediately after preparation.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Autosampler temperature:** 4°

**Flow rate:** 1.0 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 100 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_5H_{15}N_2O_3PS$  in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amifostine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 78.0%–82.0% on the as-is basis

### IMPURITIES

#### Inorganic Impurities

- HEAVY METALS**, *Method II* <231>: NMT 20 ppm

#### Organic Impurities

#### PROCEDURE

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 70 μg/mL of USP Amifostine Thiol RS and 16 μg/mL of USP Amifostine RS in water.

[NOTE—Inject immediately after preparation.]

**System suitability solution:** Use the *Standard solution* as described in the *Assay*. [NOTE—Inject immediately after preparation.]

**Sample solution:** 15 mg/mL of Amifostine in water.

[NOTE—Inject immediately after preparation.]

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 15.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amifostine thiol in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amifostine thiol from the *Sample solution*

$r_S$  = peak response of amifostine thiol from the *Standard solution*

$C_S$  = concentration of USP Amifostine Thiol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amifostine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amifostine thiol, 134.24

$M_{r2}$  = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of any other individual impurity in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity in the *Sample solution*

$r_S$  = peak response of amifostine in the *Standard solution*

$C_S$  = concentration of USP Amifostine RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of the *Sample solution* (μg/mL)

#### Acceptance criteria

**Amifostine thiol:** NMT 0.3%

**Any individual impurity, excluding amifostine thiol:** NMT 0.1%

**Total impurities including amifostine thiol:** NMT 0.3%

### SPECIFIC TESTS

- PH** <791>: 6.5–7.5, in a solution (5 in 100)

- WATER DETERMINATION**, *Method Ic* <921>

**Sample solution:** To 100.0 mg of Amifostine, contained in a stoppered centrifuge tube, add 10.0 mL of the solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.

**Acceptance criteria:** 19.2%–21.2%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.

- USP REFERENCE STANDARDS** <11>

USP Amifostine RS

USP Amifostine Thiol RS

Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.

$C_5H_{16}N_2SCL_2$  207.17

## Amifostine for Injection

### DEFINITION

Amifostine for Injection is a sterile, crystalline substance suitable for parenteral use. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amifostine ( $C_5H_{15}N_2O_3PS$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (7:18)

**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]

**Sample solution:** 3 mg/mL of amifostine from Amifostine for Injection, in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Autosampler temperature:** 4°

**Flow rate:** 1.0 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_5H_{15}N_2O_3PS$  in the portion of Amifostine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak responses from the *Sample solution*

$r_S$  = peak responses from the *Standard solution*

$C_S$  = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amifostine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE 1

**Mobile phase and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution 1:** 70 μg/mL of USP Amifostine Thiol RS in water

**Standard solution 2:** 15 μg/mL of sodium thiophosphate and 13 μg/mL of *N,N*-dimethylformamide in water. [NOTE—The retention times of sodium thiophosphate and *N,N*-dimethylformamide are about 2 min and about 3.6 min, respectively.]

**Sample solution:** 2.4 mg/mL of amifostine from Amifostine for Injection in water. [NOTE—Inject immediately after preparation.]

#### System suitability

**Samples:** *Standard solution 1* and *Standard solution 2*

#### Suitability requirements

**Relative standard deviation:** NMT 10.0%, *Standard solution 1*; NMT 4.0%, *Standard solution 2*

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of amifostine thiol in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amifostine thiol from the *Sample solution*

$r_S$  = peak response of amifostine thiol from *Standard solution 1*

$C_S$  = concentration of USP Amifostine Thiol RS in *Standard solution 1* (mg/mL)

$C_U$  = concentration of amifostine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amifostine thiol, 134.24

$M_{r2}$  = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of sodium thiophosphate or *N,N*-dimethylformamide in the portion of sample taken, if present:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of sodium thiophosphate or *N,N*-dimethylformamide from the *Sample solution*

$r_S$  = peak response of sodium thiophosphate or *N,N*-dimethylformamide from *Standard solution 2*

$C_S$  = concentration of sodium thiophosphate or *N,N*-dimethylformamide in *Standard solution 2* (mg/mL)

$C_U$  = concentration of amifostine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual, unspecified impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity in the *Sample solution*

$r_T$  = total of all peak responses in the *Sample solution*

**Acceptance criteria:** NMT 0.1% of sodium thiophosphate; NMT 0.088% of *N,N*-dimethylformamide; NMT 0.1% of any other individual unspecified impurity

##### • PROCEDURE 2

**Buffer:** 0.4 g/L of sodium 1-octanesulfonate. Adjust with trifluoroacetic acid to a pH of  $2.5 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:3)

**Standard solution:** 46 μg/mL of USP Amifostine Disulfide RS in water

**Sample solution:** Dilute a quantity of Amifostine for Injection in water to prepare a solution equivalent to 10 mg/mL. [NOTE—Inject immediately after preparation.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 247 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Autosampler temperature:** 4°

**Flow rate:** 1.0 mL/min

**Injection size:** 10 μL

**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.5**Relative standard deviation:** NMT 4.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amifostine disulfide in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of amifostine disulfide from the *Sample solution* $r_S$  = peak response of amifostine disulfide from the *Standard solution* $C_S$  = concentration of USP Amifostine Disulfide RS in the *Standard solution* (mg/mL) $C_U$  = concentration of amifostine in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of amifostine disulfide, 266.47 $M_{r2}$  = molecular weight of amifostine disulfide tetrahydrochloride, 412.31**Acceptance criteria:** NMT 2.0% of total impurities, including amifostine thiol and amifostine disulfide**SPECIFIC TESTS**

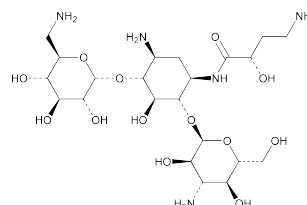
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* <1>, *Constituted Solutions*. When constituted with 0.9% *Sodium Chloride Injection*, the solution must completely dissolve in 45 s.
- **X-RAY DIFFRACTION** <941>: Its X-ray diffraction pattern conforms to that of USP Amifostine RS, similarly determined.
- **STERILITY TESTS** <71>: It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined*, *Membrane Filtration*.
- **pH** <791>: 6.5–7.5, in a solution constituted as directed in the labeling
- **WATER DETERMINATION, Method 1c** <921>  
**Sample solution:** To 100.0 mg of Amifostine for Injection, contained in a stoppered centrifuge tube, add 10.0 mL of a solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.

**Acceptance criteria:** 18.0%–22.0%

- **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** <85>: Contains NMT 0.2 USP Endotoxin Unit/mg of amifostine
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections* <1>, *Labeling*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight *Containers for Sterile Solids* as described under *Injections* <1>, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Amifostine RS  
USP Amifostine Disulfide RS  
1,3-Propanediamine, *N,N*-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride.  
 $C_{10}H_{30}N_4S_2Cl_4$  412.32  
USP Amifostine Thiol RS  
Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.  
 $C_5H_{16}N_2S_2Cl_2$  207.17  
USP Endotoxin RS

**Amikacin** $C_{22}H_{43}N_5O_{13}$  585.60D-Streptamine, O-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-O-[6-amino-6-deoxy-α-D-glucopyranosyl(1→4)]-N<sup>1</sup>-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-, O-3-Amino-3-deoxy-α-D-glucopyranosyl(1→4)-O-[6-amino-6-deoxy-α-D-glucopyranosyl(1→6)]-N<sup>3</sup>-(4-amino-L-2-hydroxybutyl)-2-deoxy-L-streptamine [37517-28-5].» Amikacin has a potency of not less than 900 μg of  $C_{22}H_{43}N_5O_{13}$  per mg, calculated on the anhydrous basis.**Packaging and storage**—Preserve in tight containers.**USP Reference standards** <11>—

USP Amikacin RS

USP Kanamycin Sulfate RS

**Identification**—**A: Thin-Layer Chromatographic Identification Test** <201>—**Test solution:** 6 mg per mL, in water. Apply 3 μL.**Standard solution:** 6 mg per mL, in water. Apply 3 μL.**Mixed solution:** a mixture of the *Test solution* and the *Standard solution* (1:1). Apply 3 μL.**Developing solvent system:** a mixture of methanol, ammonium hydroxide, and chloroform (60:35:25).**Spray reagent:** a 1 in 100 solution of ninhydrin in a mixture of butyl alcohol and pyridine (100:1).**Procedure**—Proceed as directed in the chapter, except to develop the chromatogram by continuous flow for 5.5 hours. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 minutes. Spray the plate with *Spray reagent*, and immediately locate the spots: amikacin appears as a pink spot, and the spots obtained from the *Test solution* and the *Mixed solution* correspond in distance from the origin to that obtained from the *Standard solution*.**B:** The retention time of the peak for amikacin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Specific rotation** <781S>: between +97° and +105°.**Test solution:** 20 mg per mL, in water.**Crystallinity** <695>: meets the requirements.**pH** <791>: between 9.5 and 11.5, in a solution containing 10 mg per mL.**Water, Method 1** <921>: not more than 8.5%.**Residue on ignition** <281>: not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.**Assay**—**Mobile phase**—Use 0.115 N sodium hydroxide. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).**System suitability solution**—Prepare a solution in water containing about 0.02 mg of USP Amikacin RS per mL and 0.008 mg of USP Kanamycin Sulfate RS per mL.**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Amikacin RS in water to ob-

tain a solution having a known concentration of about 0.02 mg per mL.

**Assay preparation**—Transfer about 50 mg of Amikacin, accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with an electrochemical detector, a gold working electrode, and a pH silver-silver chloride reference electrode, a guard column that contains packing L47, and a 4-mm × 25-cm analytical column that contains packing L47. The electrochemical detector is used in the integrated amperometric mode with a range of 300 nC, an output of 1 V full scale, a rise time of 0.5 second, positive polarity, potential  $E = 0.04$  V;  $t_1 = 200$  ms;  $E_2 = 0.8$  V;  $t_2 = 190$  ms;  $E_3 = -0.8$  V;  $t_3 = 190$  ms. The flow rate is about 0.5 mL per minute. Chromatograph the *System suitability solution*, and measure the peak areas as directed for *Procedure*: the relative retention times are about 0.8 for kanamycin and 1.0 for amikacin; and the resolution,  $R$ , between kanamycin and amikacin is not less than 3. Chromatograph the *Standard preparation*, and measure the peak areas as directed for *Procedure*: the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu$ g, of  $C_{22}H_{43}N_5O_{13}$  in each mg of Amikacin taken by the formula:

$$2500(CE / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amikacin RS in the *Standard preparation*;  $E$  is the designated amikacin content, in  $\mu$ g per mg, of USP Amikacin RS;  $W$  is the weight, in mg, of Amikacin taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the amikacin peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

lated on the dried basis; Amikacin Sulfate having a molar ratio of amikacin to  $H_2SO_4$  of 1:1.8 contains the equivalent of not less than 691  $\mu$ g and not more than 806  $\mu$ g of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether its molar ratio of amikacin to  $H_2SO_4$  is 1:2 or 1:1.8.

**USP Reference standards** <11>—

USP Amikacin RS

USP Kanamycin Sulfate RS

**Identification**—It responds to the *Identification* tests under *Amikacin*.

**Specific rotation** <781S>: between  $+76^\circ$  and  $+84^\circ$ .

*Test solution*: 20 mg per mL, in water.

**Crystallinity** <695>: meets the requirements.

**pH** <791>: between 2.0 and 4.0 (1:2 salt), or between 6.0 and 7.3 (1:1.8 salt), in a solution containing 10 mg per mL.

**Loss on drying** <731>—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at  $110^\circ$  for 3 hours: it loses not more than 13.0% of its weight.

**Residue on ignition** <281>: not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay**—

*Mobile phase*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Amikacin*.

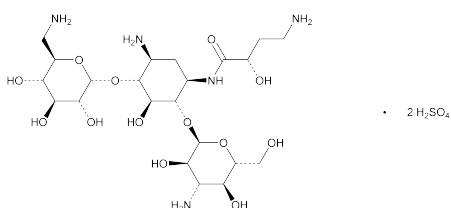
**Assay preparation**—Transfer an accurately weighed quantity of Amikacin Sulfate, equivalent to about 50 mg of amikacin ( $C_{22}H_{43}N_5O_{13}$ ), to a 250-mL volumetric flask, add about 50 mL of water, and swirl to dissolve. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Amikacin*. Calculate the quantity, in  $\mu$ g, of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) in each mg of Amikacin Sulfate taken by the formula:

$$2500(CE / W)(r_U / r_S)$$

in which  $W$  is the weight, in mg, of Amikacin Sulfate taken to prepare the *Assay preparation*; and the other terms are as defined therein.

## Amikacin Sulfate



$C_{22}H_{43}N_5O_{13} \cdot H_2SO_4$  762.15

$C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$  781.76

D-Streptamine, O-3-amino-3-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 6)-O-[6-amino-6-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 4)]-N<sup>1</sup>-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-, sulfate (1:2 or 1:1.8) (salt).  
O-3-Amino-3-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 4)-O-[6-amino-6-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 6)]-N<sup>3</sup>-(4-amino-L-2-hydroxybutyl)-2-deoxy-L-streptamine sulfate (1:2 or 1:1.8) [39831-55-5].

» Amikacin Sulfate having a molar ratio of amikacin to  $H_2SO_4$  of 1:2 contains the equivalent of not less than 674  $\mu$ g and not more than 786  $\mu$ g of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) per mg, calcu-

## Amikacin Sulfate Injection

» Amikacin Sulfate Injection is a sterile solution of Amikacin Sulfate in Water for Injection, or of Amikacin in Water for Injection prepared with the aid of Sulfuric Acid. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amikacin ( $C_{22}H_{43}N_5O_{13}$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.

**USP Reference standards** <11>—

USP Amikacin RS

USP Endotoxin RS

USP Kanamycin Sulfate RS

**Identification—**

**A:** Dilute it with water to obtain a solution containing 6 mg per mL: the resulting solution meets the requirements of *Identification test A* under *Amikacin*.

**B:** The retention time of the peak for amikacin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.33 USP Endotoxin Unit per mg of amikacin.

**pH** (791): between 3.5 and 5.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Amikacin*.

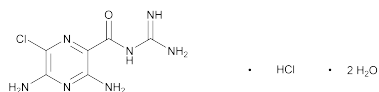
*Assay preparation*—Dilute an accurately measured volume of Injection quantitatively, and stepwise if necessary, with water to obtain a solution having a concentration of about 0.02 mg of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) per mL.

*Procedure*—Proceed as directed in the *Assay* for *Amikacin*. Calculate the quantity, in mg, of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) in each mL of the Injection taken by the formula:

$$(L / D)(CE / 1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of amikacin in each mL of Injection; *D* is the concentration, in mg per mL, of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) in the *Assay preparation*, on the basis of the labeled quantity per mL and the extent of dilution; and the other terms are as defined therein.

## Amiloride Hydrochloride



$C_6H_8ClN_7O \cdot HCl \cdot 2H_2O$  302.12  
 Pyrazinecarboxamide, 3,5-diamino-*N*-(aminoiminomethyl)-6-chloro-, monohydrochloride dihydrate;  
*N*-Amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride dihydrate [17440-83-4].

**DEFINITION**

Amiloride Hydrochloride contains NLT 98.0% and NMT 101.0% of  $C_6H_8ClN_7O \cdot HCl$ , calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197M)

- B. ULTRAVIOLET ABSORPTION** (197U)

**Sample:** 600 µg/mL of water, diluted quantitatively and stepwise with 0.1 N hydrochloric acid to a concentration of about 9.6 µg/mL

**Acceptance criteria:** Meets the requirements

- C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

**ASSAY**

- PROCEDURE**

**Sample:** 450 mg

**Analysis:** Dissolve the *Sample* in 100 mL of glacial acetic acid, add 10 mL of mercuric acetate TS and 15 mL of dioxane. Add crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N

perchloric acid is equivalent to 26.61 mg of  $C_6H_8ClN_7O \cdot HCl$ .

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES**

- RESIDUE ON IGNITION** (281): NMT 0.1%

- HEAVY METALS, Method II** (231): NMT 20 ppm

- ORGANIC IMPURITIES**

**Standard solutions:** Prepare a series of solutions, *A*, *B*, *C*, *D*, *E*, and *F*, of USP Amiloride Hydrochloride RS in a mixture of methanol and chloroform (4:1) having concentrations of 4000, 40, 20, 8, 4, and 2 µg/mL, respectively.

**Sample solution:** 4 mg/mL of Amiloride Hydrochloride in methanol and chloroform (4:1)

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel previously washed with methanol

**Application volume:** 5 µL

**Developing solvent system:** Tetrahydrofuran and 3 N ammonium hydroxide (15:2)

**Analysis**

**Samples:** *Standard solutions A*, *B*, *C*, *D*, *E*, and *F* and *Sample solution*

Proceed as directed in the chapter. Dry the spots with a stream of nitrogen, and develop the chromatograms in the solvent system, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under long-wavelength UV light: the *R<sub>f</sub>* value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*. Estimate the levels of any additional spots observed in the chromatogram of the *Sample solution* by comparison with the principal spots in the chromatograms of *Standard solutions B*, *C*, *D*, *E*, and *F*.

**Acceptance criteria:** The sum of the intensities of any additional spots observed is NMT that of the principal spot obtained from *Standard solution B* (equivalent to 1%).

**SPECIFIC TESTS**

- ACIDITY**

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* in 100 mL of a mixture of methanol and water (1:1). Titrate with 0.10 N sodium hydroxide to a potentiometric endpoint.

**Acceptance criteria:** NMT 0.30 mL is required (0.1% as HCl).

- LOSS ON DRYING**

(See *Thermal Analysis* (891).)

[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity.]

**Sample:** 10 mg

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument using the *Sample*. Heat the specimen at the rate of 10°/min between ambient temperature and 225° in an atmosphere of nitrogen at a flow rate of 40 mL/min. From the thermogram determine the accumulated loss in weight between ambient temperature and about 200° on the plateau.

**Acceptance criteria:** 11.0%–13.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers.

- USP REFERENCE STANDARDS** (11)

USP Amiloride Hydrochloride RS

## Amiloride Hydrochloride Tablets

» Amiloride Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Amiloride Hydrochloride RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer an amount of finely ground Tablets, equivalent to 5 mg of amiloride hydrochloride, to a 25-mL volumetric flask, add methanol to volume, mix, and filter. Separately apply 10  $\mu$ L each of the filtrate and a Standard solution of USP Amiloride Hydrochloride RS in methanol, containing 0.2 mg per mL, to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of tetrahydrofuran and 3 N ammonium hydroxide (22:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, and examine under short-wavelength UV light: the  $R_F$  value of the principal spot in the chromatogram of the test solution corresponds to that obtained from the Standard solution.

### Dissolution (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_6H_8ClN_7O \cdot HCl$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 363 nm on filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Amiloride Hydrochloride RS in the same *Medium*. An amount of methanol not to exceed 2% of the total volume of the Standard solution may be used to dissolve the amiloride hydrochloride.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_6H_8ClN_7O \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask, add 60 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 30 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and centrifuge a portion of the mixture. Dilute an accurately measured portion of the clear supernatant quantitatively to obtain a solution containing about 10  $\mu$ g of amiloride hydrochloride per mL. Concomitantly determine the absorbances of this solution and a Standard solution of USP Amiloride Hydrochloride RS in the same medium having a known concentration of about 10  $\mu$ g per mL, at the wavelength of maximum absorbance at about 363 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_6H_8ClN_7O \cdot HCl$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of amiloride hydrochloride in the Tablet,  $C$  is the concentration, in  $\mu$ g per mL, of USP Amiloride Hydrochloride RS, corrected for loss in

weight in the Standard solution;  $D$  is the concentration, in  $\mu$ g per mL, of amiloride hydrochloride in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablets and the Standard solution, respectively.

### Assay—

*Buffer solution*—Dissolve 136 g of monobasic potassium phosphate in 800 mL of water, and adjust by the addition of phosphoric acid, with mixing, to a pH of 3.0. Dilute with water to 1000 mL, and mix.

*Mobile phase*—Prepare a suitable degassed mixture of water, methanol, and *Buffer solution* (71:25:4).

*Standard preparation*—Dissolve a suitable quantity of USP Amiloride Hydrochloride RS in methanol to obtain a solution having a known concentration of about 1.0 mg of amiloride hydrochloride per mL. Transfer 5.0 mL of the solution to a 50-mL volumetric flask, and add 10.0 mL of methanol and 2.0 mL of 0.1 N hydrochloric acid. Dilute with water to volume, and mix. The concentration of USP Amiloride Hydrochloride RS in the *Standard preparation* is about 0.1 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of amiloride hydrochloride, to a 50-mL volumetric flask containing 15.0 mL of methanol and 2.0 mL of 0.1 N hydrochloric acid. Sonicate for 10 minutes, dilute with water to volume, sonicate for an additional 10 minutes, mix, and filter.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 286-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor of the major peak is not more than 2.0; and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amiloride Hydrochloride RS, corrected for loss in weight in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amiloride Hydrochloride and Hydrochlorothiazide Tablets

### DEFINITION

Amiloride Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- B. THIN-LAYER CHROMATOGRAPHY**  
**Standard solution A:** 0.2 mg/mL of USP Amiloride Hydrochloride RS in methanol  
**Standard solution B:** 2 mg/mL of USP Hydrochlorothiazide RS in methanol

**Sample solution:** Equivalent to 0.2 mg/mL of amiloride hydrochloride from ground Tablets in methanol. Filter.

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Tetrahydrofuran and 3 N ammonium hydroxide (22:3)

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, and examine under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  values of the amiloride hydrochloride and hydrochlorothiazide spots of the *Sample solution* correspond to those of the corresponding *Standard solutions*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.

**Mobile phase:** Methanol, water, and *Buffer* (25:71:4)

**Standard stock solution:** 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

**Standard solution:** 0.1 mg/mL of USP Amiloride Hydrochloride RS and 1 mg/mL of USP Hydrochlorothiazide RS, prepared by transferring 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 100 mg of USP Hydrochlorothiazide RS and 20.0 mL of methanol. Add 4.0 mL of 1 N hydrochloric acid, and dilute with water to volume.

**Sample solution:** Transfer an equivalent to 5 mg of amiloride hydrochloride from powdered Tablets (NLT 20) to a 50-mL volumetric flask. Add 15.0 mL of methanol and 2.0 mL of 1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and amiloride hydrochloride are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between hydrochlorothiazide and amiloride hydrochloride

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amiloride hydrochloride from the *Sample solution*

$r_S$  = peak response of amiloride hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Amiloride Hydrochloride RS, corrected for loss in weight in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amounts of  $C_6H_8ClN_7O \cdot HCl$  and  $C_7H_8ClN_3O_4S_2$

**PERFORMANCE TESTS**

• **DISSOLUTION** <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of amiloride hydrochloride and hydrochlorothiazide dissolved using *Analytical procedure 1* or *procedure 2*.

**Analytical procedure 1**

**Amiloride standard solution:** 60 mg of USP Amiloride Hydrochloride RS (equivalent to 52 mg of anhydrous amiloride hydrochloride) in a 200-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

**Hydrochlorothiazide standard solution:** Transfer 100 mg of USP Hydrochlorothiazide RS to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

**Sample solution A:** Pass a portion of the solution under test through a glass fiber filter of 0.45- $\mu$ m pore size.

**Sample solution B:** Transfer 5.0 mL of *Sample solution A* to a 25-mL volumetric flask, and dilute with *Medium* to volume.

**Detector:** UV 363 nm for amiloride hydrochloride, 270 nm for hydrochlorothiazide

**Blank:** *Medium*

**Analysis**

**Samples:** *Amiloride standard solution*, *Hydrochlorothiazide standard solution*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) dissolved:

$$\text{Result} = [(A_U \times C_S \times V)/(A_S \times L)] \times 100$$

$A_U$  = absorbance of *Sample solution A*

$C_S$  = concentration of the *Amiloride standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$A_S$  = absorbance of the *Amiloride standard solution*

$L$  = label claim of amiloride (mg/Tablet)

Calculate the correction for the interference of amiloride:

$$A_{UC} = A_{U270} - [(A_{U363} \times F)/5]$$

$A_{UC}$  = corrected absorbance of *Sample solution A*, 270 nm

$A_{U270}$  = absorbance of *Sample solution B*, 270 nm

$A_{U363}$  = absorbance of *Sample solution A*, 363 nm

$$F = A_5 \text{ at } 270 \text{ nm} / A_5 \text{ at } 363 \text{ nm}$$

$A_5$  = absorbance of the *Amiloride standard solution*  
Calculate the percentage of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = [(A_{UC} \times C_S \times V \times D) / (A_5 \times L)] \times 100$$

$A_{UC}$  = corrected absorbance of *Sample solution A*, 270 nm

$C_S$  = concentration of the *Hydrochlorothiazide standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor of *Sample solution B*, 25/5

$A_5$  = absorbance of the *Hydrochlorothiazide standard solution*

$L$  = label claim of hydrochlorothiazide (mg/Tablet)

#### Analytical procedure 2

**Buffer and Mobile phase:** Prepare as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution* from the *Assay*.

**Standard solution:** 5 µg/mL of amiloride hydrochloride and 50 µg/mL of hydrochlorothiazide from the *Standard stock solution*, in *Medium*

**Sample solution:** Pass a portion of the solution under test through a filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0% between hydrochlorothiazide and amiloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_U / r_S) \times (C_S / L) \times V \times 100$$

$r_U$  = peak response of amiloride or hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of amiloride or hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of amiloride hydrochloride or hydrochlorothiazide in the *Standard solution* (mg/mL)

$L$  = label claim of amiloride hydrochloride or hydrochlorothiazide (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_6H_8ClN_7O \cdot HCl$  and NLT 75% (Q) of the labeled amount of  $C_7H_8ClN_3O_4S_2$  are dissolved.

- **UNIFORMITY OF DOSAGE UNITS**, *Content Uniformity* <905>: Meet the requirements with respect to amiloride hydrochloride and hydrochlorothiazide

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Buffer, Mobile phase, and Sample solution:** Prepare as directed in the *Assay*.

**Standard solution:** 10 µg/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and amiloride hydrochloride are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between hydrochlorothiazide and amiloride hydrochloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Sample solution* and *Standard solution*  
Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times F \times 100$$

$r_U$  = peak response of benzothiadiazine related compound A from the *Sample solution*

$r_S$  = peak response of benzothiadiazine related compound A from the *Standard solution*

$C_S$  = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of benzothiadiazine in the *Sample solution* (mg/mL)

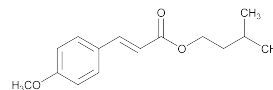
$F$  = unit conversion factor, 0.001 mg/µg

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>
  - USP Amiloride Hydrochloride RS
  - USP Benzothiadiazine Related Compound A RS
  - 4-Amino-6-chloro-1,3-benzenedisulfonamide.
  - $C_6H_8ClN_3O_4S_2$  285.73
  - USP Hydrochlorothiazide RS

## Amiloxate



$C_{15}H_{20}O_3$

248.32

4-Methoxycinnamic acid, isoamyl ester;

3-Methylbutyl 3-(4-methoxyphenyl)-(E)-2-propenoate [71617-10-2].

#### DEFINITION

Amiloxate contains NLT 98.0% and NMT 102.0% of  $C_{15}H_{20}O_3$ .

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197F>

- **B. ULTRAVIOLET ABSORPTION** <197U>

**Sample solution:** 5.0 µg/mL in alcohol

**Acceptance criteria:** Absorptivities, calculated on the as-is basis, do not differ by more than 3.0%.

#### ASSAY

- **PROCEDURE**

**Standard solution:** 20 mg/mL of USP Amiloxate RS in *tert*-butyl methyl ether



**Sample solution:** 20 mg/mL of Amiloxate in *tert*-butyl methyl ether

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 25-m, coated with a 0.1-μm film of phase G1

**Temperature**

**Injector port:** 240°

**Detector:** 260°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

**Carrier gas:** Helium

**Flow rate:** 6 mL/min

**Injection size:** 1 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amiloxate (C<sub>15</sub>H<sub>20</sub>O<sub>3</sub>) in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amiloxate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

**IMPURITIES**

• **ORGANIC IMPURITIES**

**Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all the peaks excluding solvent peak

**Acceptance criteria**

**Individual impurities:** NMT 0.5%

**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**

• **SPECIFIC GRAVITY** <841>: 1.037–1.041

• **REFRACTIVE INDEX** <831>: 1.556–1.560 at 20°

• **ACIDITY**

**Sample solution:** Transfer 50 mL of alcohol to a suitable container. Add 1 mL of phenolphthalein TS and sufficient 0.1 N sodium hydroxide to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, and add 5.0 mL of Amiloxate.

**Analysis:** Titrate with 0.1 N sodium hydroxide.

**Acceptance criteria:** NMT 0.2 mL of titrant per mL of Amiloxate is required for neutralization.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** <11>

USP Amiloxate RS

## Aminobenzoate Potassium

» Aminobenzoate Potassium contains not less than 98.5 percent and not more than 101.0 percent of C<sub>7</sub>H<sub>6</sub>KNO<sub>2</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Aminobenzoate Potassium RS

**Identification**—

**A:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 μg per mL.

*Medium:* 0.001 N sodium hydroxide.

**B:** Dissolve about 400 mg in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 hour: the *p*-aminobenzoic acid so obtained melts between 186° and 189°.

**C:** A solution (1 in 100) meets the requirements of the flame test for *Potassium* <191>.

**pH** <791>: between 8.0 and 9.0, in a solution (1 in 20).

**Loss on drying** <731>—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Volatile diazotizable substances**—

**Standard preparation**—Dissolve 10 mg of *p*-toluidine in 5 mL of methanol in a 100-mL volumetric flask, add water to volume, and mix. Transfer 1 mL to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test preparation**—Transfer 5.0 g of Aminobenzoate Potassium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the test specimen and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting about 95 mL of the distillate in a 100-mL volumetric flask. Add water to volume, and mix.

**Procedure**—Transfer 20.0-mL portions of the *Standard preparation* and the *Test preparation* to separate 100-mL beakers, and transfer 20.0 mL of water to a third 100-mL beaker to provide the blank. Treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring, allow to stand for 5 minutes for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance of the solution obtained from the *Test preparation* does not exceed that of the solution obtained from the *Standard preparation*, corresponding to not more than 0.002% of volatile diazotizable substances, as *p*-toluidine.

**Chloride** <221>—A 1.4-g portion shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%).

**Sulfate** <221>—A 1.4-g portion shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%).

**Heavy metals, Method II** (231): 0.002%.

**Assay**—Transfer about 500 mg of Aminobenzoate Potassium, accurately weighed, to a suitable vessel, add 25 mL of water and 25 mL of 3 N hydrochloric acid, mix, and cool in an ice bath. Titrate with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 17.52 mg of  $C_7H_6KNO_2$ .

## Aminobenzoate Potassium Capsules

» Aminobenzoate Potassium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Aminobenzoate Potassium RS

**Identification**—Dissolve about 1 g of the Capsule contents in 25 mL of water, add 5 mL of 3 N hydrochloric acid, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 hour: the *p*-aminobenzoic acid so obtained melts between 186° and 189°.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_7H_6KNO_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Aminobenzoate Potassium RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_6KNO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Standard preparation**—Prepare a solution of USP Aminobenzoate Potassium RS having a known concentration of about 5 µg per mL.

**Assay preparation**—Remove as completely as possible, and combine, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the combined contents, equivalent to about 100 mg of aminobenzoate potassium, to a 200-mL volumetric flask, add 150 mL of water, shake by mechanical means for 30 minutes, dilute with water to volume, mix, and filter. Pipet 2 mL of the filtrate into a 200-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 270 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of aminobenzoate potassium ( $C_7H_6KNO_2$ ) in the portion of Capsule contents taken by the formula:

$$20C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Aminobenzoate Potassium RS in the *Standard preparation*; and

$A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Aminobenzoate Potassium for Oral Solution

» Aminobenzoate Potassium for Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Aminobenzoate Potassium RS

**Identification**—

**A**: *Ultraviolet Absorption* (197U)—

*Solution*: 50 µg per mL.

*Medium*: water.

**B**: Dissolve about 400 mg in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 hour: the *p*-aminobenzoic acid so obtained melts between 186° and 189°.

**Minimum fill** (755)—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 7.0 and 9.0, in a solution (1 in 10).

**Assay**—Transfer about 100 mg of Aminobenzoate Potassium for Oral Solution, accurately weighed, to a suitable vessel, add 5 mL of hydrochloric acid and 50 mL of water, mix, cool to 15°, and add 25 g of crushed ice. Titrate with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 17.52 mg of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

## Aminobenzoate Potassium Tablets

» Aminobenzoate Potassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Aminobenzoate Potassium RS

**Identification**—Proceed as directed for *Aminobenzoate Potassium Capsules*, using 1 g of finely powdered Tablets.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_7H_6KNO_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known con-

centration of USP Aminobenzoate Potassium RS in the same Medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_6KNO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Standard preparation**—Prepare a solution of USP Aminobenzoate Potassium RS having a known concentration of about 5 µg per mL.

**Assay preparation and Procedure**—Weigh and finely powder not fewer than 20 Tablets. Using a portion of the powdered Tablets, equivalent to about 100 mg of aminobenzoate potassium, proceed as directed in the Assay under *Aminobenzoate Potassium Capsules*.

## Aminobenzoate Sodium

» Aminobenzoate Sodium contains not less than 98.5 percent and not more than 101.0 percent of  $C_7H_6NNaO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Aminobenzoate Sodium RS

**Identification**—

**A: Ultraviolet Absorption** (197U)—

**Solution:** 1 in 100,000.

**Medium:** 0.001 N sodium hydroxide.

**B:** Dissolve about 400 mg in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 hour: the *p*-aminobenzoic acid so obtained melts between 186° and 189°.

**C:** A solution (1 in 100) meets the requirements of the flame test for *Sodium* (191).

**pH** (791): between 8.0 and 9.0, in a solution (1 in 20).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Volatile diazotizable substances**—

**Standard solution**—Dissolve 10 mg of *p*-toluidine in 5 mL of methanol in a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1 mL to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Transfer 5.0 g of Aminobenzoate Sodium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the test specimen and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting about 95 mL of the distillate in a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—Transfer 20.0-mL portions of the *Standard solution* and the *Test solution* to separate 100-mL beakers, and transfer 20.0 mL of water to a third 100-mL beaker to provide the blank. Treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring, allow to stand for 5 minutes for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance of the solution

obtained from the *Test solution* does not exceed that of the solution obtained from the *Standard solution*, corresponding to not more than 0.002% of volatile diazotizable substances, as *p*-toluidine.

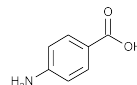
**Chloride** (221)—A 1.4-g portion shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%).

**Sulfate** (221)—A 1.4-g portion shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%).

**Heavy metals, Method II** (231): 0.002%.

**Assay**—Transfer about 500 mg of Aminobenzoate Sodium, accurately weighed, to a suitable vessel, add 25 mL of water and 25 mL of 3 N hydrochloric acid, mix, and cool in an ice bath. Titrate with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 15.91 mg of  $C_7H_6NNaO_2$ .

## Aminobenzoic Acid



$C_7H_7NO_2$  137.14

Benzoic acid, 4-amino.

*p*-Aminobenzoic acid [150-13-0].

» Aminobenzoic Acid contains not less than 98.5 percent and not more than 101.5 percent of  $C_7H_7NO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Aminobenzoic Acid RS

**Identification**—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

**Solution:** 5 µg per mL.

**Medium:** 0.001 N sodium hydroxide.

**Melting range** (741): between 186° and 189°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.2% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Volatile diazotizable substances**—

**Standard preparation**—Dissolve 10 mg of *p*-toluidine in 5 mL of methanol in a 100-mL volumetric flask, dilute water to volume, and mix. Transfer 1 mL to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test preparation**—Transfer 5.0 g of Aminobenzoic Acid to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the test specimen and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting about 95 mL of the distillate in a 100-mL volumetric flask. Add water to volume, and mix.

**Procedure**—Transfer 20.0-mL portions of the *Standard preparation* and the *Test preparation* to separate 100-mL beakers, and transfer 20.0 mL of water to a third 100-mL beaker to provide the blank. Treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring, allow to stand for 5 minutes in order for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of

guaiacol in 100 mL of 1 N sodium hydroxide), mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 450 nm, using the blank to set the instrument: the absorbance of the solution obtained from the *Test preparation* does not exceed that of the solution obtained from the *Standard preparation*, corresponding to not more than 0.002% of volatile diazotizable substances, as *p*-toluidine.

**Heavy metals, Method II** (231): 0.002%.

**Ordinary impurities** (466)—

*Test solution:* alcohol.

*Standard solution:* alcohol.

*Eluant:* a mixture of toluene, ethyl acetate, and alcohol (60:20:20), in a nonequilibrated chamber.

*Visualization:* 1.

**Assay**—Weigh accurately about 250 mg of Aminobenzoic Acid, and proceed as directed under *Nitrite Titration* (451). Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of  $C_7H_7NO_2$ .

## Aminobenzoic Acid Gel

» Aminobenzoic Acid Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoic acid ( $C_7H_7NO_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Aminobenzoic Acid RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 5 µg per mL.

*Medium:* alcohol.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 4.0 and 6.0.

**Alcohol content, Method II** (611): between 42.3% and 54.0% (w/w) of  $C_2H_5OH$ .

**Assay**—

*Mobile phase*—Mix 300 mL of methanol and 10 mL of glacial acetic acid with 690 mL of water. Allow the mixture to cool, and filter, if necessary, through a suitable micro-porous membrane filter. Degas the solution.

*Internal standard solution*—Dissolve salicylic acid in methanol, by sonicating, to obtain a solution having a concentration of about 7 mg per mL.

*Standard preparation*—Dissolve, by sonicating, an accurately weighed quantity of USP Aminobenzoic Acid RS in methanol, quantitatively dilute with methanol to obtain a solution having a known concentration of about 0.42 mg per mL, and mix. Pipet 5 mL of this solution and 5 mL of the *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through 0.6-µm filter paper. Throughout the preparation, protect against actinic light.

*Assay preparation*—Transfer an accurately weighed quantity of Gel, equivalent to about 4.2 mg of aminobenzoic acid, to a 100-mL volumetric flask, and add 10.0 mL of *Internal standard preparation* and about 50 mL of methanol. Shake or sonicate, as necessary, dilute with methanol to volume, and mix. Filter, if necessary, through filter paper (Whatman No. 41 or equivalent). Pass through 0.6-µm filter

paper. Throughout this preparation, protect against actinic light.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.0 mL per minute. Chromatograph replicate 15-µL injections of *Standard preparation* until the response ratio variability is within 1.0% of average. The resolution factor is not less than 3.0 between aminobenzoic acid and salicylic acid.

*Procedure*—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time of salicylic acid is about 3.0 relative to that of aminobenzoic acid as 1.0. Calculate the quantity, in mg, of aminobenzoic acid ( $C_7H_7NO_2$ ) in the portion of Gel taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Aminobenzoic Acid RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the aminobenzoic acid peak to the salicylic acid peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminobenzoic Acid Topical Solution

» Aminobenzoic Acid Topical Solution contains, in each mL, not less than 45 mg and not more than 55 mg of aminobenzoic acid ( $C_7H_7NO_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—

**A:** To 1 mL of Topical Solution add 1 mL of 1 N sodium hydroxide, and add, in the order named, 0.5 mL of potassium iodide TS, 0.5 mL of 3 N hydrochloric acid, and 0.5 mL of sodium hypochlorite TS: a brown precipitate is formed.

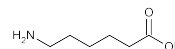
**B:** To 1 mL of Topical Solution add 2 mL of 3 N hydrochloric acid, and cool to about 10°. Add 1 mL of sodium nitrite solution (1 in 100), then add a solution prepared by mixing 50 mg of 2-naphthol with 3 mL of sodium hydroxide solution (1 in 10): a red color is produced.

**Specific gravity** (841): not less than 0.895 and not more than 0.905.

**Alcohol content** (611): between 65% and 75% of  $C_2H_5OH$ .

**Assay**—Transfer 5 mL of Topical Solution, accurately measured, to a suitable open vessel, evaporate on a steam bath to dryness, and proceed as directed under *Nitrite Titration* (451), beginning with "Add 20 mL of hydrochloric acid." Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of  $C_7H_7NO_2$ .

## Aminocaproic Acid



$C_6H_{13}NO_2$  131.17

Hexanoic acid, 6-amino-

6-Aminohexanoic acid [60-32-2].

» Aminocaproic Acid contains not less than 98.5 percent and not more than 101.5 percent of  $C_6H_{13}NO_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store at room temperature.

**USP Reference standards** (11)—

USP Aminocaproic Acid RS

**Identification**, *Infrared Absorption* (197K).

**Water**, *Method I* (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Assay**—

*Solution A*—Transfer 0.55 g of sodium 1-heptanesulfonate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Mobile phase*—Transfer 10 g of monobasic potassium phosphate to a 1000-mL beaker, dissolve in 300 mL of *Solution A*, add 250 mL of methanol, followed by another 300 mL of *Solution A*, and mix. Adjust the mixture with phosphoric acid to a pH of 2.2. Transfer the whole mixture to a 1000-mL volumetric flask, dilute with *Solution A* to volume, and mix. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of methionine in water containing 1.25 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Aminocaproic Acid RS in water to obtain a *Stock solution* having a known concentration of 12.5 mg per mL. Transfer 5.0 mL of the *Stock solution* to a 100-mL volumetric flask, add 2.0 mL of the *Internal standard solution*, dilute with water to volume, and mix.

*Assay preparation*—Transfer an accurately weighed quantity of 1.25 g of Aminocaproic Acid to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1 and is maintained at 30°. The flow rate is about 0.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.76 for aminocaproic acid and 1.0 for methionine; the resolution,  $R$ , between aminocaproic acid and methionine is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and allow the *Assay preparation* to elute for not less than two times the retention time of aminocaproic acid. Record the chromatograms, and measure all the peak responses. Calculate the quantity, in g, of  $C_6H_{13}NO_2$  in the portion of Aminocaproic Acid taken by the formula:

$$2C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aminocaproic Acid RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the aminocaproic acid peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminocaproic Acid Injection

» Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injection. It contains not less than 95.0 percent and not more than 107.5 percent of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Aminocaproic Acid RS

USP Endotoxin RS

**Identification**—Mix 2 mL of Injection, added dropwise, with 100 mL of acetone, rapidly stirring the mixture with a glass rod to induce crystallization. Allow the mixture to stand for 15 minutes, and pass through a medium-porosity, sintered-glass filter. Wash the crystals with 25 mL of acetone, apply vacuum to remove the solvent, dry at 105° for 30 minutes, and cool: the residue so obtained responds to the *Identification* test under *Aminocaproic Acid*.

**Bacterial endotoxins** (85)—It contains not more than 0.05 USP Endotoxin Unit per mg of aminocaproic acid.

**pH** (791): between 6.0 and 7.6.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*—Transfer 11 g of sodium 1-pentanesulfonate and 40 g of anhydrous sodium sulfate to a 2-L volumetric flask, and dissolve in about 500 mL of water. Add 20 mL of 1 N sulfuric acid and 30 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Aminocaproic Acid RS in *Mobile phase* to obtain a solution having a known concentration of about 2.5 mg per mL.

*Resolution solution*—Mix 20  $\mu$ L of benzyl alcohol with 100 mL of water. Dilute 1.0 mL of this solution with the *Standard preparation* to 10 mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 1.25 g of aminocaproic acid, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution* as directed for *Procedure*: the resolution,  $R$ , between benzyl alcohol and aminocaproic acid is not less than 7.0. The aminocaproic acid peak elutes prior to the benzyl alcohol peak. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminocaproic acid ( $C_6H_{13}NO_2$ ) in each mL of the Injection taken by the formula:

$$500(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aminocaproic Acid RS in the *Standard preparation*;  $V$  is the vol-

ume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the aminocaproic acid peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminocaproic Acid Oral Solution

» Aminocaproic Acid Oral Solution contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aminocaproic Acid RS

**Identification**—Mix about 1 g of ion-exchange resin (strongly acidic styrene-divinylbenzene cation-exchange resin) with about 10 mL of 1 N hydrochloric acid in a 100-mL beaker. Decant and discard the hydrochloric acid, and wash the resin with five 10-mL portions of water, decanting and discarding the liquid following each washing. Place the washed resin in a glass-stoppered, 125-mL conical flask, and add a volume of Oral Solution, equivalent to about 250 mg of aminocaproic acid, and 10 mL of water. Insert the stopper in the flask, and shake by mechanical means for 30 minutes. Transfer the resin slurry to a medium-porosity, sintered-glass funnel, wash with about 100 mL of water, apply suction to filter, and discard the washing. Place a 100-mL beaker under the stem of the funnel, add 10 mL of 1 N hydrochloric acid to the resin, stir for 4 to 5 minutes, and filter by applying suction. Evaporate the filtrate on a steam bath to dryness, dry at 105° for 1 hour, and cool: the residue so obtained meets the requirements for the *Identification* test under *Aminocaproic Acid*.

**pH** (791): between 6.0 and 6.5.

**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to about 250 mg of aminocaproic acid, to a 250-mL beaker, add 80 mL of glacial acetic acid, and mix. Add 10 drops of a 1 in 500 solution of crystal violet in chlorobenzene, and titrate with 0.1 N perchloric acid in dioxane VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid. ( $C_6H_{13}NO_2$ )

## Aminocaproic Acid Tablets

» Aminocaproic Acid Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aminocaproic Acid RS

**Identification**—Triturate 2 Tablets with 10 mL of water, and filter into 100 mL of acetone. Swirl the mixture, and allow to stand for 15 minutes to complete crystallization. Filter through a medium-porosity, sintered-glass filter, and wash the crystals with 25 mL of acetone. Apply vacuum to remove the solvent, then dry at 105° for 30 minutes, and cool: the residue so obtained responds to the *Identification* test under *Aminocaproic Acid*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

*pH 9.5 Borate buffer*—Dissolve 6.185 g of boric acid and 7.930 g of potassium chloride in about 1000 mL of water, add 60 mL of 1.0 N sodium hydroxide, and mix. Dilute with water to 2000 mL, mix, and add 1.0 N sodium hydroxide, if necessary, to adjust to a pH of  $9.5 \pm 0.1$ .

*Standard preparation*—Dissolve an accurately weighed quantity of USP Aminocaproic Acid RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of about 0.5 mg per mL.

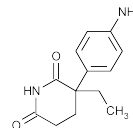
*Procedure*—Into three separate 50-mL volumetric flasks pipet (a) 1 mL of a filtered portion of the solution under test, (b) 1 mL of the *Standard preparation*, and (c) 1 mL of water to provide a blank. Add 20.0 mL of *pH 9.5 Borate buffer* and 3.0 mL of freshly prepared  $\beta$ -naphthoquinone-4-sodium sulfonate solution (1 in 500) to each, swirl to mix, and place the 3 flasks in a water bath maintained at a temperature of  $65 \pm 5^\circ$  for 45 minutes. Cool, dilute each with water to volume, and mix. Determine the amount of  $C_6H_{13}NO_2$  dissolved from absorbances, at the wavelength of maximum absorbance at about 460 nm, obtained from the test solution in comparison with those obtained from the *Standard* solution, using the blank to set the instrument.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_6H_{13}NO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of aminocaproic acid, to a beaker, add about 100 mL of glacial acetic acid, heat gently to effect solution, and cool. Add 10 drops of a 1 in 500 solution of crystal violet in chlorobenzene, and titrate with 0.1 N perchloric acid in dioxane VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid ( $C_6H_{13}NO_2$ ).

## Aminogluthethimide



$C_{13}H_{16}N_2O_2$

2,6-Piperidinedione, 3-(4-aminophenyl)-3-ethyl-; 2-(*p*-Aminophenyl)-2-ethylglutarimide [125-84-8].

232.28

### DEFINITION

Aminogluthethimide contains NLT 98.0% and NMT 102.0% of aminogluthethimide ( $C_{13}H_{16}N_2O_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. ULTRAVIOLET ABSORPTION** (197U)

**Analytical wavelength**: 242 nm

**Medium**: Methanol

**Sample solution**: 10  $\mu$ g/mL

**Acceptance criteria**: Absorptivities, calculated on a dried basis, differ by NMT 2.0%.

**ASSAY****• PROCEDURE**

**Buffer:** Add 120 mL of 0.1 N acetic acid to 100 mL of 0.1 N potassium hydroxide in a 1000-mL volumetric flask, and add 250 mL of water. Adjust by the addition of either 1 N acetic acid or 1 N potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol and *Buffer* (27:73)

**Diluent:** Methanol and *Buffer* (1:1)

**Standard solution:** 0.5 mg/mL of USP Aminoglutethimide RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Aminoglutethimide in *Diluent*. Pass through a filter of 0.45- $\mu$ m or finer pore size filter, discarding the first 5 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.3 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.7

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) in the portion of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Aminoglutethimide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminoglutethimide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

**• RESIDUE ON IGNITION** <281>: NMT 0.1%

**• HEAVY METALS**, *Method II* <231>: NMT 10 ppm

**• LIMIT OF AZO-AMINOGLUTETHIMIDE**

[NOTE—Use low-actinic glassware. Conduct this test promptly under subdued light. Wear protective gloves resistant to dimethyl sulfoxide to prevent contact with skin. Use shaking, not sonication or heat, to dissolve the USP Azo-aminoglutethimide RS and the *Sample*.]

**Buffer:** 150 mL of 0.1 N acetic acid and 50 mL of 0.1 N potassium hydroxide, diluted in water to 1000 mL

**Mobile phase:** Dissolve 100 mg of edetate disodium in 350 mL of *Buffer*. Add 650 mL of methanol, and cool to room temperature. Adjust with glacial acetic acid to a pH of  $5.0 \pm 0.1$ .

**Standard solution:** 0.5  $\mu$ g/mL of USP Azo-aminoglutethimide RS in dimethyl sulfoxide

**Sample solution:** 1 mg/mL of Aminoglutethimide in dimethyl sulfoxide

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 328 nm

**Column:** 3.9-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.2

**Capacity factor:** 2.0–5.0

**Column efficiency:** NLT 800 theoretical plates

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The aminoglutethimide elutes with the dimethyl sulfoxide.]

Calculate the percentage of azo-aminoglutethimide in the specimen of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azo-aminoglutethimide RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of Aminoglutethimide in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.03% of 3,3'-(azodi-4,1-phenylene)-3,3'-dimethylbis-[2,6-piperidinedione] (corresponding to azo-aminoglutethimide)

**• ORGANIC IMPURITIES**

**Buffer, Mobile phase, Diluent, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 10  $\mu$ g/mL of USP *m*-Aminoglutethimide RS in *Diluent*

**Sample solution:** 1 mg/mL of Aminoglutethimide in *Diluent*. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for aminoglutethimide and *m*-aminoglutethimide are about 0.8 and 1.0, respectively.]

Calculate the percentage of *m*-aminoglutethimide in the portion of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP *m*-Aminoglutethimide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminoglutethimide in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 2.0% of *m*-aminoglutethimide

Calculate the percentage of each peak, other than the main peak and the *m*-aminoglutethimide peak, in the portion of Aminoglutethimide taken, if present:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each peak

$r_T$  = sum of the responses of all the peaks from the *Sample solution*

**Acceptance criteria:** NMT 1.0% total impurities, other than *m*-aminoglutethimide

**SPECIFIC TESTS****• SULFATE**

**Sample solution:** 1 mg/mL in dilute methanol (1 in 20)

**Analysis:** To 100 mL of *Sample solution* add 1.0 mL of 3 N hydrochloric acid and 2.0 mL of barium chloride TS.

**Acceptance criteria:** No turbidity is produced.

**• pH** <791>

**Sample solution:** 1 mg/mL in dilute methanol (1 in 20)

**Acceptance criteria:** 6.2–7.3

**• LOSS ON DRYING** <731>

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** <11>  
USP Aminoglutethimide RS  
USP *m*-Aminoglutethimide RS  
USP Azo-aminoglutethimide RS

## Aminoglutethimide Tablets

### DEFINITION

Aminoglutethimide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197M>  
**Sample:** Transfer 500 mg of finely powdered Tablets to a suitable container. Add 25 mL of acetone, mix, and filter. Evaporate the filtrate at room temperature to dryness, and dry the residue under vacuum over silica gel for 2 h.  
**Acceptance criteria:** Meet the requirements

### ASSAY

- **PROCEDURE**  
**Buffer:** Add 120 mL of 0.1 N acetic acid to 100 mL of 0.1 N potassium hydroxide in a 1000-mL volumetric flask, and add 250 mL of water. Adjust by the addition of either 1 N acetic acid or 1 N potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to volume.  
**Mobile phase:** Methanol and *Buffer* (27:73)  
**Diluent:** Methanol and *Buffer* (1:1)  
**Standard solution:** 0.5 mg/mL of USP Aminoglutethimide RS in *Diluent*  
**Sample solution:** Nominally 0.5 mg/mL of aminoglutethimide in *Diluent* prepared as follows. Transfer an equivalent to 200 mg of aminoglutethimide, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 130 mL of *Diluent*, and sonicate for 5 min. Shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Centrifuge this solution, transfer 25.0 mL of the clear supernatant to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC  
**Detector:** UV 240 nm  
**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1  
**Column temperature:** 40°  
**Flow rate:** 1.3 mL/min  
**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.7  
**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Aminoglutethimide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of aminoglutethimide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **DISSOLUTION** <711>  
**Medium:** Dilute hydrochloric acid (7 in 1000); 1000 mL  
**Apparatus 1:** 100 rpm  
**Time:** 30 min  
**Instrumental conditions**  
(See *Spectrophotometry and Light-Scattering* <851>.)  
**Mode:** UV  
**Analytical wavelength:** 237 nm  
**Standard solution:** USP Aminoglutethimide RS in a mixture of dilute hydrochloric acid and pH 7.5 phosphate buffer, having a ratio similar to the *Sample solution*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with pH 7.5 phosphate buffer to a concentration that is similar to the *Standard solution*.  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
**Tolerances:** NLT 70% (Q) of the labeled amount of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

### IMPURITIES

- **ORGANIC IMPURITIES**  
**Buffer, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the *Assay*.  
**Standard solution:** 10  $\mu$ g/mL of USP *m*-Aminoglutethimide RS in *Diluent*  
**Sample solution:** Transfer an equivalent to 200 mg of aminoglutethimide, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 130 mL of *Diluent*, and sonicate for 5 min. Shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.  
**System suitability**  
**Samples:** *Standard solution* and *Sample solution*  
[NOTE—The relative retention times for aminoglutethimide and *m*-aminoglutethimide are 0.8 and 1.0, respectively.]  
**Suitability requirements**  
**Tailing factor:** NMT 1.7, *Sample solution*  
**Relative standard deviation:** NMT 2.0%, *Sample solution*

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each peak, other than the main peak and the *m*-aminoglutethimide peak, if present in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity in the *Sample solution*

$r_T$  = sum of the responses of all of the peaks, excluding that of the *m*-aminoglutethimide peak in the *Sample solution*

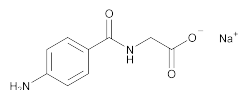
**Acceptance criteria:** NMT 2.0% total impurities, other than *m*-aminoglutethimide

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** <11>  
USP Aminoglutethimide RS  
USP *m*-Aminoglutethimide RS



## Aminohippurate Sodium Injection



$C_9H_9N_2NaO_3$  216.17

Glycine, *N*-(4-aminobenzoyl)-, monosodium salt.  
Monosodium *p*-aminohippurate [94-16-6].

» Aminohippurate Sodium Injection is a sterile solution of Aminohippuric Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_9H_9N_2NaO_3$ .

**Packaging and storage**—Preserve in single-dose or multi-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

**Identification**—

**A:** A volume of Injection, equivalent to 100 mg of aminohippuric acid, diluted to 50 mL and acidified with hydrochloric acid, responds to *Identification* test *B* under *Aminohippuric Acid*.

**B:** Transfer a volume of Injection, equivalent to about 200 mg of aminohippurate sodium, to a test tube, and add, in the order named, 2 mL of potassium iodide TS, 10 mL of water, and 5 mL of sodium hypochlorite TS: a red color is produced.

**C:** It responds to the flame test for *Sodium* (191).

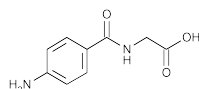
**Bacterial endotoxins** (85)—It contains not more than 0.04 USP Endotoxin Unit per mg of aminohippurate sodium.

**pH** (791): between 6.7 and 7.6.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer to a 200-mL volumetric flask an accurately measured volume of Injection, equivalent to about 1 g of aminohippurate sodium, and dilute with water to volume. Transfer 50.0 mL of the solution to a suitable container, add 5 mL of hydrochloric acid, and proceed as directed under *Nitrite Titration* (451), beginning with “cool to about 15°.” Each mL of 0.1 M sodium nitrite is equivalent to 21.62 mg of  $C_9H_9N_2NaO_3$ .

## Aminohippuric Acid



$C_9H_9N_2O_3$  194.19

Glycine, *N*-(4-aminobenzoyl)-.  
*p*-Aminohippuric acid [61-78-9].

» Aminohippuric Acid contains not less than 98.0 percent and not more than 100.5 percent of  $C_9H_9N_2O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Aminohippuric Acid RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** Dissolve about 10 mg in 5 mL of water, and add 0.5 mL of 3 N hydrochloric acid, 0.5 mL of sodium nitrite solution (1 in 10), and a solution of 0.20 g of 2-naphthol in 10 mL of 6 N ammonium hydroxide: a red color is produced.

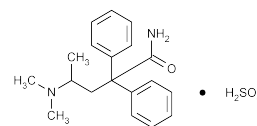
**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.25% of its weight.

**Residue on ignition** (281): not more than 0.25%.

**Heavy metals**, *Method II* (231): 0.001%.

**Assay**—Transfer about 150 mg, accurately weighed, of Aminohippuric Acid to a beaker or a casserole. Add 5 mL of hydrochloric acid and 50 mL of water, and proceed as directed under *Nitrite Titration* (451), beginning with “stir until dissolved.” Each mL of 0.1 M sodium nitrite is equivalent to 19.42 mg of  $C_9H_9N_2O_3$ .

## Aminopentamide Sulfate



$C_{19}H_{24}N_2O \cdot H_2SO_4$  394.49

$\alpha$ -[2-(Dimethylamino)propyl]- $\alpha$ -phenylbenzeneacetamide sulfate [60-46-8].

» Aminopentamide Sulfate contains not less than 95.0 percent and not more than 103.0 percent of  $C_{19}H_{24}N_2O \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Aminopentamide Sulfate RS

**Clarity and color of solution**—Dissolve 0.5 g in 10 mL of water: the solution is clear and colorless.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** It meets the requirements of the tests for *Sulfate* (191).

**Melting range** (741): between 179° and 186°.

**pH** (791): between 1.2 and 3.0, in a solution (2.5 in 100).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 4.4% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Assay**—Dissolve about 500 mg of Aminopentamide Sulfate, accurately weighed, in 100 mL of dimethylformamide in a suitable container. Add 5 drops of thymol blue TS, and titrate with 0.1 N lithium methoxide VS in toluene to a deep blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 19.72 mg of  $C_{19}H_{24}N_2O \cdot H_2SO_4$ .

## Aminopentamide Sulfate Injection

» Aminopentamide Sulfate Injection is a sterile solution of Aminopentamide Sulfate in Water for Injection. It contains not less than 90.0 percent

and not more than 110.0 percent of the labeled amount of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ).

**Packaging and storage**—Preserve in tight, single-dose or multiple-dose *Containers for Injections*, as described under *Injections* (1). Store at controlled room temperature.

**Labeling**—Label Injection to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Aminopentamide Sulfate RS

USP Endotoxin RS

**Identification**—Transfer 10 mL of the Injection to a separator, add sodium hydroxide TS until alkaline to litmus, and extract with 25 mL of chloroform. Transfer a few drops of the chloroform extract to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see *Spectrophotometry and Light-Scattering* (851)). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.

**Bacterial endotoxins** (85)—It contains not more than 25 USP Endotoxin Units per mg of aminopentamide sulfate.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 2.5 and 4.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*—Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in water to obtain a solution having a known concentration equivalent to the labeled concentration of aminopentamide sulfate in the Injection.

*Assay preparation*—Use the undiluted Injection.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ) in each mL of the Injection taken by the formula:

$$C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminopentamide Sulfate Tablets

» Aminopentamide Sulfate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label Tablets to indicate that they are for veterinary use only.

**USP Reference standards** (11)—

USP Aminopentamide Sulfate RS

**Identification**—Transfer a portion of ground Tablet powder, equivalent to about 2 mg of aminopentamide, to a separator, add 20 mL of water and 3 mL of 10 N sodium hydroxide, and mix. Extract with two 20-mL portions of methylene chloride, and evaporate the combined methylene chloride extracts to a volume of about 0.5 mL. Transfer a few drops of the chloroform concentrate to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see *Spectrophotometry and Light-Scattering* (851)). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.

**Disintegration** (701): not more than 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 1 g of powdered Tablets, accurately weighed, in vacuum at a pressure of 5 mm of mercury or less at 60° for 3 hours: it loses not more than 4.0% of its weight.

**Assay**—

*Mobile phase*—Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.2 mg of aminopentamide, to a suitable flask. Add 10.0 mL of *Mobile phase*, sonicate for 5 minutes, and stir by mechanical means for about 10 minutes. Pass this mixture through a filter having a 0.5- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1, and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 900 theoretical plates; and the relative standard deviation for replicate injections is not more than 2%.

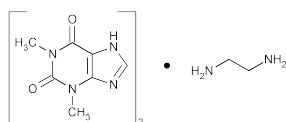
*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

mg, of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ) in the portion of Tablets taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminophylline



$C_{16}H_{24}N_4O_4$  (anhydrous) 420.43  
 1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, compd. with 1,2-ethanediamine (2:1).  
 Theophylline compound with ethylenediamine (2:1) [317-34-0].  
 Dihydrate 456.46 [5897-66-5].

» Aminophylline is anhydrous or contains not more than two molecules of water of hydration. It contains not less than 84.0 percent and not more than 87.4 percent of anhydrous theophylline ( $C_7H_8N_4O_2$ ), calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether it is anhydrous or hydrous and also to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Theophylline RS

**Identification**—

**A:** Dissolve about 500 mg in 20 mL of water, add, with constant stirring, 1 mL of 3 N hydrochloric acid, filter (retain the filtrate), wash the precipitate with small portions of cold water, and dry at 105° for 1 hour: the precipitate of theophylline so obtained melts between 270° and 274°.

**B:** To about 10 mg of the dried precipitate obtained in *Identification test A*, contained in a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide: the residue acquires a purple color, which is destroyed by solutions of fixed alkalies.

**C:** To the filtrate obtained in *Identification test A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 minutes, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 hour: the dried precipitate melts between 164° and 171°.

**Water, Method I** (921): not more than 0.75% (anhydrous form) and not more than 7.9% (hydrous form), determined on 1.5 g of it, a mixture of 25 mL of chloroform and 25 mL of methanol being used in place of the methanol solvent.

**Residue on ignition** (281): not more than 0.15%.

**Ethylenediamine content**—Dissolve about 500 mg of Aminophylline, accurately weighed, in 30 mL of water, add methyl orange TS, and titrate with 0.1 N hydrochloric acid

VS. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of  $C_2H_8N_2$ . The content of ethylenediamine ( $C_2H_8N_2$ ) is between 157 mg and 175 mg per g of  $C_7H_8N_4O_2$  found in the *Assay*.

**Assay**—

**Mobile phase**—Mix 200 mL of methanol, 960 mg of sodium 1-pentanesulfonate, and sufficient water to make 1 L. Adjust with glacial acetic acid to a pH of  $2.9 \pm 0.1$ , filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**: a mixture of water and methanol (4:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Theophylline RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL.

**Resolution solution**—Dissolve a suitable quantity of theobromine in the *Standard preparation* to obtain a solution containing about 0.08 mg per mL. Transfer 20.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 24 mg of Aminophylline, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.65 for theobromine and 1.0 for theophylline; the tailing factor for the theophylline peak is not more than 2.0; and the resolution, *R*, between theobromine and theophylline is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of theophylline ( $C_7H_8N_4O_2$ ) in the portion of Aminophylline taken by the formula:

$$250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Theophylline RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the theophylline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminophylline Injection

» Aminophylline Injection is a sterile solution of Aminophylline in Water for Injection, or is a sterile solution of Theophylline in Water for Injection prepared with the aid of Ethylenediamine. It contains, in each mL, an amount of aminophylline equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

Aminophylline Injection may contain an excess of Ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

**NOTE**—Do not use the Injection if crystals have separated.

**Packaging and storage**—Preserve in single-dose containers from which carbon dioxide has been excluded, preferably of Type I glass, protected from light.

**Labeling**—Label the Injection to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Theophylline RS

**Identification**—

**A:** Dilute a volume of Injection, equivalent to about 500 mg of aminophylline, with water to about 20 mL, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to precipitate the theophylline completely. Filter: the filtrate responds to *Identification* test C under *Aminophylline*.

**B:** Wash the precipitate of theophylline with a small portion of cold water, and dry at 105° for 1 hour: the theophylline so obtained melts between 270° and 274°, and responds to *Identification* test B under *Aminophylline*.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mg of aminophylline.

**pH** (791): between 8.6 and 9.0.

**Particulate matter** (788): meets the requirements for small-volume Injections.

**Ethylenediamine content**—Accurately measure a volume of Injection, equivalent to about 500 mg of aminophylline, and dilute with water, if necessary, to make about 30 mL. Add methyl orange TS, and titrate with 0.1 N hydrochloric acid VS. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>. The Injection contains between 166 mg and 192 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per g of C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub> found in the Assay.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Aminophylline*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of theophylline, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pipet 4 mL of this solution into a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Aminophylline*. Calculate the quantity, in mg, of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Injection taken by the formula:

$$1250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Theophylline RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the theophylline peak responses obtained from the Assay *preparation* and the *Standard preparation*, respectively.

## Aminophylline Oral Solution

» Aminophylline Oral Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

Aminophylline Oral Solution may contain an excess of ethylenediamine, but no other sub-

stance may be added for the purpose of pH adjustment.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Oral Solution to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Theophylline RS

**Identification**—

**A:** Place a volume of Oral Solution, equivalent to about 500 mg of aminophylline, in a suitable container, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or an amount sufficient to precipitate the theophylline completely. Filter (retain the filtrate), wash the precipitate with small portions of cold water until free from chloride, and dry at 105° for 1 hour: the theophylline so obtained melts between 270° and 274°.

**B:** The filtrate from *Identification* test A responds to *Identification* test C under *Aminophylline*.

**pH** (791): between 8.5 and 9.7.

**Ethylenediamine content**—Accurately measure a volume of Oral Solution, equivalent to about 500 mg of aminophylline, and dilute with water, if necessary, to make about 30 mL. Add methyl orange TS, and titrate with 0.1 N hydrochloric acid VS. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>. The Oral Solution contains between 176 mg and 283 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per g of C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub> found in the Assay.

**Assay**—

*Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the Assay under *Aminophylline*.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 18 mg of anhydrous theophylline, to a 250-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Aminophylline*. Calculate the quantity, in mg, of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in each mL of the Oral Solution taken by the formula:

$$250(C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Theophylline RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the theophylline peak responses obtained from the Assay *preparation* and the *Standard preparation*, respectively.

## Aminophylline Rectal Solution

» Aminophylline Rectal Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

Aminophylline Rectal Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

**Packaging and storage**—Preserve in tight, single-dose or multiple-dose containers, at controlled room temperature.

**Labeling**—Label the Rectal Solution to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Theophylline RS

**Identification**—Dilute a volume of Rectal Solution, equivalent to about 500 mg of aminophylline, with water to about 20 mL, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or sufficient to precipitate the theophylline completely. Filter, wash with small portions of cold water until free from chloride, and dry at 105° for 4 hours. Use the precipitate and the filtrate for the following tests.

**A:** *Infrared Absorption* (197K).

**B:** The filtrate obtained as directed above meets the requirements for *Identification* test C under *Aminophylline*.

**pH** (791): between 9.0 and 9.5.

**Ethylenediamine content**—Measure accurately a volume of Rectal Solution, equivalent to about 500 mg of aminophylline, and dilute with water, if necessary, to make about 30 mL. Add methyl orange TS, and titrate with 0.1 N hydrochloric acid VS. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>. The Rectal Solution contains between 218 mg and 267 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per g of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) found in the Assay.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Theophylline RS in dilute hydrochloric acid (1 in 100), and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Pipet an accurately measured volume of Rectal Solution, equivalent to about 500 mg of aminophylline, into a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a second 500-mL volumetric flask, add 50 mL of dilute hydrochloric acid (1 in 10), dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 270 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in each mL of the Rectal Solution taken by the formula:

$$50(C/V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Theophylline RS in the *Standard preparation*; V is the volume, in mL, of Rectal Solution taken; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Aminophylline Suppositories

» Aminophylline Suppositories contain an amount of aminophylline equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in well-closed containers, in a cold place.

**Labeling**—Label the Suppositories to state the content of anhydrous theophylline.

**Identification**—

**A:** Evaporate to about one-half its volume on a steam bath a portion, equivalent to about 500 mg of aminophyl-

line, of the water solution prepared in the Assay. Adjust with 1 N sodium hydroxide to a pH of 7.0, chill, and filter the crystals of theophylline. Save the filtrate, free from the washings, for use in *Identification* test B: the crystals, after being washed with small portions of ice-cold water and dried at 105° for 1 hour, melt between 270° and 274°, and respond to *Identification* test B under *Aminophylline*.

**B:** The filtrate from *Identification* test A responds to *Identification* test C under *Aminophylline*.

**Ethylenediamine content**—Accurately weigh a portion of the stirred, congealed mass of the Suppositories used for the Assay, equivalent to about 500 mg of aminophylline, and place in a 500-mL conical flask. Add 150 mL of a mixture of equal volumes of alcohol and ether, and warm gently under reflux for 30 minutes, with occasional swirling. Cool to room temperature, and titrate with 0.1 N hydrochloric acid VS, using a glass-modified calomel electrode system (replace the saturated potassium chloride solution of the calomel electrode with methanol saturated with lithium chloride). Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>. The Suppositories contain between 152 mg and 190 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per g of C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub> found in the Assay.

**Assay**—Tare a small dish and a glass rod, place in the dish not less than 5 Suppositories, and heat on a steam bath until melted. Mix the melt by stirring it with the rod, cool while stirring, and weigh. Weigh accurately a portion of the mass, equivalent to about 1 g of aminophylline, place it in a beaker, add 60 mL of hot water and 3 mL of nitric acid, and heat on a steam bath for 15 minutes with frequent stirring. Cool, transfer to a separator with the aid of 40 mL of ether, shake well, and allow to separate, using a few mL of alcohol, if necessary, to bring about separation of any emulsion that has formed. Draw the water layer into a 100-mL volumetric flask, wash the ether with two 15-mL portions of water, adding the washings to the volumetric flask, dilute with water to volume, and mix. Transfer an accurately measured portion of the solution, equivalent to about 250 mg of aminophylline, to a 250-mL conical flask, add 10 mL of 6 N ammonium hydroxide and about 20 mL of 0.1 N silver nitrate VS, and heat on a steam bath for 15 minutes. Cool to between 5° and 10° for 20 minutes, then filter, preferably through a filtering crucible of fine porosity under reduced pressure, and wash the precipitate with small portions of water until the last washing gives not more than a faint opalescence with hydrochloric acid. Dissolve the precipitate by pouring over it small volumes of warm 2 N nitric acid, receiving the solution in a conical flask. Wash the filtering crucible a few times with warm water acidified with nitric acid, receiving the washings in the same flask. Cool, add 2 mL of ferric ammonium sulfate TS, and titrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N ammonium thiocyanate is equivalent to 18.02 mg of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

## Aminophylline Tablets

» Aminophylline Tablets contain an amount of aminophylline equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

**NOTE**—The ammoniacal odor present in the vapor space above Aminophylline Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Tablets to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Theophylline RS

**Identification**—

**A:** Macerate a quantity of Tablets, equivalent to about 500 mg of aminophylline, with 25 mL of water, and filter: the filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and chill, if necessary, to precipitate the theophylline. Filter, and retain the filtrate, free from washings. Wash the crystals on the filter with small quantities of ice-cold water, and dry at 105° for 1 hour: the theophylline so obtained responds to *Identification* test *B* under *Aminophylline*, and when recrystallized from water and dried at 105° for 1 hour, melts between 270° and 274°.

**B:** The filtrate obtained in *Identification* test *A* responds to *Identification* test *C* under *Aminophylline*.

**Dissolution** (711)—

FOR UNCOATED OR PLAIN COATED TABLETS—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 269 nm on filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Theophylline RS in the same *Medium*.

**Tolerances**—Not less than 75% (*Q*) of the labeled amount of  $C_7H_8N_4O_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a 250-mL volumetric flask, add about 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume, and mix. Filter a portion of the mixture, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution, quantitatively diluted, if necessary, and a Standard solution of USP Theophylline RS having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of anhydrous theophylline ( $C_7H_8N_4O_2$ ) in the Tablet taken by the formula:

$$(TC/D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of anhydrous theophylline in the Tablet, *D* is the concentration, in µg per mL, of theophylline in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution, *C* is the concentration, in µg per mL, of USP Theophylline RS in the Standard solution, and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Ethylenediamine content**—Accurately weigh a portion of the powdered Tablets prepared in the *Assay*, equivalent to about 350 mg of aminophylline, transfer to a 100-mL conical flask, add 20 mL of water, and digest at 50°, with frequent shaking, for 30 minutes. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. To the combined filtrate and washings add methyl orange TS, and titrate with 0.1 N hydrochloric acid VS. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of  $C_2H_8N_2$ . The Tablets contain between 140 mg and 190 mg of ethylenediamine ( $C_2H_8N_2$ ) per g of  $C_7H_8N_4O_2$  found in the *Assay*.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer a portion of the powder, equivalent to about 2 g of aminophylline, to a 200-mL volumetric flask with the aid of a mixture of 50 mL of water and 15 mL of 6 N ammonium hydroxide, and allow to stand for 30 minutes with frequent shaking, warming to about 50°, if necessary, to dissolve the aminophylline. Cool the mixture to room temperature if it has been warmed, add water to volume, and mix. Centrifuge about 50 mL of the mixture, and pipet a portion of the clear supernatant, equivalent to about 250 mg of aminophylline, into a 250-mL conical flask, and dilute with water, if necessary, to make about 40 mL. Add 8 mL of 6 N ammonium hydroxide and 20.0 mL of 0.1 N silver nitrate VS, mix, heat to boiling, and continue boiling for 15 minutes. Cool to between 5° and 10° for 20 minutes, then filter, preferably through a filtering crucible under reduced pressure, and wash the precipitate with three 10-mL portions of water. Acidify the combined filtrate and washings with nitric acid, and add an additional 3 mL of the acid. Cool, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 18.02 mg of  $C_7H_8N_4O_2$ .

## Aminophylline Delayed-Release Tablets

» Aminophylline Delayed-Release Tablets contain an amount of aminophylline equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

**NOTE**—The ammoniacal odor present in the vapor space above Aminophylline Delayed-Release Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Tablets to state the content of anhydrous theophylline.

**USP Reference standards** (11)—

USP Theophylline RS

**Disintegration** (701): 30 minutes, determined as directed under *Delayed-Release (enteric coated) Tablets*.

**Other requirements**—Tablets respond to the *Identification* tests and meet the requirements for *Uniformity of dosage units*, *Ethylenediamine content* and *Assay* under *Aminophylline Tablets*.

## Aminosaliclylate Sodium

$C_7H_6NNaO_3 \cdot 2H_2O$  211.15

Benzoic acid, 4-amino-2-hydroxy-, monosodium salt, dihydrate.

Monosodium 4-aminosalicylate dihydrate [6018-19-5].

Anhydrous 175.12 [133-10-8].

» Aminosaliclylate Sodium contains not less than 98.0 percent and not more than 101.0 percent of  $C_7H_6NNaO_3$ , calculated on the anhydrous basis.

**Caution**—Prepare solutions of Aminosaliclylate Sodium within 24 hours of administration. Under

no circumstances use a solution if its color is darker than that of a freshly prepared solution.

**Packaging and storage**—Preserve in tight, light-resistant containers, protected from excessive heat.

**USP Reference standards** (11)—

USP Aminosalicylic Acid RS

USP *m*-Aminophenol RS

**Clarity and color of solution**—One g dissolves in 10 mL of water to give a clear solution that has not more than a faint yellow color. One g dissolves in a freshly prepared mixture of 5 mL of nitric acid and 45 mL of water to give a clear solution that has not more than a slight color.

**Identification**—

**A:** Dissolve 250 mg in 3 mL of 1 N sodium hydroxide, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 5-mL aliquot to a 250-mL volumetric flask containing 12.5 mL of pH 7 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix. This solution, when compared in a suitable spectrophotometer against a blank of the same buffer in the same concentration, exhibits absorbance maxima at  $265 \pm 2$  nm and  $299 \pm 2$  nm, and the ratio  $A_{265}/A_{299}$  is between 1.50 and 1.56.

**B:** Place about 1 g in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 minutes, add 40 mL of water, mix, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash with water, and dry at  $105^\circ$  for 1 hour: the diacetyl derivative so obtained melts between  $191^\circ$  and  $197^\circ$ .

**C:** Dissolve 50 mg in 5 mL of water, add 1 mL of 3 N hydrochloric acid, and filter if necessary. To the filtrate add 1 drop of ferric chloride TS: a violet color is produced.

**D:** A solution of it responds to the tests for *Sodium* (191).

**pH** (791): between 6.5 and 8.5, in a solution (1 in 50).

**Water, Method I** (921): between 16.0% and 18.0%.

**Chloride** (221)—Dissolve 0.50 g in a mixture of 5 mL of nitric acid and 15 mL of water: the solution shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (0.042%).

**Heavy metals, Method II** (231): 0.003%.

**Limit of *m*-aminophenol**—

*Mobile phase*—Prepare as directed in the Assay under Aminosalicylic Acid.

*Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the test for Limit of *m*-aminophenol under Aminosalicylic Acid.

*Test preparation*—Use the Assay preparation, prepared as directed in the Assay.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the Standard preparation and the Test preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.66 for sulfanilamide and 1.0 for *m*-aminophenol. Calculate the percentage of *m*-aminophenol, in relation to the quantity of aminosalicylate sodium in the portion of Aminosalicylate Sodium taken by the formula:

$$10(C / W)(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP *m*-Aminophenol RS in the Standard preparation; W is the quantity of aminosalicylate sodium, in mg, in the portion of Aminosalicylate Sodium taken, as determined in the Assay; and  $R_U$  and  $R_S$  are the ratios of the response of the *m*-aminophenol peak to the response of the sulfanilamide peak obtained from the Test preparation and the Standard preparation, respectively: not more than 0.25% of *m*-aminophenol is found.

**Hydrogen sulfide, sulfur dioxide, and amyl alcohol**—

Dissolve about 500 mg in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously: no odor of hydrogen sulfide or sulfur dioxide is perceptible, and not more than a faint odor of amyl alcohols is perceptible. A piece of moistened lead acetate test paper held over the mixture does not become discolored.

**Assay**—

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under Aminosalicylic Acid.

*Assay preparation*—Transfer about 69 mg of Aminosalicylate Sodium, accurately weighed, to a 100-mL low-actinic volumetric flask, add 50 mL of Mobile phase, and swirl to dissolve. Add 10.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

*Procedure*—Proceed as directed for Procedure in the Assay under Aminosalicylic Acid. Calculate the quantity of  $C_7H_6NNaO_3$ , in mg, in the Aminosalicylate Sodium taken by the formula:

$$(175.12 / 153.14)(100C)(R_U / R_S)$$

in which 175.12 and 153.14 are the molecular weights of anhydrous aminosalicylate sodium and aminosalicylic acid, respectively; C is the concentration, in mg per mL, of USP Aminosalicylic Acid RS in the Standard preparation; and  $R_U$  and  $R_S$  are the ratios of the response of the aminosalicylic acid peak to the response of the acetaminophen peak obtained from the Assay preparation and the Standard preparation, respectively.

## Aminosalicylate Sodium Tablets

» Aminosalicylate Sodium Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_7H_6NNaO_3 \cdot 2H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, protected from excessive heat.

**USP Reference standards** (11)—

USP Aminosalicylic Acid RS

USP *m*-Aminophenol RS

**Identification**—Digest a quantity of powdered Tablets, equivalent to about 3 g of aminosalicylate sodium, with 40 mL of water, and filter. Add to the filtrate 15 mL of 1 N acetic acid, and allow to stand until precipitation has occurred. Collect the precipitate on a filter, wash well with water, and dry at  $105^\circ$  for 30 minutes: the residue responds to the following tests.

**A:** Place about 1 g in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 minutes, add 40 mL of water, mix, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at  $105^\circ$  for 1 hour: the diacetyl derivative so obtained melts between  $191^\circ$  and  $197^\circ$ .

**B:** Shake 0.1 g with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS: a violet color is produced.

**Dissolution, Procedure for a Pooled Sample** (711)—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_7H_6NNaO_3 \cdot 2H_2O$  dissolved, employing the procedure set forth in the Assay, making any necessary modifications.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_6NNaO_3 \cdot 2H_2O$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of *m*-aminophenol—**

**Mobile phase**—Prepare as directed in the Assay under Aminosalicyclic Acid.

**Standard solution, Internal standard solution, and Chromatographic system**—Prepare as directed in the test for Limit of *m*-aminophenol under Aminosalicyclic Acid.

**Test solution**—Use the Assay preparation, prepared as directed in the Assay.

**Procedure**—Proceed as directed for Procedure in the test for Limit of *m*-aminophenol under Aminosalicyclic Acid. Calculate the percentage of *m*-aminophenol, in relation to the quantity of aminosalicilate sodium, in the portion of Tablets taken by the formula:

$$100(C / W)(R_U / R_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP *m*-Aminophenol RS in the Standard solution; W is the quantity of aminosalicilate sodium, in mg, in the portion of Tablets taken, as determined in the Assay; and  $R_U$  and  $R_S$  are the ratios of the response of the *m*-aminophenol peak to the response of the sulfanilamide peak obtained from the Test solution and the Standard solution, respectively: not more than 1.0% of *m*-aminophenol is found.

**Assay—**

**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the Assay under Aminosalicyclic Acid.

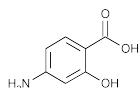
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 690 mg of aminosalicilate sodium, to a 100-mL low-actinic volumetric flask. Add 50 mL of Mobile phase, and shake for about 5 minutes. Dilute with Mobile phase to volume, and mix. Filter, and transfer 10.0 mL of the clear filtrate to a low-actinic, 100-mL volumetric flask containing 10.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

**Procedure**—Proceed as directed for Procedure in the Assay under Aminosalicyclic Acid. Calculate the quantity, in mg, of  $C_7H_6NNaO_3 \cdot 2H_2O$  in the portion of Tablets taken by the formula:

$$(211.15 / 153.14)(1000C)(R_U / R_S)$$

in which 211.15 and 153.14 are the molecular weights of aminosalicilate sodium dihydrate and aminosalicyclic acid, respectively; C is the concentration, in mg per mL, of USP Aminosalicyclic Acid RS in the Standard preparation; and  $R_U$  and  $R_S$  are the ratios of the response of the aminosalicyclic acid peak to the response of the acetaminophen peak obtained from the Assay preparation and the Standard preparation, respectively.

## Aminosalicyclic Acid



$C_7H_7NO_3$  153.14  
Benzoic acid, 4-amino-2-hydroxy-  
4-Aminosalicyclic acid [65-49-6].

» Aminosalicyclic Acid contains not less than 98.5 percent and not more than 100.5 percent of  $C_7H_7NO_3$ , calculated on the anhydrous basis.

**Caution**—Under no circumstances use a solution prepared from Aminosalicyclic Acid if its color is darker than that of a freshly prepared solution.

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.

**USP Reference standards** (11)—

USP Aminosalicyclic Acid RS

USP *m*-Aminophenol RS

**Clarity and color of solution**—One g dissolves in 10 mL of sodium bicarbonate solution (1 in 15) to form a clear solution that has not more than a faint yellow color. One g dissolves in a freshly prepared mixture of 5 mL of nitric acid and 45 mL of water to form a clear solution that has not more than a slight color.

**Identification—**

**A:** Dissolve 0.25 g in 3 mL of 1 N sodium hydroxide, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 5-mL aliquot to a 250-mL volumetric flask containing 12.5 mL of pH 7 phosphate buffer (see Buffer Solutions in the section Reagents, Indicators, and Solutions), dilute with water to volume, and mix. This solution, when compared in a suitable spectrophotometer against a blank of the same buffer in the same concentration, exhibits absorbance maxima at  $265 \pm 2$  and  $299 \pm 2$  nm, and the ratio  $A_{265}/A_{299}$  is between 1.50 and 1.56.

**B:** Place about 1 g in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 minutes, add 40 mL of water, mix, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at 105° for 1 hour: the diacetyl derivative so obtained melts between 191° and 197°.

**C:** Shake 0.1 g with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS: a violet color is produced.

**pH** (791): between 3.0 and 3.7, in a saturated solution.

**Water, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.2%.

**Chloride** (221)—Dissolve 0.50 g in a mixture of 5 mL of nitric acid and 15 mL of water: the solution shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (0.042%).

**Heavy metals, Method II** (231): 0.003%.

**Limit of *m*-aminophenol—**

**Mobile phase**—Prepare as directed in the Assay.

**Internal standard solution**—Prepare a solution of sulfanilamide in Mobile phase having a concentration of about 5  $\mu\text{g}$  per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP *m*-Aminophenol RS in Mobile phase to obtain a solution having a known concentration of about 12  $\mu\text{g}$  per mL. Transfer 10.0 mL of this solution and 10.0 mL of Internal standard solution to a 100-mL low-actinic volumetric flask, dilute with Mobile phase to volume, and mix.

**Test solution**—Transfer about 50 mg of Aminosalicyclic Acid, accurately weighed, to a 100-mL low-actinic volumetric flask, add 50 mL of Mobile phase, and swirl to dissolve. Add 10.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu\text{m}$  packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.66 for sulfanilamide and 1.0 for *m*-aminophenol;



the resolution,  $R$ , between *m*-aminophenol and sulfanilamide is not less than 2.5; and the relative standard deviation for replicate injections is not more than 7%.

**Procedure**—[NOTE—After use, wash the column for 30 minutes with a filtered and degassed mixture of methanol, water, and phosphoric acid (77:23:0.6), and then wash for 30 minutes with a filtered and degassed mixture of methanol and water (50:50).] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of *m*-aminophenol, in relation to the quantity of aminosalicyclic acid in the portion of Aminosalicyclic Acid taken by the formula:

$$10(C/W)(R_U/R_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP *m*-Aminophenol RS in the *Standard solution*,  $W$  is the quantity of aminosalicyclic acid, in mg, in the portion of Aminosalicyclic Acid taken, as determined in the *Assay*; and  $R_U$  and  $R_S$  are the ratios of the response of the *m*-aminophenol peak to the response of the sulfanilamide peak obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% of *m*-aminophenol is found.

**Hydrogen sulfide, sulfur dioxide, and amyl alcohol**—Dissolve about 500 mg in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously: no odor of hydrogen sulfide or sulfur dioxide is perceptible, and not more than a faint odor of amyl alcohols is perceptible. A piece of moistened lead acetate test paper held over the mixture does not become discolored.

#### Assay—

**Mobile phase**—Prepare a mixture of 425 mL of 0.05 M dibasic sodium phosphate, 425 mL of 0.05 M monobasic sodium phosphate, and 150 mL of methanol containing 1.9 g of tetrabutylammonium hydroxide. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of acetaminophen in *Mobile phase* having a concentration of about 5 mg per mL.

**Standard preparation**—Transfer about 12.5 mg of USP Aminosalicyclic Acid RS, accurately weighed, to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Prepare as directed for *Standard preparation*, except to use Aminosalicyclic Acid instead of USP Aminosalicyclic Acid RS.

**Chromatographic system** (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.83 for acetaminophen and 1.0 for aminosalicyclic acid; the resolution,  $R$ , between aminosalicyclic acid and acetaminophen is not less than 1.7; and the relative standard deviation of the ratios of the response of the aminosalicyclic acid peak to the response of the acetaminophen peak is not more than 1.0%.

**Procedure**—[NOTE—After use, wash the column for 30 minutes with a filtered and degassed mixture of methanol, water, and phosphoric acid (77:23:0.6), and then wash for 30 minutes with a filtered and degassed mixture of methanol and water (50:50).] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculate the quantity, in mg, of  $C_7H_7NO_3$  in the Aminosalicyclic Acid taken by the formula:

$$25C(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aminosalicyclic Acid RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the response of the aminosalicyclic acid peak to the response of the acetaminophen peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminosalicyclic Acid Tablets

» Aminosalicyclic Acid Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aminosalicyclic acid ( $C_7H_7NO_3$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.

#### USP Reference standards (11)—

USP Aminosalicyclic Acid RS

USP *m*-Aminophenol RS

**Identification**—Macerate a portion of powdered Tablets, equivalent to about 2 g of aminosalicyclic acid, with 50 mL of a mixture of 1 volume of acetone and 2 volumes of chloroform, and filter. Evaporate the filtrate with the aid of a current of warm air to dryness: the residue so obtained responds to *Identification* tests B and C under *Aminosalicyclic Acid*.

#### Dissolution (711)—

**Medium:** pH 7.5 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_7H_7NO_3$  dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_7NO_3$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Limit of *m*-aminophenol—

**Mobile phase and Internal standard solution**—Prepare as directed in the *Assay* under *Aminosalicyclic Acid*.

**Standard solution and Chromatographic system**—Prepare as directed in the test for *Limit of m-aminophenol* under *Aminosalicyclic Acid*.

**Test solution**—Use the *Assay preparation*, prepared as directed in the *Assay*.

**Procedure**—Proceed as directed for *Procedure* in the test for *Limit of m-aminophenol* under *Aminosalicyclic Acid*. Calculate the percentage of *m*-aminophenol, in relation to the quantity of aminosalicyclic acid in the portion of Tablets taken by the formula:

$$100(C/W)(R_U/R_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP *m*-Aminophenol RS in the *Standard solution*;  $W$  is the quantity of aminosalicyclic acid, in mg, in the portion of Tablets taken, as determined in the *Assay*; and  $R_U$  and  $R_S$  are the ratios of the response of the *m*-aminophenol peak to the response of the sulfanilamide peak obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% of *m*-aminophenol is found.

**Assay—**

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under Aminosalicilic Acid.

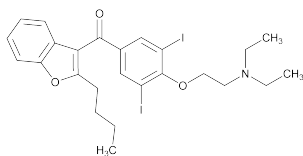
*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of aminosalicilic acid, to a 100-mL low-actinic volumetric flask. Add 50 mL of *Mobile phase*, and shake for about 5 minutes. Dilute with *Mobile phase* to volume, and mix. Filter, and transfer 10.0 mL of the clear filtrate to a 100-mL low-actinic volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Aminosalicilic Acid. Calculate the quantity, in mg, of aminosalicilic acid ( $C_7H_7NO_3$ ) in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Aminosalicilic Acid RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the response of the aminosalicilic acid peak to the response of the acetaminophen peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amiodarone Hydrochloride



$C_{25}H_{29}I_2NO_3 \cdot HCl$  681.77  
Methanone, (2-butyl-3-benzofuranyl)[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]- hydrochloride; 2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride [19774-82-4]. 2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone [1951-25-3].

**DEFINITION**

Amiodarone Hydrochloride contains NLT 98.5% and NMT 101.0% of  $C_{25}H_{29}I_2NO_3 \cdot HCl$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, Chloride (191): Meets the requirements

**ASSAY****• PROCEDURE**

**Buffer:** Dissolve 6.80 g of monobasic potassium phosphate in 900 mL of water, and add 1.0 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.00 ± 0.05, and dilute with water to 1000 mL.

**Diluent:** Acetonitrile and water (1:1)

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard stock solution:** 0.5 mg/mL of USP Amiodarone Hydrochloride RS in methanol

**Standard solution:** 0.1 mg/mL USP Amiodarone Hydrochloride RS in *Diluent* from *Standard stock solution*

**Sample stock solution:** 0.5 mg/mL of Amiodarone Hydrochloride in methanol

**Sample solution:** 0.1 mg/mL of Amiodarone Hydrochloride in *Diluent* from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm × 15-cm; 5-μm packing L26

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{25}H_{29}I_2NO_3 \cdot HCl$  in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amiodarone in the *Sample solution*

$r_S$  = peak response of amiodarone in the *Standard solution*

$C_S$  = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–101.0%, on the dried basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1% on a 1-g sample

**• HEAVY METALS**

**Buffer:** Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 70% hydrochloric acid. Adjust, if necessary, with diluted hydrochloric acid or diluted ammonia solution to a pH of 3.5. Dilute with water to 100.0 mL.

**Lead standard stock solution (1000 ppm Pb):** 1.6 mg/mL of lead nitrate in water

**Lead standard solution:** 10 ppm of lead in water from *Lead standard stock solution*. [NOTE—Prepare immediately before use.]

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.

**Thioacetamide solution:** Prepare a solution of 40 g/L of thioacetamide in water. To 0.2 mL of the freshly prepared solution, add 1 mL of a mixture of 85% glycerol, 1 M sodium hydroxide, and water (4:3:1). Heat in a water bath for 20 s.

**Sample solution:** Place about 1 g of Amiodarone Hydrochloride in a silica crucible along with 4 mL of magnesium sulfate solution (250 g/L of diluted sulfuric acid). Mix using a fine glass rod, and heat cautiously. If the mixture is a liquid, evaporate gently to dryness on a water bath. Progressively heat to ignition, and continue heating until an almost white or a mostly grayish residue is obtained. Carry out the ignition at a temperature not exceeding 800°. Allow to cool. Moisten the residue with a few drops of dilute sulfuric acid. Evaporate, ignite again, and allow to cool. The total period of ignition must not exceed 2 h. Dissolve the residue in two portions, 5 mL each, of 20% hydrochloric acid. Add 0.1 mL of *Phenolphthalein solution* followed by 25% ammonia water until a pink color is obtained. Cool, add glacial acetic acid until the solution is decolorized, and add 0.5 mL in excess. Filter if necessary, wash the filter, and dilute with water to 20 mL.

**Standard solution:** Proceed as directed for *Sample solution*, using 2 mL of *Lead standard solution* instead of

Amiodarone Hydrochloride. To 10 mL of the solution obtained, add 2 mL of the *Sample solution*.

**Monitor solution:** Proceed as directed for *Sample solution*, adding 2 mL of *Standard solution* to 1 g of Amiodarone Hydrochloride.

**Blank solution:** 10 mL of water and 2 mL of *Sample solution*

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, *Blank solution*, and *Monitor solution*

To 12 mL each of the *Standard solution*, *Sample solution*, *Blank solution*, and *Monitor solution* add 2 mL of *Buffer solution*, and mix. Add 1.2 mL of *Thioacetamide solution*, and immediately mix again. Examine the solutions after 2 min. The test is invalid if the *Standard solution* does not show a slight brown color compared to the *Blank solution* or if the *Monitor solution* is not comparable with the *Standard solution*.

**Acceptance criteria:** Any brown color in the *Sample solution* is not more intense than that in the *Standard solution* (20 ppm). [NOTE—If the result is difficult to judge, pass the solutions through a membrane filter having a porosity of 3 µm. Carry out the filtration slowly and uniformly, applying moderate and constant pressure. Compare the spots on the filters obtained from the different solutions.]

#### Organic Impurities

[NOTE—The product meets the requirements for both *Procedure 1* and *Procedure 2*.]

#### • PROCEDURE 1

**Potassium iodobismuthate solution:** Dissolve 100 g of tartaric acid in 400 mL of water, and add 8.5 g of bismuth subnitrate. Shake for 1 h, add 200 mL of a 400 g/L solution of potassium iodide, and shake well. Allow to stand for 24 h, filter, and protect from light.

**Standard solution A:** 0.02 mg/mL of USP Amiodarone Related Compound H RS in methylene chloride

**Standard solution B:** *Standard solution A* and *Sample solution* (1:1).

**Sample solution:** 100 mg/mL of Amiodarone Hydrochloride in methylene chloride

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.5-mm layer of chromatographic silica gel and fluorescent indicator with maximum absorbance at 254 nm

#### Application volume

**Standard solution A:** 50 µL

**Standard solution B:** 100 µL

**Sample solution:** 50 µL

**Developing solvent system:** Methylene chloride, methanol, and anhydrous formic acid (17:2:1)

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the plate in the *Developing solvent system* until the solvent front has moved NLT two-thirds the length of the plate, and dry in a current of cold air. Spray the plate with *Potassium iodobismuthate solution* and then with 3% hydrogen peroxide solution. Examine immediately in daylight: the spot from *Standard solution B* due to amiodarone related compound H is clearly visible.

**Acceptance criteria:** Any spot with the same  $R_f$  as the spot due to amiodarone related compound H from *Standard solution B* is not more intense than the spot from *Standard solution A* (0.02%).

#### • PROCEDURE 2

**Buffer:** Add 3 mL of glacial acetic acid to 800 mL of water. Adjust with diluted ammonia solution to a pH of 4.9, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile: methanol: *Buffer* (4:3:3 v/v/v).

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** Dissolve equal quantities of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS in a known amount of methanol.

**Standard solution:** 0.01 mg/mL each of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS, in *Diluent* from *Standard stock solution*

**Sample solution:** 5 mg/mL of Amiodarone Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**Run time:** 2 times the retention time of amiodarone

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between amiodarone related compound D and amiodarone related compound E

#### Analysis

[NOTE—Disregard any peak that is less than 0.05%.]

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity in the *Sample solution*

$r_S$  = peak response of amiodarone in the *Standard solution*

$C_S$  = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.5%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone related compound A <sup>a</sup>	0.26	0.2
Amiodarone related compound D <sup>b</sup>	0.29	0.2
Amiodarone related compound E <sup>c</sup>	0.37	0.2
Amiodarone related compound B <sup>d</sup>	0.49	0.2
Amiodarone related compound C <sup>e</sup>	0.55	0.2
Amiodarone related compound G <sup>f</sup>	0.62	0.2
Amiodarone related compound F <sup>g</sup>	0.69	0.2

<sup>a</sup> (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]phenyl}methanone.

<sup>b</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.

<sup>c</sup> (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.

<sup>d</sup> (2-Butylbenzofuran-3-yl){4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl}methanone.

<sup>e</sup> (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3-iodophenyl}methanone.

<sup>f</sup> [2-[(1RS)-1-Methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.

<sup>g</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone hydrochloride	1.00	—
Any other individual impurity	—	0.10

<sup>a</sup> (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]phenyl}methanone.<sup>b</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.<sup>c</sup> (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.<sup>d</sup> (2-Butylbenzofuran-3-yl){4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl}methanone.<sup>e</sup> (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3-iodophenyl}methanone.<sup>f</sup> [2-[(1*R*S)-1-Methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.<sup>g</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone.**SPECIFIC TESTS****• LIMIT OF IODIDES**

**Solution A:** Add 1.50 g of Amiodarone Hydrochloride to 40 mL of water at 80°, and shake until completely dissolved. Cool, and dilute with water to 50.0 mL.

**Standard solution:** To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid, 1.0 mL of an 88.2 mg/L solution of potassium iodide, and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.

**Sample solution:** To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.

**Analysis:** Measure the absorbances of the *Standard solution* and the *Sample solution* at 420 nm, using a mixture of 15.0 mL of *Solution A* and 1.0 mL of 0.1 M hydrochloric acid diluted with water to 20.0 mL to serve as the blank. The absorbance of the *Sample solution* is NMT half the absorbance of the *Standard solution*.

**Acceptance criteria:** NMT 150 ppm

- PH (791):** 3.2–3.8. Dissolve 1 g of Amiodarone Hydrochloride in water by heating at 80°. Cool, and dilute with water to 20 mL.
- LOSS ON DRYING (731):** Use 1 g of sample, and dry under vacuum (NMT 0.3 kPa) at 50° for 4 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Amiodarone Hydrochloride RS
  - USP Amiodarone Related Compound D RS  
(2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.  
C<sub>19</sub>H<sub>16</sub>I<sub>2</sub>O<sub>3</sub> 546.14
  - USP Amiodarone Related Compound E RS  
(2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.  
C<sub>19</sub>H<sub>18</sub>O<sub>3</sub> 294.34
  - USP Amiodarone Related Compound H RS  
2-Chloro-*N,N*-diethylethanamine.  
C<sub>6</sub>H<sub>14</sub>ClN 135.64

**Add the following:****▲Amiodarone Hydrochloride Oral Suspension****DEFINITION**

Amiodarone Hydrochloride Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride (C<sub>25</sub>H<sub>29</sub>I<sub>2</sub>NO<sub>3</sub> · HCl).

Prepare Amiodarone Hydrochloride Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Amiodarone Hydrochloride tablets <sup>a</sup> equivalent to	600 mg
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> (regular or sugar-free) and Ora-Plus <sup>b</sup> , a sufficient quantity to make	120 mL

<sup>a</sup> Cordarone 200-mg tablets, Wyeth-Ayerst Laboratories, Philadelphia, PA.<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar and comminute to a fine powder with a pestle. Adjust the pH of the *Vehicle* to 6.5 ± 0.5 with a sodium bicarbonate 50-mg/mL solution prepared in Purified Water. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an amiodarone hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume and mix well.

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol, water, and 50 mM monobasic ammonium phosphate (0.5: 0.5: 99)

**Standard solution:** 2.5 mg/mL of USP Amiodarone Hydrochloride RS in *Mobile phase*

**Sample solution:** Shake thoroughly by hand each bottle of the Oral Suspension. Prepare 2.5 mg/mL of amiodarone hydrochloride from Oral Suspension and *Mobile phase*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 10-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for amiodarone is about 3.6 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.1% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiodarone hydrochloride (C<sub>25</sub>H<sub>29</sub>I<sub>2</sub>NO<sub>3</sub> · HCl) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of amiodarone hydrochloride in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

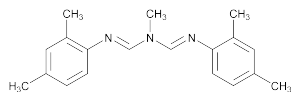
#### SPECIFIC TESTS

- **PH** (791): 5.8–6.8

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature or controlled room temperature.
- **LABELING:** Label it to state that it is to be well-shaken before use and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled cold temperature; NMT 30 days when stored at controlled room temperature
- **USP REFERENCE STANDARDS** (11)  
USP Amiodarone Hydrochloride RS▲<sup>USP36</sup>

## Amitraz



$C_{19}H_{23}N_3$  293.41

Methanimidamide, *N'*-(2,4-dimethylphenyl)-[[*N*-(2,4-dimethylphenyl)imino]methyl-*N*]-methyl-

*N*-Methyl-*N'*-2,4-xylyl-*N*-(*N*-2,4-xylylformimidoyl)formamidine.

*N*-Methylbis(2,4-xylyliminomethyl)amine [33089-61-1].

» Amitraz contains not less than 95.0 percent and not more than 101.5 percent of  $C_{19}H_{23}N_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Amitraz RS

**Labeling**—Label it to indicate that it is for veterinary use only.

#### Identification—

**A:** *Infrared Absorption* (197M).

**B:** Proceed as directed in the test for *Related compounds*, except to prepare a test solution of Amitraz in toluene containing 2 mg per mL and a *Standard solution* of USP Amitraz RS in toluene containing 2 mg per mL: the  $R_f$  value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the *Standard solution*.

**C:** The retention time of the major peak in the chromatogram of the *Reference solution* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water, Method I** (921): not more than 0.1%, anhydrous pyridine being used in place of methanol in the titration vessel.

**Residue on ignition** (281): not more than 0.2%.

**Related compounds**—Prepare a test solution of Amitraz in toluene containing 100 mg per mL. Prepare a solution of USP Amitraz RS in toluene having a concentration of 2.0 mg per mL (*Standard solution 1*). Prepare a solution of 2,4-dimethylaniline in toluene having a concentration of 0.30 mg per mL (*Standard solution 2*). Prepare a thin-layer

chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture as follows. Stand the plate to a depth of 3.5 cm in a solution prepared by dissolving 35 g of acetamide in 100 mL of methanol, adding 100 mL of triethylamine, and diluting to 250 mL with methanol. Allow to stand the wet plate in a current of cold air for about 30 seconds. Immediately apply separately to the plate, at a level about 1 cm below the top of the impregnated zone, 2  $\mu$ L each of the test solution, *Standard solution 1*, and *Standard solution 2*. Promptly develop the chromatogram in a solvent system consisting of a mixture of cyclohexane, ethyl acetate, and triethylamine (5:3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, allow it to air-dry, and examine it under short-wavelength UV light. Any secondary spot in the chromatogram obtained from the test solution is not more intense than the spot in the chromatogram obtained from *Standard solution 1* (2.0%). Expose the plate to the vapor of hydrochloric acid for about 10 minutes, then expose it to the vapor of nitrogen dioxide (prepared by the reaction of nitric acid and zinc) for 10 minutes, remove any excess nitric oxide by air exhaust, and spray the plate with a 0.5% solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in methanol, and examine the plate. Any secondary spot in the chromatogram obtained from the test solution corresponding to 2,4-dimethylaniline is not more intense than the spot in the chromatogram obtained from *Standard solution 2* (0.30%).

#### Assay—

**Internal standard solution**—Prepare a solution of squalane in methyl acetate containing 10 mg per mL.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Amitraz RS in *Internal standard solution* to obtain a solution having a known concentration of about 8 mg of USP Amitraz RS per mL.

**Assay preparation**—Transfer about 200 mg of Amitraz, accurately weighed, to a 25-mL volumetric flask, add about 20 mL of *Internal standard solution*, and swirl to dissolve. Dilute with *Internal standard solution* to volume, and mix.

**Reference solution**—Prepare a solution of Amitraz in methyl acetate containing about 8 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.5-m column packed with 3% liquid phase G1 on support S1A. The column and detector block temperatures are maintained at about 250°. Dry nitrogen is used as the carrier gas at a flow rate of about 60 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between squalane and amitraz is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation*, the *Assay preparation*, and the *Reference solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{19}H_{23}N_3$  in the portion of Amitraz taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amitraz RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the response of the amitraz peak to the response of the squalane peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amitraz Concentrate for Dip

» Amitraz Concentrate for Dip contains Amitraz in a suitable vehicle. It may contain a suitable stabilizing agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amitraz ( $C_{19}H_{23}N_3$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only. The label states also that it is to be diluted before use and states the name and quantity of diluent to be used, the directions for dilution, and the conditions for storage of the constituted Dip.

**USP Reference standards** (11)—

USP Amitraz RS

**Identification**—

**A:** Proceed as directed in the test for *Related compounds* under *Amitraz*, except to prepare a test solution by diluting the Concentrate with toluene to obtain a solution containing about 5 mg of amitraz per mL and to prepare a Standard solution of USP Amitraz RS in toluene containing 5 mg per mL: the  $R_f$  value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the Standard solution.

**B:** The retention time of the major peak in the chromatogram of the *Reference solution* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method I* (921): not more than 0.15%, anhydrous pyridine being used in place of methanol in the titration vessel.

**Assay**—

*Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Amitraz*.

*Assay preparation*—Transfer an accurately measured volume of Concentrate, equivalent to about 80 mg of amitraz, to a 10-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

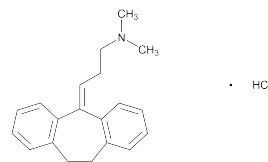
*Reference solution*—Transfer an accurately measured volume of Concentrate, equivalent to about 80 mg of amitraz, to a 10-mL volumetric flask, dilute with methyl acetate to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Amitraz*. Calculate the quantity, in mg, of amitraz ( $C_{19}H_{23}N_3$ ) in each mL of the Concentrate taken by the formula:

$$10(C/V)(R_U/R_S)$$

in which  $V$  is the volume, in mL, of Concentrate taken to prepare the *Assay preparation*; and the other terms are as defined therein.

## Amitriptyline Hydrochloride



$C_{20}H_{23}N \cdot HCl$

313.86

1-Propanamine, 3-(10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-, hydrochloride; 10,11-Dihydro-*N,N*-dimethyl-5H-dibenzo[*a,d*]cycloheptene- $\Delta^{5,7}$ -propylamine hydrochloride [549-18-8].

### DEFINITION

Amitriptyline Hydrochloride contains NLT 98.0% and NMT 102.0% of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

### ASSAY

#### Change to read:

#### • PROCEDURE

**Diluted phosphoric acid:** Phosphoric acid and water (1:10)

**Buffer:** 1.42 g/L of dibasic sodium phosphate ( $Na_2HPO_4$ ) in water, adjusted with *Diluted phosphoric acid* to a pH of 7.7

**Mobile phase:** Methanol and *Buffer* (7:3)

**System suitability stock solution A:** 1 mg/mL of USP Amitriptyline Related Compound A RS in methanol

**System suitability stock solution B:** 0.4 mg/mL of USP Amitriptyline Hydrochloride RS, 0.6 mg/mL each of USP Amitriptyline Related Compound B RS, USP Cyclobenzaprine Hydrochloride RS, and USP Nortriptyline Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

**System suitability solution:** 1  $\mu$ g/mL of amitriptyline hydrochloride, 0.5  $\mu$ g/mL of amitriptyline related compound A, and 1.5  $\mu$ g/mL each of amitriptyline related compound B, cyclobenzaprine hydrochloride, and nortriptyline hydrochloride from suitable volumes of *Standard solution*, *System suitability stock solution A*, and *System suitability stock solution B* in *Mobile phase*

**Sample solution:** 0.2 mg/mL of Amitriptyline Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature:** 45°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** 1.5 times the retention time of amitriptyline<sup>▲USP36</sup>

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—For relative retention times, see Table 1.]

#### Suitability requirements

**Resolution:** NLT 1.5 between amitriptyline related compound B and nortriptyline, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the amitriptyline peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, *Method II* (231): NMT 10 ppm

#### Change to read:

#### • ORGANIC IMPURITIES

**Diluted phosphoric acid, Buffer, Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** Use the *System suitability solution*, prepared as directed in the *Assay*.

**Sample solution:** 1 mg/mL of Amitriptyline Hydrochloride in *Mobile phase*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of the individual amitriptyline related compounds in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each amitriptyline related compound from the *Sample solution*

$r_S$  = peak response of the corresponding amitriptyline related compound from the *Standard solution*

$C_S$  = concentration of the corresponding amitriptyline related compound in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

▲ Calculate the percentage of each unspecified impurity in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of USP Amitriptyline

Hydrochloride RS from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)▲<sup>USP36</sup>

[NOTE—Discard any peak with a relative retention time less than 0.22.]

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amitriptyline related compound A	0.35	0.05
Amitriptyline related compound B	0.52	0.15
Nortriptyline ▲ <sup>USP36</sup>	0.60	0.15
Cyclobenzaprine ▲ <sup>USP36</sup>	0.76	0.15
Amitriptyline ▲ <sup>USP36</sup>	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

#### SPECIFIC TESTS

##### Delete the following:

▲ **MELTING RANGE OR TEMPERATURE** (741): 195°–199°▲<sup>USP36</sup>

• **PH** (791)

**Sample:** 10 mg/mL in water

**Acceptance criteria:** 5.0–6.0, in a solution (1 in 100)

• **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at a pressure not exceeding 5 mm of mercury at 60° to constant weight.

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Amitriptyline Hydrochloride RS

USP Amitriptyline Related Compound A RS

10,11-Dihydro-5H-dibenzo[a,d]cyclohepten-5-one; (also known as dibenzosuberone).

$C_{15}H_{12}O$  208.26

USP Amitriptyline Related Compound B RS

5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ol; (also known as amitriptynol).

$C_{20}H_{25}NO$  295.42

USP Cyclobenzaprine Hydrochloride RS

USP Nortriptyline Hydrochloride RS

## Amitriptyline Hydrochloride Injection

#### DEFINITION

Amitriptyline Hydrochloride Injection is a sterile solution of Amitriptyline Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ).

#### IDENTIFICATION

• **A.**

**Sample solution:** Pipet 1 mL of Injection into a 125-mL separator containing 10 mL of water and 1 mL of 1 N sodium hydroxide, mix, extract with two 10-mL portions of methylene chloride, and evaporate the extracts on a steam bath just to dryness. Dissolve the residue in methanol, add 1 mL of 1.2 N hydrochloric acid, and then add methanol to make 100 mL. Dilute 10 mL of this solution with methanol to 100 mL.

**Acceptance criteria:** The UV absorption spectrum of this solution exhibits a maximum at the same wavelength as

that of a similar solution of USP Amitriptyline Hydrochloride RS, concomitantly measured.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### Delete the following:

#### ▲ C. IDENTIFICATION—ORGANIC NITROGENOUS BASES <181>

**Analysis:** Pipet a volume of Injection, equivalent to 50 mg of amitriptyline hydrochloride, into a separator containing 25 mL of water. Proceed as directed in the chapter, beginning with "In a second separator," and using water in place of 0.01 N hydrochloric acid in the Reference Standard solution.

**Acceptance criteria:** The *Sample solution* so obtained meets the requirements of the test.▲<sup>USP36</sup>

#### ASSAY

##### • PROCEDURE

**Buffer:** Dissolve 11.04 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of  $2.5 \pm 0.5$ , and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer* (42:58)

**Standard solution:** 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in water

**Sample solution:** Nominally 0.2 mg/mL of amitriptyline hydrochloride from a suitable volume of the Injection in water

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

##### • PYROGEN TEST <151>

**Sample:** Amitriptyline Hydrochloride Injection, diluted with Sodium Chloride Injection containing 0.9% of sodium chloride to a concentration of 2.5 mg of amitriptyline hydrochloride/mL

**Acceptance criteria:** Meets the requirements for a test dose of 1 mL/kg

##### • pH <791>: 4.0–6.0

##### • OTHER REQUIREMENTS: Meets the requirements in *Injections* <1>

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

#### • USP REFERENCE STANDARDS <11>

USP Amitriptyline Hydrochloride RS

## Amitriptyline Hydrochloride Tablets

#### DEFINITION

Amitriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ).

#### IDENTIFICATION

##### • A.

**Sample solution:** Nominally 0.01 mg/mL of amitriptyline hydrochloride in methanol from a suitable amount of finely powdered Tablets. Filter a portion of the solution, and use the filtrate for analysis.

**Acceptance criteria:** The UV absorption spectrum of this solution exhibits a maximum at the same wavelength as that of a similar solution of USP Amitriptyline Hydrochloride RS, concomitantly measured.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 11.04 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.5$ , and dilute to make 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer* (42:58)

**Standard solution:** 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of amitriptyline hydrochloride in *Mobile phase* prepared as follows.

Transfer NLT 20 Tablets to a suitable volumetric flask, add 50% of the flask volume of *Mobile phase*, and shake the mixture for 1 h or until the Tablets have disintegrated. Dilute with *Mobile phase* to volume, and filter. Dilute the clear filtrate with *Mobile phase* to obtain a solution with a nominal concentration of 0.2 mg/mL of amitriptyline hydrochloride.

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

#### Instrumental conditions

Analytical wavelength: UV 239 nm

Standard solution: USP Amitriptyline Hydrochloride RS in Medium

#### Analysis

Samples: Standard solution and Sample solution

Determine the percentage of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) dissolved from UV absorbances of the Sample solution, suitably diluted with Medium if necessary, in comparison with a Standard solution having a known concentration.

Tolerances: NLT 75% (Q) of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers.

### • USP REFERENCE STANDARDS <11>

USP Amitriptyline Hydrochloride RS

Add the following:

## ▲Amlodipine Oral Suspension

### DEFINITION

Amlodipine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ).

Prepare Amlodipine Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Amlodipine tablets <sup>a</sup> equivalent to	100 mg
Vehicle: A 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Norvasc 5-mg tablets, Pfizer, Inc., Groton, CT.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar and comminute to a fine powder. Add the Vehicle in small portions, and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make an amlodipine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the Vehicle to bring to final volume, and mix well. [NOTE—To ensure component uniformity, homogenization is recommended.]

## ASSAY

### • PROCEDURE

Mobile phase: Acetonitrile, methanol, and 40 mM ammonium acetate, (50:15:35). Filter through a nylon 66 filter of 0.45-μm pore size, and degas.

**Standard stock solution:** Dissolve an appropriately weighed amount of USP Amlodipine Besylate RS in methanol, equivalent to 1.0 mg/mL of amlodipine (approximately equal to 1.4 mg/mL of amlodipine besylate).

**Standard solution:** Transfer 1.0 mL of the Standard stock solution into a 50-mL volumetric flask, and dilute with Mobile phase to volume to obtain a solution with a nominal concentration of about 20 μg/mL of amlodipine. Centrifuge.

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 50-mL volumetric flask, rinse the pipet three times with Mobile phase, and dilute with Mobile phase to volume to obtain a solution with a nominal concentration of about 20 μg/mL of amlodipine. Centrifuge.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.0-mm × 15-cm; 5-μm packing L10

Flow rate: 0.4 mL/min

Injection size: 10 μL

### System suitability

Sample: Standard solution

[NOTE—The retention time for amlodipine is about 10.1 min.]

### Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amlodipine from the Sample solution

$r_S$  = peak response of amlodipine from the Standard solution

$C_S$  = concentration of amlodipine in the Standard solution (μg/mL)

$C_U$  = nominal concentration of amlodipine in the Sample solution (μg/mL)

Acceptance criteria: 90.0%–110.0%

## SPECIFIC TESTS

### • PH <791>: 4.0–5.0

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.

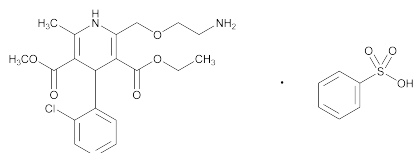
### • LABELING: Label it to indicate that it is to be well-shaken before use, and to state the Beyond-Use Date.

### • BEYOND-USE DATE: NMT 90 days after the date on which it was compounded when stored at controlled cold temperature; NMT 60 days when stored at controlled room temperature

### • USP REFERENCE STANDARDS <11>

USP Amlodipine Besylate RS▲<sup>USP36</sup>

## Amlodipine Besylate



$C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$  567.05

3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl-5-methyl ester, (±)-, monobenzenesulfonate.

3-Ethyl 5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(o-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulfonate [111470-99-6].

Monohydrate 585.07

» Amlodipine Besylate is anhydrous or hydrated and contains not less than 97.0 percent and not more than 102.0 percent of  $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, protected from light. Store at room temperature.

**USP Reference standards** (11)—

USP Amlodipine Besylate RS

**Labeling**—Where it is the hydrated form, the label so indicates.

**Identification**—

**A: Infrared Absorption** (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Optical rotation** (781A): between  $-0.10^\circ$  and  $+0.10^\circ$ , measured at  $20^\circ$ .

*Test solution*: 10 mg per mL, in methanol.

**Water**, *Method I* (921): not more than 0.5% for the anhydrous form. If labeled as the hydrated form, the limit is between 3.1% and 5.0%.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals**, *Method II* (231): 0.002%.

**Related compounds**—

TEST 1—

**Adsorbent**: 0.25-mm layer of chromatographic silica gel mixture.

*Test solution*—Transfer 140 mg of Amlodipine Besylate to a 2-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

*System suitability solution*—Transfer about 14 mg of USP Amlodipine Besylate RS to a suitable container, dissolve in 0.2 mL of methanol, and mix.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in methanol to obtain a solution containing 7.0 mg per mL.

*Standard solution 1*—Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

*Standard solution 2*—Transfer 1.0 mL of the *Standard stock solution* to another 100-mL volumetric flask, dilute with methanol to volume, and mix.

*Application volume*: 10  $\mu$ L.

*Developing solvent system*—Use the upper layer of a mixture of methyl isobutyl ketone, water, and glacial acetic acid (50:25:25).

*Procedure*—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate for 15 minutes at  $80^\circ$ . Examine the plate under UV light at 254 nm and 365 nm. The chromatogram from the *System suitability solution* shows two clearly separated minor spots with  $R_F$  values of about 0.18 and 0.22. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*. Any spot obtained from the *Test solution*, except for the principal spot, is not greater in size than the spot obtained from *Standard solution 1* (0.3%), and at most two spots are more intense than the spot obtained from *Standard solution 2* (0.1%).

TEST 2—

*pH 3.0 Buffer* and *Mobile phase*—Prepare as directed in the *Assay*.

*System suitability solution*—Dissolve about 5 mg of Amlodipine Besylate in 5 mL of hydrogen peroxide, and heat at  $70^\circ$  for 45 minutes.

*Standard solution*—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.003 mg per mL.

*Test solution*—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between amlodipine impurity A and amlodipine is not less than 4.5. [NOTE—For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate.] Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the standard deviation for replicate injections is not more than 10.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is about 3 times the retention time of amlodipine, and measure the peak responses. Calculate the percentage of each impurity in the portion of Amlodipine Besylate taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_s)$$

in which  $F$  is the relative response factor, which is equal to 0.5 for amlodipine impurity A and to 1.0 for other impurities;  $C_S$  and  $C_T$  are the concentrations, in mg per mL, of amlodipine besylate in the *Standard solution* and the *Test solution*, respectively;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for amlodipine besylate obtained from the *Standard solution*: not more than 0.3% of amlodipine impurity A is found, and not more than 0.3% of total other impurities is found. Disregard any peak less than 0.03%, and disregard any peak due to benzene sulfonate.

**Assay**—

*pH 3.0 Buffer*—Dissolve 7.0 mL of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and dilute with water to 1 L.

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 3.0 Buffer*, methanol, and acetonitrile (50:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay preparation**—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 237-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$  in the portion of Amlodipine Besylate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of amlodipine besylate in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amlodipine Besylate Tablets

### DEFINITION

Amlodipine Besylate Tablets contain NLT 90% and NMT 110% of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ).

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION <197U>

**Standard solution and Sample solution:** Prepare as directed in the test for *Dissolution*.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Add 7.0 mL of triethylamine into a 1000-mL flask containing 900 mL of water. Adjust the solution with phosphoric acid to a pH of  $3.0 \pm 0.1$ . Dilute with water to volume, and mix well.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (35:15:50)

**System suitability solution:** 0.02 mg/mL of USP Amlodipine Besylate RS and 0.002 mg/mL of USP Amlodipine Related Compound A RS in *Mobile phase*

**Standard solution:** 0.02 mg/mL of amlodipine prepared from USP Amlodipine Besylate RS in *Mobile phase*

**Sample stock solution:** Place 5 Tablets into a 500-mL volumetric flask. Add 50 mL of *Mobile phase* to the flask, and swirl to disintegrate the Tablets. Add 300 mL of *Mobile phase*, insert the stopper into the flask, and shake on a reciprocating shaker for 30 min. Dilute with *Mobile phase* to volume, and mix well.

**Sample solution:** 0.02 mg/mL of amlodipine from the *Sample stock solution* in *Mobile phase*. Pass the sample through a syringe tip filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 3.9-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The run time is about three times the retention of the amlodipine peak.]

#### Suitability requirements

**Resolution:** NLT 8.5 between amlodipine and amlodipine related compound A

**Tailing factor:** NMT 2.0 for both amlodipine and amlodipine related compound A

**Relative standard deviation:** NMT 1.0% for amlodipine and NMT 5.0% for amlodipine related compound A

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90%–110% of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ )

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

[NOTE—Do not expose any of the solutions to stainless steel because of the degradation of amlodipine.]

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 2:** 75 rpm. [NOTE—Use paddles covered with Teflon or made of any inert material except stainless steel.]

**Time:** 30 min

**Standard solution:** Make appropriate dilutions of USP Amlodipine Besylate RS in *Medium* to obtain the following concentrations: 0.00695 mg/mL for Tablets labeled to contain 2.5 mg; 0.0139 mg/mL for Tablets labeled to contain 5 mg; 0.0278 mg/mL for Tablets labeled to contain 10 mg. These solutions are stable for one day.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

**Analysis:** Determine the amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) dissolved by using UV absorption at the wavelength of maximum absorbance at about 239 nm on portions of the *Sample solution* in comparison with the *Standard solution*, using a 1-cm quartz cell and the *Medium* as blank.

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times (M_{r1}/M_{r2}) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$D$  = dilution factor of the *Sample solution*

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.06

$V$  = volume of *Medium*, 500 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard solution:** Use the *System suitability solution*.

**Sample solution:** Place a suitable number of Tablets into a 25-mL volumetric flask to obtain a solution having a final nominal concentration of 0.4 mg/mL of amlodipine. Add about 10 mL of *Mobile phase* to the flask. Swirl to disintegrate the Tablet(s), followed by sonication for 5 min to completely dissolve, and then cool the sample to room temperature. Dilute with *Mobile phase* to volume. Stir for an additional 15 min using a magnetic stir bar, and pass the sample through a syringe tip filter of 0.45-μm pore size, discarding the first 5 mL.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of amlodipine related compound A from the *Sample solution*  
 $r_S$  = peak response of amlodipine related compound A from the *Standard solution*  
 $C_S$  = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of amlodipine related compound A, 406.86  
 $M_{r2}$  = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of amlodipine glucose/galactose adduct or amlodipine lactose adduct, if present, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of the amlodipine glucose/galactose adduct or amlodipine lactose adduct in the *Sample solution*  
 $r_S$  = peak response of amlodipine in the *Standard solution*  
 $C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of amlodipine, 408.9  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of amlodipine from the *Standard solution*  
 $C_S$  = concentration of amlodipine in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amlodipine related compound A <sup>a</sup>	0.50	1.0
Amlodipine lactose adduct <sup>b</sup>	0.80	0.5
Amlodipine glucose/galactose adduct <sup>b</sup>	0.90	0.5
Amlodipine besylate	1.0	—
Any other individual unspecified degradation product	—	0.20

<sup>a</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

<sup>b</sup> Formulation-specific impurities.

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Amlodipine Besylate RS  
 USP Amlodipine Related Compound A RS  
 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate fumarate.  
 $C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$  522.93

**Aromatic Ammonia Spirit**

» Aromatic Ammonia Spirit is a hydroalcoholic solution that contains, in each 100 mL, not less than 1.7 g and not more than 2.1 g of total  $NH_3$ , and Ammonium Carbonate corresponding to not less than 3.5 g and not more than 4.5 g of  $(NH_4)_2CO_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.

**Alcohol content, Method I** (611): between 62.0% and 68.0% of  $C_2H_5OH$ .

**Assay for total  $NH_3$** —Transfer 10.0 mL to a 250-mL conical flask containing about 50 mL of water. Add 30.0 mL of 0.5 N sulfuric acid VS, and boil until the solution becomes clear. Cool, add methyl red TS, and titrate the excess acid with 0.5 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N sulfuric acid is equivalent to 8.515 mg of  $NH_3$ .

**Assay for ammonium carbonate**—Transfer 10.0 mL to a flask of about 300-mL capacity. Add 30 mL of 0.5 N sodium hydroxide, and boil the mixture, replacing the water lost by evaporation, until the vapors no longer turn moistened red litmus paper blue. Cool, dilute with 100 mL of cold, carbon dioxide-free water, add about 6 drops of phenolphthalein TS, then add just enough 0.5 N sulfuric acid VS to discharge the color of the phenolphthalein. Add methyl orange TS, and titrate with 0.5 N sulfuric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N sulfuric acid consumed in the titration with methyl orange TS is equivalent to 48.04 mg of  $(NH_4)_2CO_3$ .

## Ammonium Chloride

NH<sub>4</sub>Cl 53.49

Ammonium chloride.

Ammonium chloride [12125-02-9].

» Ammonium Chloride contains not less than 99.5 percent and not more than 100.5 percent of NH<sub>4</sub>Cl, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A solution (1 in 10) responds to the tests for *Ammonium* <191> and for *Chloride* <191>.

**pH** <791>: between 4.6 and 6.0, in a solution (1 in 20).

**Loss on drying** <731>—Dry it over silica gel for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>—Add 1 mL of sulfuric acid to about 2 g, accurately weighed, and heat the mixture gently until volatilization is complete: the residue is white, and when ignited, not more than 0.1% of nonvolatile substance remains.

**Limit of thiocyanate**—Acidify 10 mL of a solution (1 in 10) with hydrochloric acid, and add a few drops of ferric chloride TS: no orange-red color is produced.

**Heavy metals, Method I** <231>: 0.001%.

**Assay**—Transfer about 100 mg of Ammonium Chloride, accurately weighed, to a conical flask, add 10 mL of water, and swirl to dissolve. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.349 mg of NH<sub>4</sub>Cl.

## Ammonium Chloride Injection

» Ammonium Chloride Injection is a sterile solution of Ammonium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NH<sub>4</sub>Cl. Hydrochloric acid may be added to adjust the pH.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling**—The label states the content of ammonium chloride in terms of weight and of milliequivalents in a given volume. The label states also the total osmolar concentration in mOsmol per L or per mL. The label states that the Injection is not for direct injection but is to be diluted with Sodium Chloride Injection to the appropriate strength before use.

**USP Reference standards** <11>—

USP Endotoxin RS

**Identification**—It responds to the tests for *Ammonium* <191> and for *Chloride* <191>.

**Bacterial endotoxins** <85>—It contains not more than 1.72 USP Endotoxin Units per mEq of chloride.

**pH** <791>: between 4.0 and 6.0, in a concentration of not more than 100 mg of ammonium chloride per mL.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Chloride content**—Transfer an accurately measured volume of Injection, evaporated, if necessary, equivalent to about 2 g of ammonium chloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a conical flask, add 10 mL of

glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. The content of Cl is between 63.0% and 70.3% of the labeled amount of ammonium chloride.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, dilute with water to 200 mL, mix, and add 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of NH<sub>4</sub>Cl.

## Ammonium Chloride Delayed-Release Tablets

» Ammonium Chloride Delayed-Release Tablets contain not less than 94.0 percent and not more than 106.0 percent of the labeled amount of NH<sub>4</sub>Cl. Ammonium Chloride Delayed-Release Tablets are enteric-coated.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A filtered solution of finely powdered Tablets, equivalent to ammonium chloride solution (1 in 10), responds to the tests for *Ammonium* <191> and for *Chloride* <191>.

**Disintegration** <701>: 2 hours, determined as directed for *Enteric-Coated Tablets*.

**Limit of thiocyanate**—Powder and dissolve in water a sufficient number of Tablets to make about 25 mL of ammonium chloride solution (1 in 10), and filter. Acidify 10 mL of the solution with hydrochloric acid, and add a few drops of ferric chloride TS: no reddish orange color is produced.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, and add 200 mL of water and 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of NH<sub>4</sub>Cl.

## Ferric Ammonium Citrate

» Ferric Ammonium Citrate contains not less than 16.5 percent and not more than 18.5 percent of iron (Fe).

**Packaging and storage**—Preserve in tight, light-resistant containers, in a cool place.

**Identification—**

**A:** Ignite about 0.5 g; it chars, and leaves a residue of iron oxide.

**B:** To 10 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 6 N ammonium hydroxide dropwise: the solution darkens, but no precipitate forms.

**C:** To 5 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 0.3 mL of potassium permanganate TS and 4 mL of mercuric sulfate TS, and heat the mixture to boiling: a white precipitate forms.

**Ferric citrate—**To a solution of Ferric Ammonium Citrate (1 in 100) add potassium ferrocyanide TS: no blue precipitate is formed.

**Sulfate** (221)—Dissolve 100 mg in 1 mL of 2.7 N hydrochloric acid, and dilute with water to 30 mL. Add 3 mL of barium chloride TS, dilute with water to 50 mL, and mix: any turbidity formed after 10 minutes is not greater than that produced in a similarly treated control solution containing 0.31 mL of 0.020 N sulfuric acid (0.3%).

**Oxalate—**Transfer 1 g to a 125-mL separator, dissolve in 10 mL of water, add 2 mL of hydrochloric acid, and extract successively with one 50-mL portion and one 20-mL portion of ether. Transfer the combined ether extracts to a 150-mL beaker, add 10 mL of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 mL of calcium acetate solution (1 in 20): no turbidity is produced within 5 minutes.

**Mercury—**

*Mercury Stock Solution and Standard Mercury Solution—*Proceed as directed for *Method I* under *Mercury* (261).

*Mercury Detection Instrument, Aeration Apparatus, and Stannous Chloride Solution—*Proceed as directed for *Method IIa* and *Method IIb* under *Mercury* (261).

*Standard solutions—*Transfer 0.25, 0.50, 1.0, and 3.5 mL of *Standard Mercury Solution* to four separate glass-stoppered bottles, such as biological oxygen-demand bottles, of about 300-mL capacity. Dilute the contents of each bottle with water to 100 mL, and mix. These solutions contain the equivalent of 2.5, 5.0, 10.0, and 35.0 µg of mercury per mL, respectively.

*Test solution—*Transfer about 1.000 g of Ferric Ammonium Citrate, accurately weighed, to a 200-mL centrifuge bottle with a polytetrafluoroethylene-lined screw cap, and add 5 mL of nitric acid and 5 mL of hydrochloric acid. Close the bottle tightly, digest on a steam bath for 1 hour, and cool. Quantitatively transfer the solution to a suitable glass-stoppered bottle, dilute with water to 100 mL, and bubble air through the solution for 2 minutes. Prepare a reagent blank in the same manner.

*Procedure—*Add 5 mL of stannous chloride solution (1 in 10) to each solution, and immediately insert the bubbler of the *Aeration Apparatus*. Obtain the absorbances as directed by the instrument manufacturer's operating instructions. Perform a blank determination, and make any necessary correction. Plot the absorbances of the *Standard solutions* versus concentrations, in µg per mL, of mercury, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, in µg per g, of mercury in the *Test solution*: not more than 10 µg per g is found.

**Limit of lead—**

*Standard stock solution—*Dissolve about 159.8 mg of lead nitrate, accurately weighed, in 100 mL of water containing 1 mL of nitric acid. Dilute with water to 1000.0 mL, and mix.

*Standard solution—*[NOTE—Prepare this solution on the day of use.] Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, dilute with water to volume, and mix. Each mL contains the equivalent of 2 µg of lead (Pb).

*Test solution—*Transfer about 15 g of Ferric Ammonium Citrate, accurately weighed, to a 100-mL volumetric flask

(previously rinsed with nitric acid and water), dissolve in a mixture of 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.

*Procedure—*Using a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a deuterium arc background corrector, a digital readout device, and a burner head capable of handling 15% solids content, perform a blank determination with water, following the manufacturer's operating instructions. Separately aspirate portions of the *Standard solution* and the *Test solution*, and record the absorbances. Calculate the lead content, in µg per g, in the portion of Ferric Ammonium Citrate taken by the formula:

$$100(C / W)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of lead in the *Standard solution*; *W* is the weight, in g, of Ferric Ammonium Citrate taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 10 µg per g is found.

**Assay—**Transfer about 1 g of Ferric Ammonium Citrate, accurately weighed, to a 250-mL conical flask, and dissolve in 25 mL of water and 5 mL of hydrochloric acid. Add 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

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## Ferric Ammonium Citrate for Oral Solution

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» Ferric Ammonium Citrate for Oral Solution contains Ferric Ammonium Citrate and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Fe. It may contain one or more suitable flavors, colors, or stabilizing agents.

**Packaging and storage—**Preserve in tight, light-resistant containers, and store in a cool place.

**Identification—**A 6-g portion dissolves in 600 mL of water with effervescence. The collected gas meets the requirements of the test for *Bicarbonate* (191), and the resulting solution meets the requirements of the tests for *Iron* (191) and for *Citrate* (191).

**Uniformity of dosage units** (905)—

FOR POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR POWDER PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay—**Transfer about 6 g of Ferric Ammonium Citrate for Oral Solution, accurately weighed, to a 250-mL conical flask, and dissolve in 100 mL of water. Allow the gas to escape, add 5 mL of hydrochloric acid and 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 25 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe.

## Ammonium Molybdate

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  1236.00  
Molybdate ( $\text{Mo}_7\text{O}_{24}^{6-}$ ), hexaammonium, tetrahydrate;  
Hexaammonium molybdate tetrahydrate [12054-85-2].

### DEFINITION

Ammonium Molybdate contains NLT 99.3% and NMT 101.8% of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ .

### IDENTIFICATION

#### • PROCEDURE

**Sample:** 0.6 g

**Analysis:** Dissolve the *Sample* in 1.4 mL of water and 1.45 mL of ammonium hydroxide. Cool this mixture, and add slowly, with mixing, 7.2 mL of a well-cooled mixture of 3.2 mL of nitric acid and 4 mL of water. Allow to stand for 24–48 h, and pass through a sintered-glass filter. To 5 mL of the filtrate add 2 mL of dibasic sodium phosphate TS.

**Acceptance criteria:** A yellow precipitate is formed, and it is soluble in an excess of 6 N ammonium hydroxide.

### ASSAY

#### • PROCEDURE

**Sample solution:** Dissolve 0.7 g of Ammonium Molybdate in 100 mL water. Adjust with dilute nitric acid to a pH of 4.0. Add saturated hexamethylenetetramine solution to achieve a pH of 5–6.

**Analysis:** Heat the *Sample solution* to 60°, and add 0.2 mL of 0.1% 4-[2-pyridylazo]resorcinol solution in alcohol. Titrate with 0.1 M lead nitrate VS from the yellow color to the first permanent pink endpoint. Carry out a blank titration. Each mL of 0.1 M lead nitrate is equivalent to 17.66 mg of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ .

**Acceptance criteria:** 99.3%–101.8%

### IMPURITIES

#### Inorganic Impurities

#### • ARSENATE, PHOSPHATE, AND SILICATE

**Sample solution:** Dissolve 2.5 g of the analyte in 70 mL of water in a container other than glass.

**Control solution:** Dissolve 0.5 g of the analyte in 70 mL of water in a container other than glass, and add an amount of sodium silicate solution equivalent to 0.02 mg of silica ( $\text{SiO}_2$ ).

#### Analysis

**Samples:** *Sample solution* and *Control solution*  
Adjust with 1.2 N hydrochloric acid to a pH of between 3 and 4, transfer to a glass container, add 2 mL of bromine TS, and adjust with 1.2 N hydrochloric acid to a pH of  $1.8 \pm 0.1$ . Heat almost to boiling, and cool to room temperature. Dilute with water to 90 mL, add 10 mL of hydrochloric acid, and transfer to a separator. Add 1 mL of butyl alcohol and 30 mL of 4-methyl-2-pentanone, shake vigorously, and allow the phases to separate. Discard the aqueous phase, and wash the ketone phase with three successive 10-mL portions of 1.2 N hydrochloric acid, discarding the washings. To the washed ketone phase add 10 mL of 1.2 N hydrochloric acid to which has just been added 0.2 mL of a freshly prepared solution (1 in 50) of stannous chloride in hydrochloric acid.

**Acceptance criteria:** Any blue color in the *Sample solution* does not exceed that in the *Control solution* (NMT 10 ppm).

- **CHLORIDE AND SULFATE, Chloride <221>:** A 0.5-g portion shows no more chloride than 0.30 mL of 0.001 N hydrochloric acid (NMT 20 ppm).
- **CHLORIDE AND SULFATE, Sulfate <221>:** A 0.25-g portion shows no more sulfate than corresponds to 1.0 mL of 0.001 N sulfuric acid (NMT 200 ppm).

#### • HEAVY METALS <231>

**Sample stock solution:** Dissolve 2.0 g of Ammonium Molybdate in 20 mL of water, add 10 mL of 2.5 N sodium hydroxide and 2 mL of ammonium hydroxide, and dilute with water to 40 mL.

**Control solution:** To 10 mL of *Sample stock solution* add 1.0 mL of *Standard Lead Solution*, prepared as directed under *Heavy Metals <231>*, and dilute with water to 40 mL.

**Sample solution:** Dilute the remaining 30-mL portion of the *Sample stock solution* with water to 40 mL.

**Analysis:** To both the *Sample solution* and the *Control solution* add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, and allow to stand for 5 min.

**Acceptance criteria:** Any color in the *Sample solution* does not exceed that in the *Control solution* (NMT 10 ppm).

#### • INSOLUBLE SUBSTANCES

**Sample:** 20 g

**Analysis:** Dissolve the *Sample* in 200 mL of water in a beaker, heat to boiling, cover, and heat on a steam bath for 1 h. Pass the hot solution through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.

**Acceptance criteria:** The weight of the residue is NMT 1 mg (0.005%).

#### • NITRATE

**Sample:** 1 g

**Analysis:** Dissolve the *Sample* in 10 mL of water containing 5 mg of sodium chloride, and add 0.10 mL of a solution (1 in 1000) of indigo carmine in 3.6 N sulfuric acid.

**Acceptance criteria:** The blue color is not completely discharged in 5 min.

#### • MAGNESIUM AND ALKALI SALTS

**Sample:** 5.0 g

**Analysis:** Dissolve the *Sample* in 50 mL of water, and filter. To the filtrate add 0.5 g of sodium carbonate and 25 mL of 2.5 N sodium hydroxide. Boil the solution gently for 5 min, cool, and pass through an ignited and tared filter. Wash the filter with 1 N ammonium hydroxide. Ignite the filter at  $800 \pm 25^\circ$  for 30 min.

**Acceptance criteria:** The weight of the residue does not exceed 1 mg (NMT 0.02%).

#### • PHOSPHATE

**Standard solution:** Dissolve 143.3 mg of dried monobasic potassium phosphate in water to make 1000 mL, and then dilute 1.0 mL of this solution with 3 N ammonium hydroxide to 100 mL.

**Sample solution:** Dissolve 20 g of the analyte in 100 mL of 3 N ammonium hydroxide.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Add 3.5 mL of ferric nitrate solution (1 in 10), and allow to stand for 15 min. Warm gently to coagulate the precipitate, and filter. Wash the filter several times with 1.5 N ammonium hydroxide, then wash the filter with 60 mL of warm 4 N nitric acid to dissolve the residue on the filter, collecting the filtrate in a glass-stoppered, 250-mL conical flask. Add 13 mL of ammonium hydroxide, warm to 40°, add 50 mL of ammonium molybdate TS, shake for 5 min, and allow to stand at 40° for 2 h.

**Acceptance criteria:** Any yellow precipitate formed from the *Sample solution* does not exceed that from the *Standard solution* (5 ppm).

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Ammonium Molybdate Injection

» Ammonium Molybdate Injection is a sterile solution of Ammonium Molybdate in Water for Injection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of molybdenum (Mo).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling**—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

### Identification—

**A:** The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 313 nm when tested as directed for *Procedure* in the *Assay*.

**B:** Add 0.3 mL of alkaline mercuric-potassium iodide TS to 5 mL of Injection: a reddish-brown color develops.

**C:** Evaporate 50 mL of Injection on a steam bath to a volume of about 0.3 mL, and add 0.3 mL of ammonium hydroxide. Cool, and add slowly, with mixing, a well-cooled mixture of 1 mL of nitric acid and 1.2 mL of water. Allow to stand for 24 to 48 hours, and pass through a sintered-glass filter. To the filtrate add 0.5 mL of dibasic sodium phosphate TS: a yellow precipitate is formed, and it dissolves in an excess of 6 N ammonium hydroxide.

**Pyrogen** <151>—It meets the requirements, the test dose being 10 mL of Injection per kg.

**pH** <791>: between 3.0 and 6.0.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay—

**Ammonium hydroxide diluent**—Dilute 40 mL of ammonium hydroxide with water to 1000 mL. Store in a plastic bottle.

**Sodium sulfate solution**—Dissolve 1 g of sodium sulfate in water to make 100 mL.

**Molybdenum stock solution**—Transfer about 1.84 g of previously assayed Ammonium Molybdate, accurately weighed, to a 1000-mL volumetric flask, dilute with *Ammonium hydroxide diluent* to volume, and mix. This solution contains the equivalent of 1000 µg of molybdenum per mL.

**Standard preparations**—Transfer 0, 1.0, 2.0, 3.0, and 4.0 mL, respectively, of *Molybdenum stock solution* to separate 100-mL volumetric flasks, and to the respective flasks add 5.0, 4.0, 3.0, 2.0, and 1.0 mL of *Ammonium hydroxide diluent*. To each flask add 10 mL of *Sodium sulfate solution*, dilute with water to volume, and mix. These *Standard preparations* contain, respectively, 0, 10, 20, 30, and 40 µg of molybdenum per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 500 µg of molybdenum, to a 25-mL volumetric flask, add 1.25 mL of *Ammonium hydroxide diluent* and 2.5 mL of *Sodium sulfate solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the molybdenum emission line of 313.3 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>) equipped with a molybdenum hollow-cathode lamp and a nitrous oxide-acetylene reducing flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of molybdenum, and draw the straight line best fitting the five plotted points. From the graph so obtained, deter-

mine the concentration, in µg per mL, of molybdenum in the *Assay preparation*. Calculate the quantity, in µg, of molybdenum (Mo) in each mL of the Injection taken by the formula:

$$25C / V$$

in which C is the concentration, in µg per mL, of molybdenum in the *Assay preparation*; and V is the volume, in mL, of Injection taken.

## Amobarbital Sodium

$C_{11}H_{17}N_2NaO_3$  248.25

2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(3-methylbutyl)-, monosodium salt.

Sodium 5-ethyl-5-isopentylbarbiturate [64-43-7].

» Amobarbital Sodium contains not less than 98.5 percent and not more than 100.5 percent of  $C_{11}H_{17}N_2NaO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards <11>—

USP Amobarbital RS

USP Endotoxin RS

**Completeness of solution** <641>—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

### Identification—

**A:** *Infrared Absorption* <197K>: of residue obtained in the *Assay*.

**B:** Ignite about 200 mg: the residue effervesces with acid and responds to the tests for *Sodium* <191>.

**pH** <791>: not more than 11.0, in the solution prepared for the test for *Completeness of solution*.

**Loss on drying** <731>—Dry about 1 g, accurately weighed, at 105° for 4 hours: it loses not more than 2.0% of its weight.

**Heavy metals, Method II** <231>: 0.003%.

**Other requirements**—Where the label states that Amobarbital Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Amobarbital Sodium for Injection*. Where the label states that Amobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Amobarbital Sodium for Injection*.

**Assay**—Dissolve about 500 mg of Amobarbital Sodium, accurately weighed, in about 15 mL of water in a separator. To the solution add 2 mL of hydrochloric acid, shake, and completely extract the liberated amobarbital with 25-mL portions of chloroform. Test for completeness of extraction by extracting with an additional 10-mL portion of chloroform and evaporating the solvent: not more than 0.5 mg of residue remains. Filter the combined extract through a glass filter funnel into a tared beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate the combined filtrate and washings on a steam bath with the aid of a current of air, dry the residue at 105° for 30 minutes, cool, and weigh. The weight of the residue, multiplied by 1.097, represents the weight of  $C_{11}H_{17}N_2NaO_3$ .



## Amobarbital Sodium for Injection

» Amobarbital Sodium for Injection is Amobarbital Sodium suitable for parenteral use.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

**USP Reference standards** ⟨11⟩—

USP Amobarbital RS

USP Endotoxin RS

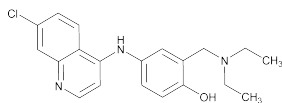
**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

**Bacterial endotoxins** ⟨85⟩—It contains not more than 0.4 USP Endotoxin Unit per mg of amobarbital sodium.

**Loss on drying** ⟨731⟩—Dry about 1 g, accurately weighed, at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Other requirements**—It conforms to the Definition, responds to the *Identification* tests, and meets the requirements for *Completeness of solution*, *pH*, *Heavy metals*, and *Assay* under *Amobarbital Sodium*. It meets also the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* under *Injections* ⟨1⟩.

## Amodiaquine



$C_{20}H_{22}ClN_3O$  355.86  
Phenol, 4-[(7-chloro-4-quinolyl)amino]-2-[(diethylamino)methyl]-;  
4-[(7-Chloro-4-quinolyl)amino]-α-(diethylamino)-o-cresol [86-42-0].

### DEFINITION

Amodiaquine contains NLT 97.0% and NMT 103.0% of amodiaquine ( $C_{20}H_{22}ClN_3O$ ), calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION ⟨197K⟩

**Standard:** Dissolve 20 mg of USP Amodiaquine Hydrochloride RS in 10 mL of water in a separator, add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.

**Acceptance criteria:** Meets the requirements

#### • B. ULTRAVIOLET ABSORPTION ⟨197U⟩

**Sample solution:** 10 µg/mL in 0.1 N hydrochloric acid

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Standard solution:** 15 µg/mL of USP Amodiaquine Hydrochloride RS in 0.1 N hydrochloric acid

**Sample solution:** 15 µg/mL of Amodiaquine in 0.1 N hydrochloric acid

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

**Mode:** UV

**Analytical wavelength:** 342 nm

**Cell:** 1 cm

**Blank:** 0.1 N hydrochloric acid

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amodiaquine ( $C_{20}H_{22}ClN_3O$ ) in the portion of Amodiaquine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Amodiaquine in the *Sample solution* (µg/mL)

$M_{r1}$  = molecular weight of amodiaquine, 355.86

$M_{r2}$  = molecular weight of anhydrous aminodiaquine hydrochloride, 428.79

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

### IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

#### • ORGANIC IMPURITIES

**Standard solution A:** To 20 mg of USP Amodiaquine Hydrochloride RS in a glass-stoppered test tube add 1.0 mL of chloroform (saturated with ammonium hydroxide), and shake vigorously for 2 min. Allow the solids to settle, and decant the liquid into a second test tube.

**Standard solution B:** *Standard solution A* and chloroform (saturated with ammonium hydroxide) (1 in 200)

**Sample solution:** 15 mg/mL of Amodiaquine in chloroform (saturated with ammonium hydroxide)

#### Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Chloroform (saturated with ammonium hydroxide) and dehydrated alcohol (9:1)

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.

**Acceptance criteria:** The chromatograms show principal spots at about the same  $R_f$  value; and no secondary spot, if present in the chromatogram from the *Sample solution*, is more intense than the principal spot obtained from *Standard solution B*.

### SPECIFIC TESTS

• **WATER DETERMINATION**, *Method I* ⟨921⟩: NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amodiaquine Hydrochloride RS

## Amodiaquine Hydrochloride

$C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$

464.81

$C_{20}H_{22}ClN_3O \cdot 2HCl$

428.79

Phenol, 4-[(7-chloro-4-quinoliny)amino]-2-[(diethylamino)-methyl]-, dihydrochloride, dihydrate;  
4-[(7-Chloro-4-quinolyl)amino]- $\alpha$ -(diethylamino)-*o*-cresol dihydrochloride dihydrate [6398-98-7].  
Anhydrous [69-44-3].

**DEFINITION**

Amodiaquine Hydrochloride contains NLT 97.0% and NMT 103.0% of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl$ ), calculated on the anhydrous basis.

**IDENTIFICATION****A. INFRARED ABSORPTION** (197K)

**Sample:** Dissolve 20 mg of Amodiaquine Hydrochloride in 10 mL of water in a separator. Add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.

**Acceptance criteria:** Meets the requirements

**B. ULTRAVIOLET ABSORPTION** (197U)

**Sample solution:** 10  $\mu$ g/mL in dilute hydrochloric acid (1 in 100)

**Acceptance criteria:** Meets the requirements

**C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements**D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****PROCEDURE**

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of  $2.5 \pm 0.5$ , and pass through a filter of 0.45- $\mu$ m pore size.

**Mobile phase:** Methanol and *Buffer* (22:78)

**System suitability solution:** 0.15 mg/mL of USP

Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Standard solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS in water

**Sample solution:** 0.15 mg/mL in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Column temperature:** 25°  $\pm$  5°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate

**Relative standard deviation:** NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl$ ) in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response from the *Sample solution*

$r_S$  = response from the *Standard solution*

$C_S$  = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amodiaquine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

**IMPURITIES****RESIDUE ON IGNITION** (281): NMT 0.2%**ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each impurity from the *Sample solution*

$r_T$  = sum of the responses from the *Sample solution*

**Acceptance criteria**

**Individual impurity:** NMT 0.5%

**SPECIFIC TESTS****WATER DETERMINATION, Method I** (921): 7.0%–9.0%**COMPLETENESS OF SOLUTION** (641): A solution of 200 mg in 10 mL of water is clear.**ADDITIONAL REQUIREMENTS****PACKAGING AND STORAGE:** Preserve in tight containers.**USP REFERENCE STANDARDS** (11)

USP Amodiaquine Hydrochloride RS

USP Chloroquine Phosphate RS

## Amodiaquine Hydrochloride Tablets

**DEFINITION**

Amodiaquine Hydrochloride Tablets contain an amount of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$ ) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of amodiaquine ( $C_{20}H_{22}ClN_3O$ ).

**IDENTIFICATION****A. INFRARED ABSORPTION** (197K)

**Sample:** Powder 1 or more Tablets, and transfer a portion of the powder, equivalent to 50 mg of amodiaquine, to a 125-mL separator. Add 20 mL of water, and shake for 1 min. Add 25 mL of chloroform and 1 mL of ammonium hydroxide, shake for 2 min, and when settled, filter the chloroform extract through cotton that previously has been rinsed with chloroform, collecting the extract in a vessel suitable for evaporation. Evaporate the chloroform, and dry the residue at 105° for 1 h.

**Acceptance criteria:** Meet the requirements

**B.** The retention time of the amodiaquine hydrochloride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****Change to read:****PROCEDURE**

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45- $\mu$ m pore size.

**Diluent:** 1% (v/v) hydrochloric acid in water

**Mobile phase:** Methanol and *Buffer* (22:78)

**Standard solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water ▲USP36

**Sample solution:** Transfer a quantity equivalent to 7.5 mg of amodiaquine hydrochloride from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume. Sonicate for 25 min at 29°. Pass 10 mL through a nylon filter of 0.2-μm pore size, discarding the first 4 mL. Use 2 mL for the analysis.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** ▲Standard solution ▲USP36

[NOTE—The relative retention times for the chloroquine and amodiaquine peaks are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate

**Relative standard deviation:** NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of amodiaquine (C<sub>20</sub>H<sub>22</sub>ClN<sub>3</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of ▲amodiaquine in ▲USP36 USP Amodiaquine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amodiaquine ▲USP36 in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 342 nm

**Standard solution:** USP Amodiaquine Hydrochloride RS in *Medium*

**Sample solution:** Filter portions of the solution under test, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Amodiaquine Hydrochloride RS.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Determine the amount of amodiaquine hydrochloride (C<sub>20</sub>H<sub>22</sub>ClN<sub>3</sub>O · 2HCl · 2H<sub>2</sub>O) dissolved from UV absorbances.

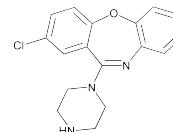
**Tolerances:** An amount of amodiaquine hydrochloride (C<sub>20</sub>H<sub>22</sub>ClN<sub>3</sub>O · 2HCl · 2H<sub>2</sub>O) equivalent to NLT 75% (Q) of the labeled amount of amodiaquine (C<sub>20</sub>H<sub>22</sub>ClN<sub>3</sub>O) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS <11>**  
USP Amodiaquine Hydrochloride RS  
USP Chloroquine Phosphate RS

## Amoxapine



C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O 313.78  
Dibenz[*b,f*][1,4]oxazepine, 2-chloro-11-(1-piperazinyl)-;  
2-Chloro-11-(1-piperazinyl)dibenz[*b,f*][1,4]oxazepine  
[14028-44-5].

#### DEFINITION

Amoxapine contains NLT 98.5% and NMT 101.0% of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION <197K>**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** 11.3 g of tetramethylammonium chloride in 100 mL of water

**Solution B:** Phosphoric acid (1 in 5)

**Buffer:** 1.4 g/L of monobasic sodium phosphate

**Mobile phase:** Dilute a mixture of 360 mL of acetonitrile, 20 mL of *Solution A*, and 2 mL of *Solution B* with *Buffer* to 1 L.

**System suitability solution:** 0.1 mg/mL of USP Amoxapine RS and 0.1 mg/mL of USP Loxapine Succinate RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**Standard solution:** 0.1 mg/mL of USP Amoxapine RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**Sample solution:** 0.1 mg/mL of Amoxapine in *Mobile phase*. Sonication may be used to aid in dissolution.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

[NOTE—The relative retention times for amoxapine and loxapine are 1.0 and 1.2, respectively.]

**Resolution:** NLT 2.5 between amoxapine and loxapine, *System suitability solution*

**Tailing factor:** NMT 1.8, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O) in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of amoxapine from the *Sample solution*  
 $r_S$  = peak response of USP Amoxapine RS from the *Standard solution*  
 $C_S$  = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Amoxapine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–101.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **ORGANIC IMPURITIES**

**Standard solution A:** 0.5 mg/mL of USP Amoxapine RS in chloroform

**Standard solution B:** 0.25 mg/mL of USP Amoxapine RS in chloroform, from *Standard solution A*

**Sample solution:** 50 mg/mL of Amoxapine in chloroform

**Developing solvent system:** Chloroform, methanol, and ammonium hydroxide (18: 2: 0.1)

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
**Mode:** TLC

**Adsorbent:** 0.2-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, examine it under short-wave-length UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatogram of the *Standard solutions*.

**Acceptance criteria:** No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B* (0.5%), and the sum of the intensities of the secondary spots of the *Sample solution* corresponds to NMT 1.0%.

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Amoxapine RS

USP Loxapine Succinate RS

### Amoxapine Tablets

» Amoxapine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amoxapine ( $C_{17}H_{16}ClN_3O$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Amoxapine RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Triturate a quantity of finely ground Tablets, equivalent to about 50 mg of amoxapine, with 10 mL of chloroform, and filter. Evaporate the filtrate on a steam bath to dryness (about 30 minutes).

#### Dissolution (711)—

**Medium:** simulated gastric fluid (without enzyme); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{16}ClN_3O$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 294 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Amoxapine RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{16}ClN_3O$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**0.01 M Monobasic sodium phosphate**—Dissolve 2.76 g of monobasic sodium phosphate in 2000 mL of water, and mix.

**1 M Tetramethylammonium chloride**—Dissolve 11.3 g of tetramethylammonium chloride in 100 mL of water, and mix.

**Mobile phase**—Transfer 40.0 mL of 1 M Tetramethylammonium chloride, 4.0 mL of dilute phosphoric acid (1 in 5), and 720 mL of acetonitrile to a 2000-mL volumetric flask. Dilute with 0.01 M Monobasic sodium phosphate to volume, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 50 mg of USP Amoxapine RS, accurately weighed, to a 50-mL volumetric flask, add 30 mL of acetonitrile, and shake by mechanical means to dissolve. Dilute with acetonitrile to volume, and mix. Quantitatively dilute a portion of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of amoxapine, to a 50-mL volumetric flask, add 40 mL of *Mobile phase*, and shake vigorously by mechanical means for 20 minutes. Dilute with *Mobile phase* to volume, mix, and filter. Pipet 5.0 mL of the filtrate into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

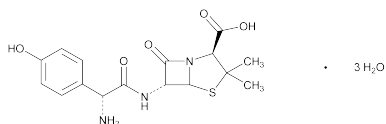
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 1.8, the column efficiency determined from the analyte peak is not less than 1200 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of amoxapine ( $C_{17}H_{16}ClN_3O$ ) in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Amoxapine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the amoxapine peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amoxicillin



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$  419.45  
 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-, trihydrate [2S-[2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ (S\*)]]-; (2S,5R,6R)-6-[(R)-(-)-2-Amino-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [61336-70-7].  
 Anhydrous 365.41  
 [26787-78-0].

### DEFINITION

Amoxicillin contains NLT 900  $\mu$ g and NMT 1050  $\mu$ g of  $C_{16}H_{19}N_3O_5S$  per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** <197K>

### ASSAY

#### • PROCEDURE

**Diluent:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Diluent* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]

**Sample solution:** 1.2 mg/mL of Amoxicillin in *Diluent*. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g/mg, of  $C_{16}H_{19}N_3O_5S$  in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

**Acceptance criteria:** 900–1050  $\mu$ g of  $C_{16}H_{19}N_3O_5S$  per mg on the anhydrous basis

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**Solution A:** 2.72 g/L of monobasic potassium phosphate. Adjust with 1 N potassium hydroxide or 20% phosphoric acid to a pH of  $5.0 \pm 0.1$ .

#### Solution B: Methanol

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	97	3
10	97	3
22	75	25
26	97	3

**Standard solution:** 12.5  $\mu$ g/mL of USP Amoxicillin RS in *Solution A*

**System suitability solution:** 12.5  $\mu$ g/mL each of USP Amoxicillin Related Compound A RS and USP Amoxicillin Related Compound D RS in *Solution A*

**Sample solution:** 1.25 mg/mL of Amoxicillin in *Solution A*. [NOTE—Store this solution at 4° and use within 4 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

**Autosampler temperature:** 4°

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

[NOTE— Identify peaks by the relative retention times in *Impurity Table 1*.]

**Resolution:** NLT 1.5 between amoxicillin related compound A and the second peak for amoxicillin related compound D, *System suitability solution*

**Relative standard deviation:** NMT 10%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of Amoxicillin in the *Sample solution* (mg/mL)

$F$  = unit conversion factor (0.001 mg/ $\mu$ g)

#### Acceptance criteria

[NOTE—The reporting limit is 0.03% of the amoxicillin peak from the *Standard solution*.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 5.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I <sup>a</sup> (D-hydroxyphenylglycine)	0.32	1.0
Amoxicillin related compound D <sup>b,c</sup> (amoxicillin open ring)	0.53	1.0
	0.68	1.0
Amoxicillin related compound A <sup>d</sup> (6-aminopenicillanic acid)	0.78	0.5
Amoxicillin related compound B <sup>e,f</sup> (L-amoxicillin)	0.87	—
Amoxicillin	1.0	—
Amoxicillin related compound G <sup>g</sup> (D-hydroxyphenyl-glycylamoxicillin)	2.9	1.0
Amoxicillin related compound E <sup>h,i</sup> (amoxicillin penilloic derivative)	4.5	1.0
Amoxicillin related compound M <sup>i</sup> (N-(penicillan-6-yl) open ring amoxicillinamide)	6.0	1.0
Amoxicillin related compound F <sup>e,k</sup> (phenylpyrazinediol)	6.3	—
Amoxicillin related compound C <sup>l</sup> (amoxicillin rearrangement product)	6.4	1.0
Amoxicillin related compound E <sup>h,i</sup> (amoxicillin penilloic derivative)	6.7	1.0
Amoxicillin related compound J <sup>m</sup> (amoxicillin open ring dimer)	8.8	1.0
Amoxicillin related compound L <sup>n</sup> (N-(penicillan-6-yl) amoxicillinamide)	9.0	1.0
Any unspecified individual impurity	—	1.0

<sup>a</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> The chromatographic system resolves two penicilloic acids from each other.

<sup>c</sup> (4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>e</sup> These compounds are listed for information only and are not to be reported.

<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> The chromatographic system resolves two penilloic acids from each other.

<sup>i</sup> (4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>j</sup> (2S,5R,6R)-6-(2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>k</sup> 3-(4-Hydroxyphenyl)pyrazin-2-ol.

<sup>l</sup> (4S)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>m</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>n</sup> (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

## SPECIFIC TESTS

- **CRYSTALLINITY** <695>: Meets the requirements
- **DIMETHYLANILINE** <223>: Meets the requirement
- **PH** <791>: 3.5–6.0  
Sample solution: 2 mg/mL
- **WATER DETERMINATION, Method I** <921>: 11.5%–14.5%
- **STERILITY TESTS** <71>: Where the label states that Amoxicillin is sterile, it meets the requirements when tested as

directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

- **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Amoxicillin is sterile or Amoxicillin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.25 USP Endotoxin Unit/mg of amoxicillin.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is intended for veterinary use only and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Label all other Amoxicillin to indicate that it is to be used in the manufacture of nonparenteral drugs only.
- **USP REFERENCE STANDARDS** <11>
  - USP Amoxicillin RS
  - USP Amoxicillin Related Compound A RS  
(2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; 6-aminopenicillanic acid.  
C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S 216.26
  - USP Amoxicillin Related Compound D RS  
(4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid; amoxicillin open ring.  
C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>S 383.42
  - USP Endotoxin RS

## Amoxicillin Boluses

» Amoxicillin Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amoxicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label Boluses to indicate that they are for veterinary use only.

**USP Reference standards** <11>—

USP Amoxicillin RS

**Identification**—

*Test solution*—To a portion of powdered Boluses add 0.1 N hydrochloric acid to obtain a *Test solution* containing about 4 mg of amoxicillin per mL. Use within 10 minutes after preparation.

*Application volume, Developing solvent system, Procedure*—Proceed as directed for the *Identification* test under *Amoxicillin Tablets*.

**Disintegration** <701>: 30 minutes, simulated gastric fluid being used instead of water.

**Water, Method I** (921): not more than 7.5%.

#### Assay—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under Amoxicillin.

*Assay preparation*—Weigh and finely powder not fewer than 5 Boluses. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of amoxicillin, to a 250-mL volumetric flask, add *Diluent* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the amoxicillin. Pass a portion of this solution through a filter of 1- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*. [NOTE—Use this solution within 6 hours.]

*Procedure*—Proceed as directed for *Procedure* in the Assay under Amoxicillin. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the portion of Boluses taken by the formula:

$$0.25CP(r_U / r_S)$$

in which the terms are as defined therein.

## Amoxicillin Capsules

### DEFINITION

Amoxicillin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity, equivalent to 200 mg of anhydrous amoxicillin, to a 200-mL volumetric flask. Add *Buffer* to volume. Sonicate if necessary to ensure complete dissolution. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in the portion of Capsules taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

##### Test 1

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm, for Capsules containing 250 mg

**Apparatus 2:** 75 rpm, for Capsules containing 500 mg

**Time:** 60 min

**Analytical wavelength:** UV 272 nm

**Standard solution:** USP Amoxicillin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 90 min

**Analytical wavelength:** UV 272 nm

**Standard solution:** USP Amoxicillin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- USP REFERENCE STANDARDS (11)**  
USP Amoxicillin RS

## Amoxicillin and Clavulanate Potassium for Oral Suspension

### DEFINITION

Amoxicillin and Clavulanate Potassium for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $4.4 \pm 0.1$ , and dilute with water to 1000 mL.

**Mobile phase:** Methanol and *Buffer* (1:19). Pass through a suitable filter.

**Standard solution:** 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

**Sample solution:** Nominally 0.5 mg/mL of amoxicillin in water, prepared as follows. Constitute Amoxicillin and Clavulanate Potassium for Oral Suspension with water using the volume specified in the labeling. Stir by mechanical means for 10 min, and filter. Use within 1 h.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks

**Tailing factor:** NMT 1.5 for each analyte peak

**Relative standard deviation:** NMT 2.0% for each analyte peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the *Sample solution*

$r_S$  = peak response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Calculate the percentage of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response of clavulanic acid from the *Sample solution*

$r_S$  = peak response of clavulanic acid from the *Standard solution*

$C_S$  = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)

$P$  = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

**Acceptance criteria:** 90.0%–120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and 90.0%–125.0% of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ )

**PERFORMANCE TESTS****• DELIVERABLE VOLUME <698>**

For powder packaged in multiple-unit containers: Meets the requirements

**• UNIFORMITY OF DOSAGE UNITS <905>**

For powder packaged in single-unit containers: Meets the requirements

**SPECIFIC TESTS****• pH <791>**

**Sample solution:** Constitute as directed in the labeling, and perform the test immediately after constitution.

**Acceptance criteria:** 3.8–6.6

**• MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>**

The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:**

Preserve in tight containers, at controlled room temperature.

**• USP REFERENCE STANDARDS <11>**

USP Amoxicillin RS

USP Clavulanate Lithium RS

## Amoxicillin and Clavulanate Potassium Tablets

**DEFINITION**

Amoxicillin and Clavulanate Potassium Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amounts of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and clavulanic acid ( $C_8H_9NO_5$ ).

**IDENTIFICATION**

The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $4.4 \pm 0.1$ , and dilute with water to 1000 mL.

**Mobile phase:** Methanol and *Buffer* (1:19). Pass through a suitable filter.

**Standard solution:** 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

**Sample stock solution:** Dissolve NLT 10 Tablets in water with the aid of mechanical stirring. Transfer to a suitable volumetric flask, and dilute with water to volume.

**Sample solution:** Dilute a suitable volume of the *Sample stock solution* with water to obtain a solution containing 0.5 mg/mL of amoxicillin. [NOTE—Use the *Sample solution* within 1 h.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks



**Tailing factor:** NMT 1.5 for each analyte peak  
**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the *Sample solution*

$r_S$  = peak response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of USP Amoxicillin RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

Calculate the percentage of  $C_8H_9NO_5$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response of clavulanic acid from the *Sample solution*

$r_S$  = peak response of clavulanic acid from the *Standard solution*

$C_S$  = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)

$P$  = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701): Tablets labeled for veterinary use only; 30 min, simulated gastric fluid TS being substituted for water in the test

- **DISSOLUTION** (711)

[NOTE—Tablets labeled for veterinary use only are exempt from this requirement.]

##### Test 1

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min; or 45 min where the Tablets are labeled as chewable

**Analysis:** Determine the amount of  $C_{16}H_{19}N_3O_5S$  and  $C_8H_9NO_5$  dissolved, using the *Analysis* set forth in the *Assay*, making any necessary volumetric adjustments.

**Tolerances:** NLT 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  and NLT 80% (Q) of the labeled amount of  $C_8H_9NO_5$  are dissolved.

**For Tablets labeled as chewable:** NLT 80% (Q) of the labeled amounts of  $C_{16}H_{19}N_3O_5S$  and  $C_8H_9NO_5$  is dissolved in 45 min.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus 2, and Analysis:** Proceed as directed for *Test 1*.

**Times:** 45 min for amoxicillin, and 30 min for clavulanic acid

**Tolerances:** NLT 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  and NLT 80% (Q) of the labeled amount of  $C_8H_9NO_5$  are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921):

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤250	7.5
>250 and ≤500	10.0
>500	11.0

For products labeled as chewable Tablets:

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤125	6.0
>125	8.0

For Tablets labeled for veterinary use only: NMT 10.0%

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label chewable Tablets to include the word “chewable” in juxtaposition to the official name. The labeling indicates that chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labeled. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
 USP Amoxicillin RS  
 USP Clavulanate Lithium RS

### Amoxicillin Intramammary Infusion

» Amoxicillin Intramammary Infusion is a suspension of Amoxicillin in a suitable vegetable oil vehicle. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ). It contains a suitable dispersing agent and preservative.

**Packaging and storage**—Preserve in well-closed disposable syringes.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards** (11)—  
 USP Amoxicillin RS

**Identification**—Transfer a quantity of Intramammary Infusion, equivalent to about 60 mg of amoxicillin, to a 50-mL centrifuge tube, add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for about 30 seconds after each addition of toluene. Dry the residue in vacuum over silica gel. Add 15 mL of 0.1 N hydrochloric acid to the residue, and mix. The solution obtained responds to the *Identification* test under *Amoxicillin Capsules*.

**Water**, *Method I* (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed for amoxicillin under *Antibiotics—Microbial Assays* (81). Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of Buffer No. 3 and 1.0 mL of polysorbate 80, and blend for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with Buffer No. 3 to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amoxicillin for Injectable Suspension

» Amoxicillin for Injectable Suspension is a sterile mixture of Amoxicillin and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Amoxicillin RS

USP Endotoxin RS

**Identification**—Prepare a test solution containing the equivalent of 4 mg of amoxicillin per mL by adding 0.1 N hydrochloric acid to Amoxicillin for Injectable Suspension. Allow the solution to stand for 5 minutes before use: the solution responds to the *Identification* test under *Amoxicillin Capsules*.

**Bacterial endotoxins** (85)—It contains not more than 0.25 Endotoxin Unit per mg of amoxicillin.

**Sterility** (71)—It meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

**pH** (791): between 5.0 and 7.0, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): between 11.0% and 14.0%.

**Assay**—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Amoxicillin*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and quantitatively dilute with *Diluent* to obtain a solution containing about 1 mg of anhydrous amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1- $\mu$ m or finer porosity, and use the filtrate as *Assay preparation 1*. Use this solution within 6 hours.

*Assay preparation 2* (where the label states the quantity of amoxicillin in a given volume of constituted suspension)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Quantitatively dilute an accurately measured volume of the constituted suspension with *Diluent* to obtain a solution containing about 1 mg of anhydrous

amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1- $\mu$ m or finer porosity, and use the filtrate as *Assay preparation 2*. Use this solution within 6 hours.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Amoxicillin*. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the container, or in the portion of constituted Suspension taken by the formula:

$$(L / D)(CP / 1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of anhydrous amoxicillin in the container, or in the volume of constituted suspension taken; *D* is the concentration, in mg of anhydrous amoxicillin per mL, of *Assay preparation 1* or of *Assay preparation 2* on the basis of the labeled quantity in the container or in the portion of constituted suspension taken, respectively, and the extent of dilution; and the other terms are as defined therein.

## Amoxicillin Oral Suspension

» Amoxicillin Oral Suspension is a suspension of Amoxicillin in Soybean Oil. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

**Packaging and storage**—Preserve in multiple-dose containers equipped with a suitable dosing pump.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Amoxicillin RS

**Identification**—Shake a portion of Oral Suspension with a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing about 1 mg of amoxicillin per mL. The solution responds to the *Identification* test under *Amoxicillin Capsules*.

**Water, Method I** (921): not more than 2.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Amoxicillin RS.

*Assay preparation*—Using the dosing pump, deliver a number of doses of Oral Suspension, equivalent to about 250 mg of amoxicillin, to a separator containing 100 mL of hexanes, and shake vigorously. Add 140 mL of water, and shake for 5 minutes. Allow the layers to separate, and drain the lower, aqueous layer into a 250-mL volumetric flask. Repeat the extraction with two 50-mL portions of water. Combine the aqueous extracts in the volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed with Oral Suspension as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), using USP Amoxicillin RS. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in each dose of Oral Suspension taken by the formula:

$$(250 / N)(F / 2000)(B - I)$$

in which *N* is the number of doses taken, and the other terms are as defined therein.

## Amoxicillin for Oral Suspension

### DEFINITION

Amoxicillin for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Dilute a measured volume of Amoxicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, quantitatively and stepwise in *Buffer* to obtain a solution containing nominally 1 mg/mL of anhydrous amoxicillin. Pass a portion of this solution through a suitable filter. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in the Amoxicillin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

#### UNIFORMITY OF DOSAGE UNITS (905)

- For solids packaged in single-unit containers: Meets the requirements

- DELIVERABLE VOLUME** (698): Meets the requirements

### SPECIFIC TESTS

- PH** (791): 5.0–7.5, in the suspension constituted as directed in the labeling

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS** (11)  
USP Amoxicillin RS

## Amoxicillin Tablets

### DEFINITION

Amoxicillin Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Place NLT 5 Tablets in a high-speed glass blender jar containing *Buffer* sufficient to yield a concentration of 1 mg/mL of anhydrous amoxicillin. Blend for  $4 \pm 1$  min, allow to stand for 5 min, and centrifuge a portion of the mixture. [NOTE—Where the volume of *Buffer* required would exceed 500 mL, place 5 Tablets in a volumetric flask of such capacity that when finally diluted to volume, a concentration of 1 mg of anhydrous amoxicillin per mL would be obtained. Add a volume of *Buffer* equivalent to three-fourths of the capacity of the volumetric flask, and sonicate for 5 min. Dilute with *Buffer* to volume, add a magnetic stirring bar, and stir for 30 min. Centrifuge a portion of this solution.]

Pass a portion of the clear supernatant through a suitable filter. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the *Sample solution*

$r_S$  = peak response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS**• **DISSOLUTION** <711>**Medium:** Water; 900 mL**Apparatus 2:** 75 rpm**Time:** 30 minDetermine the amount of  $C_{16}H_{19}N_3O_5S$  dissolved by using the following method.**Buffer:** 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to obtain 4 L of solution.**Mobile phase:** Acetonitrile and *Buffer* (1:39)**Standard solution:** 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]**Sample solution:** Pass a portion of the sample through a suitable filter of 0.5- $\mu$ m pore size. Quantitatively dilute a volume of the filtrate with water to obtain an estimated concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column****Analytical:** 3.9-mm  $\times$  30-cm; packing L1**Guard:** 2-mm  $\times$  2-cm; packing L2**Column temperature:** The analytical column is maintained at a constant temperature of  $40 \pm 1^\circ$ .**Flow rate:** 0.7 mL/min**Injection size:** 10  $\mu$ L**System suitability****Sample:** *Standard solution***Suitability requirements****Capacity factor:** 1.1–2.8**Column efficiency:** NLT 1700 theoretical plates**Tailing factor:** NMT 2.5**Relative standard deviation:** NMT 1.5%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (D/V) \times P \times F \times 100$$

 $r_U$  = peak response of amoxicillin from the *Sample solution* $r_S$  = peak response of amoxicillin from the *Standard solution* $C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL) $L$  = label claim (mg/Tablet) $D$  = dilution factor for the *Sample solution* $V$  = volume of the dissolution medium, 900 mL $P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg) $F$  = conversion factor, 0.001 mg/ $\mu$ g**Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.**For products labeled as chewable Tablets:** Proceed as directed above.**For chewable Tablets labeled to contain 200 or 400 mg****Time:** 20 min**Tolerances:** NLT 70% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.**For chewable Tablets labeled to contain 125 or 250 mg****Time:** 90 min**Tolerances:** NLT 70% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.**For veterinary products:** Proceed as directed above, except use *Apparatus 2* at 100 rpm.**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended solely for veterinary use are so labeled.
- **USP REFERENCE STANDARDS** <11>  
USP Amoxicillin RS

**Amoxicillin Tablets for Oral Suspension****DEFINITION**Amoxicillin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>**Standard solution:** 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid. Use within 10 min of preparation.**Sample solution:** An aqueous dispersion of Tablets for Oral Suspension in 0.1 N hydrochloric acid containing 4 mg/mL of amoxicillin. Use within 10 min of preparation.**Chromatographic system****Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volume:** 5  $\mu$ L**Developing solvent system:** Methanol, chloroform, pyridine, and water (90:80:1:30)**Spray reagent:** 3 mg/mL of ninhydrin in alcohol**Analysis****Samples:** *Standard solution* and *Sample solution*Proceed as directed in the chapter. Dry the plate with the aid of a current of warm air for 10 min. Spray lightly with *Spray reagent*, and dry at  $110^\circ$  for 15 min.**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.**ASSAY**• **PROCEDURE****Diluent:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .**Mobile phase:** Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. Use this solution within 6 h.**Sample solution:** Prepare a dispersion of 20 Tablets for Oral Suspension using a suitable aliquot of water. Dilute a portion of the dispersion with *Diluent* to obtain a solution containing 1.2 mg/mL of amoxicillin. Pass a portion of the solution through a filter of 1- $\mu$ m or finer pore size. Use this solution within 6 h.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)

Mode: LC  
 Detector: UV 230 nm  
 Column: 4-mm × 25-cm; packing L1  
 Flow rate: 1.5 mL/min  
 Injection volume: 10 µL

**System suitability**

Sample: *Standard solution*  
**Suitability requirements**  
 Capacity factor: 1.1–2.8  
 Column efficiency: NLT 1700 theoretical plates  
 Tailing factor: NMT 2.5  
 Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of amoxicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)  
 $P$  = potency of amoxicillin in USP Amoxicillin RS (µg/mg)  
 $F$  = conversion factor, 0.001 mg/µg  
 Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISINTEGRATION** <701>  
 Medium: Water at 20 ± 5°  
 Time: 3 min  
 Acceptance criteria: Meet the requirements
- **DISSOLUTION** <711>  
 Medium: Water; 900 mL  
 Apparatus 2: 75 rpm  
 Time: 30 min  
 Buffer: 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1, and dilute with water to obtain 4 L of solution.  
 Mobile phase: Acetonitrile and *Buffer* (10:390). Pass through a filter of 0.5-µm or finer pore size.  
 Standard solution: 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. Use this solution within 6 h.  
 Sample solution: Pass a portion of the sample through a filter of 0.5-µm or finer pore size. Dilute a suitable aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC  
 Detector: UV 230 nm  
 Columns  
 Guard: 2-mm × 2-cm; packing L2  
 Analytical: 3.9-mm × 30-cm; packing L1  
 Column temperature: 40 ± 1°  
 Flow rate: 0.7 mL/min  
 Injection volume: 10 µL

**System suitability**

Sample: *Standard solution*  
**Suitability requirements**  
 Capacity factor: 1.1–2.8  
 Column efficiency: NLT 1700 theoretical plates  
 Tailing factor: NMT 2.5  
 Relative standard deviation: NMT 1.5%

**Analysis**

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of amoxicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times P \times F \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)  
 $V$  = volume of medium, 900 mL  
 $D$  = dilution factor  
 $P$  = potency of amoxicillin in USP Amoxicillin RS (µg/mg)  
 $F$  = conversion factor, 0.001 mg/µg  
 $L$  = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of amoxicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

**SPECIFIC TESTS**

- **DISPERSION FINENESS**: Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion that passes through a No. 25 sieve is obtained.

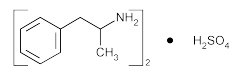
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
 USP Amoxicillin RS

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**Amphetamine Sulfate**


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(C<sub>9</sub>H<sub>13</sub>N)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> 368.49  
 Benzeneethanamine, α-methyl-, sulfate (2:1), (±)-;  
 (±)-α-Methylphenethylamine sulfate (2:1) [60-13-9].

**DEFINITION**

Amphetamine Sulfate contains NLT 98.0% and NMT 102.0% of (C<sub>9</sub>H<sub>13</sub>N)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197M>
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>: Meets the requirements  
 Sample solution: 100 mg/mL

**ASSAY****PROCEDURE**

**Solution A:** Add 5.0 mL of trifluoroacetic acid to 900 mL of water, adjust with ammonium hydroxide to a pH of 2.2 ± 0.1, and add 100 mL of acetonitrile.

**Solution B:** Use degassed acetonitrile.  
**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	65	35
20	0	100
22	0	100
23	100	0
30	100	0

**Standard solution:** 2.0 mg/mL of USP Dextroamphetamine Sulfate RS in *Solution A*

**System suitability solution:** Transfer 40 mL of the *Standard solution* to a 50-mL volumetric flask. Using a microliter syringe, add 1 µL each of USP Dextroamphetamine Related Compound A RS and USP Dextroamphetamine Related Compound B RS. Dilute with *Standard solution* to volume, and mix.

**Sample solution:** 2.0 mg/mL of Amphetamine Sulfate in *Solution A*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Identify the peaks by the relative retention times in *Impurity Table 1* under *Organic Impurities*. Amphetamine and dextroamphetamine have exactly the same retention time.]

#### Suitability requirements

**Resolution:** NLT 3.0 between dextroamphetamine related compound A and dextroamphetamine related compound B, *System suitability solution*

**Tailing factor:** NMT 3.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of (C<sub>9</sub>H<sub>13</sub>N)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for amphetamine sulfate from the *Sample solution*

$r_S$  = peak response for dextroamphetamine sulfate from the *Standard solution*

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amphetamine Sulfate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

##### Inorganic Impurities

• **RESIDUE ON IGNITION** <281>: NMT 0.2%

##### Organic Impurities

##### • PROCEDURE

*Solution A*, *Solution B*, *Mobile phase*, *System suitability solution*, *Standard solution*, *Sample solution*,

**Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Identify the impurities by the relative retention times in *Impurity Table 1*.]

Calculate the percentage of each impurity in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for dextroamphetamine from the *Standard solution*

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amphetamine Sulfate in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cathinone	0.81	55.6	0.25
Amphetamine	1.0	1.0	—
Benzaldehyde	1.73	105.3	0.25
Dextroamphetamine related compound A	1.88	1.5	0.25
Dextroamphetamine related compound B	2.05	1.8	0.25
Individual unspecified impurity	—	1.0	0.1

#### SPECIFIC TESTS

- **LOSS ON DRYING** <731>: Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.
- **DEXTROAMPHETAMINE:** A solution (20 mg/mL) is optically inactive.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>
  - USP Dextroamphetamine Sulfate RS
  - USP Dextroamphetamine Related Compound A RS
  - 1-Phenyl-2-propanol.
  - C<sub>9</sub>H<sub>12</sub>O 136.20 [CAS-14898-87-4]
  - USP Dextroamphetamine Related Compound B RS
  - Phenyl acetone.
  - C<sub>9</sub>H<sub>10</sub>O 134.18 [CAS-103-79-7]

## Amphetamine Sulfate Tablets

### DEFINITION

Amphetamine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ .

### IDENTIFICATION

#### • A. MELTING RANGE OR TEMPERATURE, Class I (741)

**Sample:** Macerate a quantity of powdered Tablets, equivalent to 50 mg of amphetamine sulfate, with 10 mL of water for 30 min, and filter into a small flask. To the filtrate add 3 mL of 1 N sodium hydroxide. Cool to 10°–15°, add 1 mL of a mixture of absolute ether and benzoyl chloride (2:1), insert the stopper, and shake well for 3 min. Filter the precipitate, wash with 15 mL of cold water, and recrystallize twice from diluted alcohol. Dry the residue at 80° for 2 h.

**Acceptance criteria:** The crystals of the benzoyl derivative of amphetamine melt between 131° and 135°.

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluted acetic acid:** 14 mL of glacial acetic acid in 100 mL of water

**Mobile phase:** Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of diluted acetic acid and 450 mL of methanol. Adjust with glacial acetic acid to a pH of  $3.3 \pm 0.1$ . Pass through a 0.5- $\mu$ m membrane filter.

**Standard solution:** 0.3 mg/mL of USP Dextroamphetamine Sulfate RS in 0.12 N phosphoric acid

**Sample solution:** Nominally 0.3 mg/mL of amphetamine sulfate from NLT 20 finely powdered Tablets prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 80% of the flask volume of 0.12 N phosphoric acid, and sonicate for 15 min. Dilute with 0.12 N phosphoric acid to volume. Pass through a 0.5- $\mu$ m membrane filter, discarding the first 20 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min. [NOTE—A 4.6-mm  $\times$  25-cm column; 5- $\mu$ m packing L1 may be used with a flow rate of 1 mL/min.]

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 3

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amphetamine sulfate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

### PERFORMANCE TESTS

#### • DISSOLUTION, Procedure for a Pooled Sample (711)

**Medium:** Water; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:** USP Dextroamphetamine Sulfate RS in *Medium* having a known concentration of USP Dextroamphetamine Sulfate RS similar to the concentration expected in the sample.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Diluted acetic acid:** 14 mL of glacial acetic acid in 100 mL of water

**Mobile phase:** 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of *Diluted acetic acid* and 400 mL of methanol. Adjust with glacial acetic acid to a pH of  $3.3 \pm 0.1$ .

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 500  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

#### Analysis

Calculate the percentage of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 500 mL

**Tolerances:** NLT 75% (Q) of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

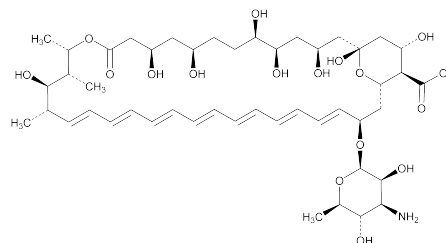
### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Dextroamphetamine Sulfate RS

## Amphotericin B



$C_{47}H_{73}NO_{17}$  924.08

Amphotericin B.

Amphotericin B.

[1R-(1R\*,3S\*,5R\*,6R\*,9R\*,11R\*,15S\*,16R\*,17R\*,18S\*,19E,21E,23E,25E,27E,29E,31E,33R\*,35S\*,36R\*,37S\*)]-33-[(3-Amino-3,6-dideoxy- $\beta$ -D-mannopyranosyl)oxy]-1,3,5,6,

9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,-39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [1397-89-3].

» Amphotericin B has a potency of not less than 750 µg of C<sub>47</sub>H<sub>73</sub>NO<sub>17</sub> per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store in a cold place.

**Labeling**—Label it to state whether it is intended for use in preparing dermatological and oral dosage forms or parenteral dosage forms.

**USP Reference standards** (11)—

USP Amphotericin B RS

USP Nystatin RS

**Identification**, *Ultraviolet Absorption* (197U)—

*Spectral range 1:* 240 to 320 nm.

*Solution 1:* prepared as directed for *Test preparation* in the *Limit of amphotericin A*, and compare its absorbance to that of the *Amphotericin B standard preparation*. An extra peak may occur at 304 nm in the spectrum of this solution.

*Spectral range 2:* 320 to 400 nm.

*Solution 2:* prepared as directed for *Test preparation* in the *Limit of amphotericin A* and then diluted with 9 volumes of methanol. Compare its absorbance to that of a similar dilution of the *Amphotericin B standard preparation*.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, yields not more than 3.0%.]

**Limit of amphotericin A**—

*Test preparation*—Dissolve about 50 mg of Amphotericin B, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Nystatin standard preparation*—Dissolve about 20 mg of USP Nystatin RS, accurately weighed, in 40.0 mL of dimethyl sulfoxide in a 200-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Amphotericin B standard preparation*—Dissolve about 50 mg of USP Amphotericin B RS, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Prepare this solution fresh daily.

*Procedure*—Concomitantly determine the absorbances of the *Nystatin* and *Amphotericin B standard preparations* and the *Test preparation* in 1-cm cells at 304 nm and at 282 nm, with a suitable spectrophotometer, using a 1 in 62.5 solution of dimethyl sulfoxide in methanol as the blank. Calculate the percentage of amphotericin A taken by the formula:

$$25W_N[(A_{B_{282}} \times A_{U_{304}}) - (A_{B_{304}} \times A_{U_{282}})] / [(A_{B_{282}} \times A_{N_{304}}) - (A_{B_{304}} \times A_{N_{282}})]W_U$$

in which  $W_N$  is the weight, in mg, of USP Nystatin RS taken,  $A_{B_{282}}$  and  $A_{B_{304}}$  are the absorbances of the *Amphotericin B standard preparation* at 282 nm and 304 nm, respectively,  $A_{N_{282}}$  and  $A_{N_{304}}$  are the absorbances of the *Nystatin standard*

*preparation* at 282 nm and 304 nm, respectively,  $A_{U_{282}}$  and  $A_{U_{304}}$  are the absorbances of the *Test preparation* at 282 nm and 304 nm, respectively, and  $W_U$  is the weight, in mg, of the Amphotericin B taken: not more than 5%, calculated on the dried basis, is found. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, contains not more than 15% of amphotericin A, calculated on the dried basis.]

**Assay**—Proceed with amphotericin B as directed under *Antibiotics—Microbial Assays* (81).

## Amphotericin B Cream

» Amphotericin B Cream contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of Amphotericin B.

**Packaging and storage**—Preserve in collapsible tubes, or other well-closed containers.

**USP Reference standards** (11)—

USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), blending a suitable accurately weighed portion of Cream in a high-speed blender with a sufficient accurately measured volume of dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer No. 10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B for Injection

» Amphotericin B for Injection is a sterile complex of Amphotericin B and deoxycholate sodium and one or more suitable buffers. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of C<sub>47</sub>H<sub>73</sub>NO<sub>17</sub>.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), in a refrigerator and protected from light.

**Labeling**—Label it to indicate that it is intended for use by intravenous infusion to hospitalized patients only, and that the solution should be protected from light during administration.

**USP Reference standards** (11)—

USP Amphotericin B RS

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of amphotericin B. For products used or labeled for intrathecal injection, it contains not more than 0.9 USP Endotoxin Unit per mg of amphotericin B.

**Sterility** (71)—It meets the requirements when tested as directed in the section *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 50 mg from each container being tested.



**pH** (791): between 7.2 and 8.0, in an aqueous solution containing 10 mg of amphotericin B per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling under Injections* (1).

**Assay—**

*Assay preparation 1* (where it is packaged as a single-dose container)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

*Assay preparation 2* (where the labeling states the quantity of amphotericin B in a given volume of constituted solution)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw an accurately measured volume of the resultant solution, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

*Procedure*—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer No. 10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B Lotion

» Amphotericin B Lotion contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

**pH** (791): between 5.0 and 7.0.

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), quantitatively dissolving a suitable accurately measured volume of Lotion in sufficient dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer No. 10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B Ointment

» Amphotericin B Ointment is Amphotericin B in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

**Packaging and storage**—Preserve in collapsible tubes, or other well-closed containers.

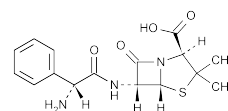
**USP Reference standards** (11)—  
USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment, equivalent to about 30 mg of amphotericin B, mixed with 10.0 mL of ether in a suitable glass-stoppered conical flask and allowed to stand, with intermittent shaking, for 1 hour. Add 20.0 mL of dimethyl sulfoxide and shake by mechanical means for 10 minutes. Dilute quantitatively and stepwise with dimethyl sulfoxide to a concentration of approximately 20 µg per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer No. 10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Ampicillin



C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S (anhydrous) 349.40  
4-Thia-1-azabicyclo[3.2.0]heptane-2 carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-,  
[2S-[2α,5α,6β(S\*)]]-;  
(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid  
[69-53-4].  
Trihydrate 403.46  
[7177-48-2].

### DEFINITION

Ampicillin is anhydrous or contains three molecules of water of hydration. It contains NLT 900 µg and NMT 1050 µg of C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** (197K): Except that where the specimen under test is the trihydrate, both it and the USP Ampicillin Trihydrate RS are undried.

### ASSAY

#### • PROCEDURE

[NOTE—The *Standard solution* and the *Sample solution* should be analyzed immediately after preparation.]

**Solution A:** 6.54 mg/mL of monobasic potassium phosphate and 0.34 mg/mL of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 5.5 before final dilution

**Solution B:** Acetonitrile and *Solution A* (2:23)

**Solution C:** Acetonitrile and *Solution A* (3:7)

**Mobile phase:** See gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
15	0	100
16	0	100
18	100	0
20	100	0

**Solution D:** 46.3 mg/mL of monobasic potassium phosphate and 27.8 mg/mL of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 6.5 before final dilution

**System suitability solution:** 0.5 mg/mL of USP Ampicillin RS and 0.1 mg/mL of USP Amoxicillin RS in acetonitrile, water, and *Solution D* (4:91:5). [NOTE—Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.]

**Standard solution:** 0.5 mg/mL of USP Ampicillin RS in acetonitrile, water, and *Solution D* (4:91:5). [NOTE—Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.]

**Sample solution:** 0.5 mg/mL of Ampicillin in acetonitrile, water, and *Solution D* (4:91:5). [NOTE—Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10 between the ampicillin and amoxicillin peaks, *System suitability solution*

**Tailing factor:** NMT 1.4, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in μg, of C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S in each mg of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin in the *Sample solution* (mg/mL)

$P$  = potency of USP Ampicillin RS (μg/mg)

**Acceptance criteria:** 900 μg–1050 μg on the anhydrous basis

5% of the final volume, and dilute with water to volume.]

#### System suitability

**Samples:** *Sensitivity solution*, *System suitability solution*, and *Standard solution*

#### Suitability requirements

**Signal-to-noise ratio:** NLT 3, *Sensitivity solution*

**Resolution:** NLT 10 between ampicillin and amoxicillin, *System suitability solution*

**Tailing factor:** NMT 1.4, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of ampicillin from the *Standard solution*

$C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin in the *Sample solution* (mg/mL)

$P$  = potency of USP Ampicillin RS (μg/mg)

$F$  = conversion factor, 0.001 mg/μg

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 3.0%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine <sup>a</sup>	0.27	0.5
6-Aminopenicillanic acid <sup>b</sup>	0.31	0.5
Ampicilloic acid <sup>c</sup>	0.45	1.0
Ampicillin thiazepine analog <sup>d</sup>	0.65	0.3
Ampicillin	1.0	—
Ampicillin rearrangement product (isomer 1) <sup>e</sup>	1.8	0.4
Ampicillin rearrangement product (isomer 2) <sup>e</sup>	2.0	0.3
Ampicillin oligomer 2 <sup>f</sup>	2.2	0.6
D-Phenylglycylampicillins <sup>g</sup>	2.5	0.8
Ampicillin oligomer 1 (dimer) <sup>h</sup>	2.6	1.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.

<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxy)methyl-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> (S)-6-[(R)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.

<sup>e</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>f</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>i</sup> (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-penta-oxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).

## IMPURITIES

### Organic Impurities

#### • PROCEDURE 1

[NOTE— The *Standard solution* and the *Sample solution* should be analyzed immediately after preparation.]

**Solution A, Solution B, Solution C, Mobile phase, Solution D, System suitability solution, Sample solution, and Chromatographic system:** Prepare as directed in the *Assay*.

**Standard stock solution:** Prepare as directed for *Standard solution* in the *Assay*.

**Standard solution:** 0.005 mg/mL of ampicillin in *Solution D* and water (1:19) from *Standard stock solution*.

[NOTE—Transfer an aliquot of the *Standard stock solution* to a suitable volumetric flask, add *Solution D*, using about 5% of the final volume, dilute with water to volume.]

**Sensitivity solution:** 0.0005 mg/mL of ampicillin in *Solution D* and water (1:19) from *Standard solution*.

[NOTE—Transfer an aliquot of the *Standard solution* to a suitable volumetric flask, add *Solution D*, using about

**Impurity Table 1** (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ampicillin oligomer 1 (trimer) <sup>i</sup>	2.9	0.4
Any individual unspecified impurity	—	0.25

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxy)methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>d</sup> (S)-6-[(R)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.<sup>e</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>f</sup> (4S)-2-{1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>h</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>i</sup> (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-penta-oxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).

- **PROCEDURE 2: DIMETHYLANILINE** <223>: Meets the requirements

**SPECIFIC TESTS**

- **STERILITY TESTS** <71>: Where the label states that Ampicillin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*, except to dissolve 6 g in 800 mL of *Fluid D* containing sufficient sterile penicillinase to inactivate the ampicillin and to swirl the vessel until solution is complete before filtering.
- **CRYSTALLINITY** <695>: Meets the requirements
- **PH** <791>: 3.5–6.0  
Sample solution: 10 mg/mL
- **WATER DETERMINATION, Method I** <921>: NMT 2.0% where it is labeled as Ampicillin (anhydrous); between 12.0% and 15.0% where it is labeled as Ampicillin (tri-hydrate)
- **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Ampicillin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Label to indicate whether it is anhydrous or is the trihydrate. Where the quantity of ampicillin is indicated in the labeling of any preparation containing Ampicillin, this shall be understood to be in terms of anhydrous ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S). Where it is intended for use in preparing injectable dosage forms, the label states that it is the trihydrate and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** <11>  
USP Amoxicillin RS  
USP Ampicillin RS  
USP Ampicillin Trihydrate RS  
USP Endotoxin RS

**Ampicillin Boluses**

» Ampicillin Boluses contain an amount of ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Boluses to indicate that they are for veterinary use only.

**USP Reference standards** <11>—  
USP Ampicillin RS

**Identification**—Powder 1 or more Boluses, and prepare a solution containing the equivalent of 10 mg of ampicillin per mL in a mixture of acetone and 0.1 N hydrochloric acid (4:1); the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**Uniformity of dosage units** <905>: meet the requirements.

**Loss on drying** <731>: not more than 5.0%.

**Assay—**

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* <425>, using USP Ampicillin RS.

*Assay preparation*—Place not fewer than 5 Boluses in a high-speed glass blender jar containing an accurately measured volume of water, and blend for 4 ± 1 minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

*Procedure*—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* <425>. Calculate the quantity, in mg, of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S) in each Bolus taken by the formula:

$$(T / D)(F / 2000)(B - I)$$

in which *T* is the labeled quantity, in mg, of ampicillin in each Bolus; and *D* is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in each Bolus and the extent of dilution.

**Ampicillin Capsules**

» Ampicillin Capsules contain an amount of ampicillin (anhydrous or as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Capsules to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.

**USP Reference standards** <11>—  
USP Ampicillin RS

**Identification**—Prepare a solution containing about 5 mg of ampicillin per mL, using powder from Ampicillin Capsules, in a mixture of acetone and 0.1 N hydrochloric acid (4:1). Prepare a Standard solution of USP Ampicillin RS to contain 5 mg per mL in the same solvent mixture. Apply separately 2 µL of each solution on a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place

the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of acetone, water, toluene, and glacial acetic acid (650:100:25). When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with a solution of ninhydrin in alcohol containing 3 mg per mL, and dry at 90° for 15 minutes: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Dissolution, Procedure for a Pooled Sample** (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ampicillin RS in water to obtain a solution having a known concentration of about L/900 mg per mL,  $L$  being the labeled amount, in mg, of ampicillin per Capsule.

**Procedure**—Proceed as directed for *Procedure* in the section *Antibiotics—Hydroxylamine Assay under Automated Methods of Analysis* (16), using a filtered portion of the solution under test as the *Assay preparation*. Calculate the quantity, in mg, of  $C_{16}H_{19}N_3O_4S$  dissolved by the formula:

$$0.9CP(A_U / A_S).$$

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 4.0% where the Capsules contain anhydrous Ampicillin, or between 10.0% and 15.0% where the Capsules contain Ampicillin trihydrate.

**Assay—**

**Standard preparation**—Prepare as directed for *Standard Preparation under Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

**Assay preparation**—Place not fewer than 5 Capsules in a high-speed glass blender jar containing an accurately measured volume of water, and blend for  $4 \pm 1$  minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

**Procedure**—Proceed as directed for *Procedure under Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each Capsule taken by the formula:

$$(T / D)(F / 2000)(B - I)$$

in which  $T$  is the labeled quantity, in mg, of ampicillin in each Capsule; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in each Capsule and the extent of dilution.

## Ampicillin for Injection

### DEFINITION

Ampicillin for Injection contains an amount of Ampicillin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

**Diluent:** Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

**Standard solution:** 1 mg/mL of USP Ampicillin RS in *Diluent*. Shake and sonicate, if necessary, to dissolve. Use this solution promptly after preparation.

**System suitability solution:** 0.12 mg/mL of caffeine in the *Standard solution*

**Sample solution 1** (where it is represented as being in a single-dose container): 1 mg/mL of ampicillin in *Diluent*. Constitute Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Diluent*. Use this solution promptly after preparation.

**Sample solution 2** (where the label states the quantity of ampicillin in a given volume of constituted solution): 1 mg/mL of ampicillin in *Diluent*. Constitute 1 container of Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Dilute a suitable aliquot of the constituted solution with *Diluent*. Use this solution promptly after preparation.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Columns**

**Precolumn:** 4-mm  $\times$  5-cm; 5- to 10- $\mu$ m packing L1

**Analytical:** 4-mm  $\times$  30-cm; 5- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

### Suitability requirements

**Resolution:** NLT 2.0 between caffeine and ampicillin, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution*

**Capacity factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the container or in the volume of constituted solution taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P \times (1 / F) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)

$P$  = potency of ampicillin in USP Ampicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Where the test for *Uniformity of Dosage Units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

**Acceptance criteria:** 90.0%–115.0%

### PERFORMANCE TESTS

#### • UNIFORMITY OF DOSAGE UNITS (905)

**Procedure for content uniformity**

**Analysis:** Perform the *Assay* on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.

Acceptance criteria: Meets the requirements

#### SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements. Freeze-dried products are exempt from this requirement.
- **pH** (791)  
Sample solution: 10.0 mg/mL of ampicillin  
Acceptance criteria: 8.0–10.0
- **WATER DETERMINATION, Method I** (921): NMT 2.0%
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **STERILITY TESTS** (71): Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.15 USP Endotoxin Units/mg of ampicillin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* (1), *Constituted Solutions*.
- **OTHER REQUIREMENTS:** It meets the requirements of the tests for *Identification* in *Ampicillin Sodium*. It also meets the requirements in *Injections* (1), *Labeling*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Injections* (1), *Containers for Sterile Solids*. Protect the constituted solution from freezing.
- **USP REFERENCE STANDARDS** (11)  
USP Ampicillin RS  
USP Ampicillin Sodium RS  
USP Endotoxin RS

### Ampicillin Soluble Powder

» Ampicillin Soluble Powder is a dry mixture of Ampicillin (as the trihydrate) and one or more suitable diluents and stabilizing agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—  
USP Ampicillin RS

**Identification**—Dissolve a quantity of it in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 10 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**pH** (791): between 3.5 and 6.0, in an aqueous solution containing the equivalent of 20 mg of ampicillin per mL.

**Water, Method I** (921): not more than 5.0%.

#### Assay—

**Standard preparation**—Prepare as directed for *Standard Preparation under Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

**Assay preparation**—Transfer an accurately weighed quantity of Soluble Powder, equivalent to about 125 mg of ampicillin, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure under Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the portion of Soluble Powder taken by the formula:

$$(F/20)(B - I).$$

### Ampicillin for Injectable Suspension

» Ampicillin for Injectable Suspension is a dry mixture of ampicillin trihydrate and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Ampicillin RS  
USP Endotoxin RS

**Identification**—Dissolve a quantity in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 5 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**Bacterial endotoxins** (85)—It contains not more than 0.15 Endotoxin Unit per mg of ampicillin.

**pH** (791): between 5.0 and 7.0, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): between 11.4% and 14.0%.

**Sterility** (71)—It meets the requirements when tested as directed for *Antibiotic Solids, Bulks, and Blends* in the section *Membrane Filtration under Test for Sterility of the Product to be Examined*, except to use *Fluid D*, to which has been added sufficient sterile penicillinase to inactivate the ampicillin and to swirl the vessel until solution is complete before filtering. If it does not dissolve completely, proceed as directed for *Solids* in the section *Direct Inoculation of the Culture Medium under Test for Sterility of the Product to be Examined*, except to use *Fluid Thioglycollate Medium* and *Soybean-Casein Digest Medium* containing sufficient penicillinase to inactivate the ampicillin in each vessel.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905), and for *Labeling* under *Injections* (1).

#### Assay—

**Phosphate buffer solution**—Accurately weigh 68 g of monobasic potassium phosphate, and transfer to a 500-mL volumetric flask. Dissolve in and dilute with water to volume.

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer solution*, and glacial acetic acid (3600:360:40:4). Pass through a 0.45- $\mu$ m nylon filter, and degas.

**Standard preparation**—Dissolve, with sonication, an accurately weighed quantity of USP Ampicillin RS in water to prepare a solution having 0.5 mg per mL. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**Caffeine solution**—Transfer about 30 mg of caffeine, accurately weighed, to a 50-mL volumetric flask. Add 25 mL of water, sonicate to dissolve, and dilute with water to volume. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**System suitability solution**—Prepare a solution of 1.0 mL of *Caffeine solution* and 9.0 mL of *Standard preparation*, and mix.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Ampicillin for Injectable Suspension, constituted as directed in the labeling, with water to obtain a solution containing about 0.5 mg per mL. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm analytical column that contains 10- $\mu$ m packing L1. The flow rate is about 2.0 mL per min-

ute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the order of elution is ampicillin followed by caffeine; the resolution,  $R$ , between ampicillin and caffeine is greater than 2; the column efficiency is not less than 2000 theoretical plates for the ampicillin peak; the tailing factor is not greater than 1.4; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each mL of the constituted solution of Ampicillin for Injectable Suspension taken by the formula:

$$CD(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ampicillin RS in the *Standard preparation*;  $D$  is the dilution factor used in preparing the *Assay preparation*; and  $r_U$  and  $r_S$  are the average peak responses of the ampicillin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ampicillin for Oral Suspension

» Ampicillin for Oral Suspension contains an amount of Ampicillin (anhydrous or as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{16}H_{19}N_3O_4S$ , when constituted as directed. It contains one or more suitable buffers, colors, flavors, preservatives, and sweetening ingredients.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.

**USP Reference standards** (11)—  
USP Ampicillin RS

**Identification**—Dissolve a quantity in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 5 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 7.5, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 2.5%, or not more than 5.0% if it contains ampicillin trihydrate and contains the equivalent of 100 mg of ampicillin per mL when constituted as directed in the labeling.

**Assay**—

**Standard preparation**—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

**Assay preparation**—Dilute an accurately measured volume of Ampicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, quantitatively and stepwise with water to obtain a solution containing about 1.25 mg of ampicillin per mL.

**Procedure**—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of  $C_{16}H_{19}N_3O_4S$  in each mL of the constituted suspension prepared from Ampicillin for Oral Suspension taken by the formula:

$$(T / D)(F / 2000)(B - I)$$

in which  $T$  is the labeled quantity, in mg per mL, of ampicillin in the constituted suspension; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in the constituted suspension and the extent of dilution.

## Ampicillin Tablets

» Ampicillin Tablets contain an amount of Ampicillin (anhydrous form or trihydrate form) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label the Tablets to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate. Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended for veterinary use only are so labeled.

**USP Reference standards** (11)—  
USP Ampicillin RS

**Identification**—Powder 1 or more Tablets, and prepare a solution containing 5 mg of ampicillin per mL in a mixture of acetone and 0.1 N hydrochloric acid (4:1): the resulting solution meets the requirements for the *Identification* test under *Ampicillin Capsules*.

**Dissolution, Procedure for a Pooled Sample** (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ampicillin RS in water to obtain a solution having a known concentration of about  $L/900$  mg per mL,  $L$  being the labeled amount, in mg, of ampicillin per Tablet.

**Procedure**—Proceed as directed for *Procedure* in the section *Antibiotics—Hydroxylamine Assay* under *Automated Methods of Analysis* (16), using a filtered portion of the solution under test as the *Assay preparation*. Calculate the quantity, in mg, of  $C_{16}H_{19}N_3O_4S$  dissolved by the formula:

$$0.9CP(A_U / A_S).$$

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 4.0% where nonchewable Tablets contain anhydrous Ampicillin; between 9.5% and 12.0% where nonchewable Tablets contain Ampicillin trihydrate; not more than 3.0% where chewable Tablets contain anhydrous Ampicillin; not more than 5.0% where chewable Tablets contain Ampicillin trihydrate; and not more than 13.0% where Tablets are labeled for veterinary use only and contain Ampicillin trihydrate.

**Assay—**

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

*Assay preparation*—Place not fewer than 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, and blend for  $4 \pm 1$  minutes. Dilute an accurately measured volume of this stock solution with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

*Procedure*—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each Tablet taken by the formula:

$$(T/D)(F/2000)(B - I)$$

in which  $T$  is the labeled quantity, in mg, of ampicillin in each Tablet; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

## Ampicillin and Probenecid for Oral Suspension

» Ampicillin and Probenecid for Oral Suspension contains an amount of Ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of probenecid ( $C_{13}H_{19}NO_4S$ ). It contains one or more suitable colors, flavors, and suspending agents.

**Packaging and storage**—Preserve in tight, unit-dose containers.

**USP Reference standards** (11)—

USP Ampicillin RS  
USP Probenecid RS

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements for *Content Uniformity* with respect to ampicillin and probenecid.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 7.5, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 5.0%.

**Assay for ampicillin—**

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

*Assay preparation*—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Transfer the resulting suspension to a high-speed glass blender jar containing sufficient water to make 500.0 mL, and blend for about 10 minutes. Quantitatively dilute an accurately measured volume of this stock solution with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

*Procedure*—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in

mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$(L/D)(F/2000)(B - I)$$

in which  $L$  is the labeled quantity, in mg, of ampicillin in the Ampicillin and Probenecid for Oral Suspension; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution.

**Assay for probenecid—**

*Standard preparation*—Dissolve an accurately weighed portion of USP Probenecid RS in sodium carbonate solution (1 in 100) to obtain a solution having a known concentration of about 1 mg per mL.

*Assay preparation*—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Quantitatively dilute the resulting suspension with sodium carbonate solution (1 in 100) to obtain a solution containing about 1 mg of probenecid per mL, mix, and filter.

*Procedure*—Transfer 2.0 mL of the clear *Assay preparation* to a 125-mL separator, and add 8.0 mL of 1.0 N hydrochloric acid. Extract this solution with four 20-mL portions of chloroform, filtering each extract through a glass wool pledget and 6 g of chloroform-washed anhydrous sodium sulfate into a 100-mL volumetric flask. Wash the pledget and the sodium sulfate with chloroform, collecting the washings in the 100-mL volumetric flask, dilute with chloroform to volume, and mix. Treat 2.0 mL of the *Standard preparation* in the same manner. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 257 nm, with a suitable spectrophotometer, using chloroform washed with sodium carbonate solution (1 in 100) as the blank. Calculate the quantity, in mg, of probenecid ( $C_{13}H_{19}NO_4S$ ) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$C(L/D)(A_U/A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Probenecid RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of probenecid in the Ampicillin and Probenecid for Oral Suspension;  $D$  is the concentration, in mg per mL, of probenecid in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ampicillin Sodium

$C_{16}H_{18}N_3NaO_4S$  371.39  
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2S-[2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ (S\*)]]-;  
Monosodium *D*-(-)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3].

**DEFINITION**

Ampicillin Sodium has a potency equivalent to NLT 845  $\mu$ g and NMT 988  $\mu$ g of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) per mg, calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197M>
- **B. IDENTIFICATION TESTS—GENERAL**, Sodium <191>

**ASSAY**• **PROCEDURE**

**Diluent:** Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

**Mobile phase:** Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

**Standard solution:** 1 mg/mL of USP Ampicillin RS in *Diluent* using shaking and sonication, if necessary, to dissolve. Use this solution promptly after preparation.

**System suitability solution:** 0.12 mg/mL of caffeine in *Standard solution*

**Sample solution:** [NOTE—Ampicillin Sodium is hygroscopic. Minimize exposure to the atmosphere, and weigh promptly.] Equivalent to 1 mg/mL of anhydrous ampicillin in *Diluent*. [NOTE—Use this solution promptly after preparation.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column**

**Pre-column:** 4-mm × 5-cm; 5- to 10-μm packing L1

**Analytical column:** 4-mm × 30-cm; 5- to 10-μm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20 μL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 2.0 between the caffeine and the ampicillin peaks, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution*

**Capacity factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in μg, of C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S in each mg of Ampicillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

$P$  = potency of USP Ampicillin RS (μg/mg)

**Acceptance criteria:** 845–988 μg/mg on the anhydrous basis

**IMPURITIES****Organic Impurities**• **PROCEDURE 1: LIMIT OF METHYLENE CHLORIDE**

**Internal standard solution:** 2.1 mg/mL of dioxane in dimethyl sulfoxide

**Standard solution:** 0.33 mg/mL of methylene chloride in *Internal standard solution*

**Sample solution:** 166.7 mg/mL of Ampicillin Sodium in *Internal standard solution*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.8-m × 4-mm glass column packed with a 10% phase G39 on unsilanized support S1A

**Temperature**

**Column:** 65°

**Injector:** 100°

**Detector block:** 260°

**Carrier gas:** Nitrogen

**Flow rate:** 60 mL/min

**Injection size:** 1 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE— The relative retention times for methylene chloride and dioxane are 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4 between methylene chloride and dioxane

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of methylene chloride in the portion of Ampicillin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of methylene chloride to dioxane from the *Sample solution*

$R_S$  = peak response ratio of methylene chloride to dioxane from the *Standard solution*

$C_S$  = concentration of methylene chloride in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%.

- **PROCEDURE 2: DIMETHYLANILINE** <223>: Meets the requirement

**SPECIFIC TESTS**

- **CRYSTALLINITY** <695>: Meets the requirements. [NOTE— Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]
- **PH** <791>: 8.0–10.0  
**Sample solution:** 10.0 mg/mL
- **WATER DETERMINATION, Method I** <921>: NMT 2.0%
- **STERILITY TESTS** <71>: Where the label states that Ampicillin Sodium is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Ampicillin Sodium is sterile or the label states that Ampicillin Sodium must be subjected to further processing during the processing of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** <11>  
USP Ampicillin RS  
USP Ampicillin Sodium RS  
USP Endotoxin RS

**Ampicillin and Sulbactam for Injection**

» Ampicillin and Sulbactam for Injection is a sterile, dry mixture of Ampicillin Sodium and Sulbactam Sodium. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S) and sulbactam (C<sub>8</sub>H<sub>11</sub>NO<sub>5</sub>S), the labeled amounts representing proportions of ampicillin to sulbactam of 2:1. It contains not



less than 563 µg of ampicillin and 280 µg of sulbactam per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Ampicillin RS

USP Endotoxin RS

USP Sulbactam RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.17 USP Endotoxin Unit in a portion equivalent to 1 mg of a mixture of ampicillin and sulbactam (0.67 and 0.33 mg, respectively).

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 8.0 and 10.0, in a solution containing 10 mg of ampicillin and 5 mg of sulbactam per mL.

**Water, Method I** (921): not more than 2.0%.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay**—

*0.005 M Tetrabutylammonium hydroxide*—Dilute 6.6 mL of a 40% solution of tetrabutylammonium hydroxide with water to obtain 1800 mL of solution. Adjust with 1 M phosphoric acid to a pH of  $5.0 \pm 0.1$ , dilute with water to 2000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of 0.005 M Tetrabutylammonium hydroxide and acetonitrile (1650:350). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve accurately weighed quantities of USP Ampicillin RS and USP Sulbactam RS in *Mobile phase* to obtain a solution having known concentrations of about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Resolution solution*—Prepare a solution of USP Sulbactam RS in 0.01 N sodium hydroxide containing 0.3 mg per mL, and allow to stand for 30 minutes. Adjust with phosphoric acid to a pH of  $5.0 \pm 0.1$ . Transfer 5 mL of the solution to a 25-mL volumetric flask, add 4.25 mL of acetonitrile, dilute with 0.005 M Tetrabutylammonium hydroxide to volume, and mix. Transfer 1 mL of this solution to a second 25-mL volumetric flask, add 15 mg of USP Ampicillin RS, dilute with *Mobile phase* to volume, and mix. [NOTE—Inject this solution promptly.]

*Assay preparation 1*—Mix the contents of a container of Ampicillin and Sulbactam for Injection. Quantitatively dissolve an accurately weighed portion of the powder in *Mobile phase* to obtain a solution having a concentration of about 1 mg of the powder per mL. [NOTE—Inject this solution promptly.]

*Assay preparation 2* (where it is represented as being in a single-dose container)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent

specified in the labeling. Withdraw the total withdrawable contents from the container, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Assay preparation 3* (where the label states the quantities of ampicillin and sulbactam in a given volume of constituted solution)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for ampicillin and 1.0 for sulbactam alkaline degradation product; and the resolution,  $R$ , between ampicillin and sulbactam alkaline degradation product is not less than 4.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative retention times are about 0.35 for ampicillin and 1.0 for sulbactam; the column efficiency determined from the sulbactam peak is not less than 3500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in µg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and of sulbactam ( $C_8H_{11}NO_5S$ ) in the portion of Ampicillin and Sulbactam for Injection taken by the same formula:

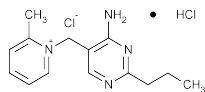
$$(C_S P / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*;  $P$  is the assigned content, in µg per mg, of the appropriate USP Reference Standard;  $C_U$  is the concentration, in mg per mL, of Ampicillin and Sulbactam for Injection in *Assay preparation 1*, based on the weight, in mg, of powder removed from the container and the extent of dilution; and  $r_U$  and  $r_S$  are the peak areas for the appropriate analyte obtained from *Assay preparation 1* and the *Standard preparation*, respectively. Calculate the quantities of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and of sulbactam ( $C_8H_{11}NO_5S$ ) withdrawn from the container, or in the volume of constituted solution taken by the same formula:

$$(L / D)(C_S P)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container or in the volume of constituted solution taken;  $D$  is the concentration, in mg per mL, of ampicillin or sulbactam in *Assay preparation 2* or *Assay preparation 3*, on the basis of the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container and the extent of dilution;  $r_U$  and  $r_S$  are the peak areas for the appropriate analyte obtained from *Assay preparation 2* or *Assay preparation 3* and the *Standard preparation*, respectively; and the other terms are as defined above.

## Amprolium



$C_{14}H_{19}ClN_4 \cdot HCl$  315.24

1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride monohydrochloride.

1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-picolinium chloride monohydrochloride [137-88-2].

» Amprolium contains not less than 97.0 percent and not more than 101.0 percent of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Amprolium RS

**Identification**—

**A:** *Infrared Absorption* (197K): previously dried.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* 0.1 N hydrochloric acid.

Absorptivities at 246 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at 100° for 3 hours: it loses not more than 1.0% of its weight.

**Assay**—

*Diluent*—Prepare a mixture of 500 mL of water, 450 mL of methanol, and 50 mL of acetonitrile.

*Mobile phase*—Dissolve 6 g of sodium 1-heptanesulfonate in 500 mL of water, add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile, and mix. Pass through a suitable filter of 0.5 µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Amprolium RS in *Diluent* to obtain a solution having a known concentration of about 0.5 mg per mL.

*Assay preparation*—Transfer about 50 mg of Amprolium, accurately weighed, to a 100-mL volumetric flask, add *Diluent* to volume, and mix.

*Resolution solution*—Prepare a solution in *Diluent* containing about 0.5 mg of USP Amprolium RS and 0.2 mg of 2-picoline per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column containing packing L13. The flow rate is about 0.6 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the resolution,  $R$ , between amprolium and 2-picoline is not less than 7; the column efficiency determined from the amprolium peak is not less than 6500 theoretical plates; the tailing factor for the analyte peak is not more than 2.3; and the relative standard deviation of the amprolium responses for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the areas for the major peaks. Calculate the quantity, in mg, of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ) in the portion of Amprolium taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Amprolium RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the amprolium peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amprolium Soluble Powder

» Amprolium Soluble Powder contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Amprolium RS

**Identification, Ultraviolet Absorption** (197U)—

*Solution:* 10 µg per mL, filtered.

*Medium:* 0.1 N hydrochloric acid.

**Assay**—

*Diluent, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Amprolium*.

*Assay preparation*—Transfer an accurately weighed portion of Soluble Powder, equivalent to about 50 mg of amprolium, to a 100-mL volumetric flask, add about 75 mL of *Diluent*, and sonicate for about 10 minutes. Allow to cool to room temperature, dilute with *Diluent* to volume, and mix. Pass through a suitable filter of 0.5-µm or finer porosity, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Amprolium*. Calculate the quantity, in mg, of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ) in the portion of Soluble Powder taken by the formula:

$$100C(r_U / r_S)$$

in which the terms are as defined therein.

## Amprolium Oral Solution

» Amprolium Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, protected from light. Store at a temperature between 5° and 30°, in a dry place.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Amprolium RS

**Identification, Ultraviolet Absorption** (197U)—

*Solution:* 10 µg per mL, filtered.

*Medium:* 0.1 N hydrochloric acid.

**pH** (791): between 2.5 and 3.0.

**Assay—**

*Mobile phase*—To 4.5 g of sodium 1-hexanesulfonate add 1500 mL of water, 400 mL of methanol, and 100 mL of acetonitrile, mix, and allow to cool to room temperature. Adjust with phosphoric acid to a pH of 5.1, and pass through a filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Amprolium RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 960 mg of amprolium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 268-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The column is maintained at a constant temperature of about 45°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for amprolium. Calculate the quantity, in mg, of amprolium (C<sub>14</sub>H<sub>19</sub>ClN<sub>4</sub> · HCl) in each mL of the Oral Solution taken by the formula:

$$(2000C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Amprolium RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the amprolium peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Amyl Nitrite**

C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub> 117.15

Mixture of nitrous acid, 2-methylbutyl ester, and nitrous acid, 3-methylbutyl ester [8017-89-8; 110-46-3].

» Amyl Nitrite is a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 85.0 percent and not more than 103.0 percent of C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>.

**Caution**—Amyl Nitrite is very flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in tight containers, and store in a cool place, protected from light.

**USP Reference standards** (11)—

USP Benzyl Benzoate RS

**Identification—**

A: The NMR spectrum recorded as directed in the *Assay* exhibits, among other peaks, a doublet with a band centered

at about 1 ppm and a multiplet with a band centered at about 4.8 ppm representing methyl protons and methylene protons alpha to the nitrite group, respectively, both relative to the tetramethylsilane singlet at 0 ppm.

B: To a few drops of it add a mixture of 1 mL of ferrous sulfate TS and 5 mL of 3 N hydrochloric acid: a greenish brown color is produced.

**Specific gravity** (841): between 0.870 and 0.876.

**Acidity**—To 0.30 mL in a glass-stoppered cylinder add a mixture of 0.60 mL of 0.1 N sodium hydroxide, 10 mL of water, and 1 drop of phenolphthalein TS, and invert the cylinder three times: the red tint of the water layer is still perceptible.

**Limit of nonvolatile residue**—Allow 10 mL to evaporate at room temperature in a tared evaporating dish, in a well-ventilated hood, and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 2 mg (0.02%).

**Content of total nitrites**—Inject a portion of Amyl Nitrite of suitable volume, but not more than 2 µL, into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a thermal conductivity detector. Under typical conditions, the instrument contains a 3-mm × 2-m column packed with a methyl polysiloxane oil, 25% by weight on suitable calcined diatomite, the column is maintained at about 80°, the injection port and detector block are maintained about 10° above the temperature of the column, and helium is used as a carrier gas at a flow rate of about 60 mL per minute. From the area under the curve, calculate the percentage (a/a) of total nitrites, represented by the area under the main peak of the chromatogram, in the Amyl Nitrite taken: not less than 97.0% is found.

**Assay—**

*Solvent:* carbon tetrachloride.

*Internal standard*—USP Benzyl Benzoate RS.

*Procedure*—Transfer 4 to 5 mEq of *Internal standard*, accurately weighed, to a semimicro sampling tube, add 2 to 3 mL of carbon tetrachloride, apply a sampling valve and septum,\* thereby sealing the tube, and determine the weight of the sealed assembly. Open the valve, introduce about 500 µL of Amyl Nitrite with a syringe, close the valve, and determine the weight of the sealed assembly when it has attained constant weight. Shake the sampling tube and valve assembly, and transfer about 500 µL of the solution to a precision NMR tube as directed for *Absolute Method of Quantitation* under *Nuclear Magnetic Resonance* (761). With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as  $A_S$  the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as  $A_U$  the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub> in the Amyl Nitrite taken, using 58.57 as the equivalent weight of amyl nitrite ( $EW_U$ ) and 106.12 as that of benzyl benzoate ( $EW_S$ ).

**Amyl Nitrite Inhalant**

» Amyl Nitrite Inhalant contains a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 80.0 percent

\* Suitable sampling tubes, sampling valves, and septums are available, respectively, as catalog Nos. K-749000, K-749100, and K-749102 (50 septums) or K-749101 (100 septums), from Kontes Glass Company, Vineland, NJ 08360.

and not more than 105.0 percent of  $C_5H_{11}NO_2$ . It contains a suitable stabilizer.

**Caution**—Amyl Nitrite Inhalant is very flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in tight, unit-dose glass containers, wrapped loosely in gauze or other suitable material, and store in a cool place, protected from light.

**USP Reference standards** (11)—

USP Benzyl Benzoate RS

**Specific gravity** (841): between 0.870 and 0.880.

**Content of total nitrites**—Remove the gauze or other covering, place the glass container of Inhalant upright in a dry ice-acetone slurry, and cool for 10 minutes. Dry the container of Inhalant, place it in a pointed glass tube, and break the container with a glass rod. Proceed as directed for *Total nitrites* under *Amyl Nitrite*: not less than 95.0% is found.

**Other requirements**—It responds to the *Identification* tests and meets the requirements of the test for *Acidity* under *Amyl Nitrite*.

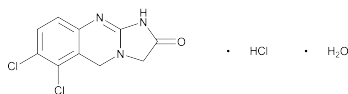
**Assay**—

**Solvent:** carbon tetrachloride.

**Internal standard**—USP Benzyl Benzoate RS.

**Procedure**—Remove the gauze or other covering from 1 or more Inhalant ampuls containing a total of 300 to 400  $\mu$ L of amyl nitrite. Weigh accurately the clean and dry intact glass ampul(s), and place the weighed specimen in a freezer for not less than 15 minutes. Transfer the chilled specimen to a glass-stoppered, 25-mL conical flask containing a solution of 4 to 5 mEq of *Internal standard*, accurately weighed, in 1 to 2 mL of carbon tetrachloride. Break the ampul(s) with a glass rod, and rinse any sample or glass fragments adhering to the glass rod with 1 mL of carbon tetrachloride into the main assay solution. Insert the stopper in the flask immediately, mix, and proceed as directed for *Absolute Method of Quantitation under Nuclear Magnetic Resonance* (761), beginning with "When dissolution has been completed." With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as  $A_s$  the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as  $A_U$  the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of  $C_5H_{11}NO_2$  in the Inhalant taken, using 58.57 as the equivalent weight of amyl nitrite ( $EW_U$ ) and 106.12 as that of benzyl benzoate ( $EW_s$ ). Rinse the flask containing the assay preparation with three 5-mL portions of ether, decanting each rinsing carefully to avoid loss of glass fragments, and evaporate any remaining ether with the aid of a current of dry air. Transfer the dry glass fragments to a tared watch glass, weigh, and subtract the weight of the glass fragments from that of the intact ampul(s) to obtain the weight of the Inhalant taken.

## Anagrelide Hydrochloride



$C_{10}H_7Cl_2N_3O \cdot HCl \cdot H_2O$   
Anhydrous

310.56  
292.55

[58579-51-4].

Imidazo[2,1-*b*]quinazolin-2(3*H*)-one, 6,7-dichloro-1,5-dihydro-, monohydrochloride, monohydrate;  
6,7-Dichloro-1,5-dihydroimidazo[2,1-*b*]quinazolin-2(3*H*)-one monohydrochloride, monohydrate [823178-43-4].

**DEFINITION**

Anagrelide Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{10}H_7Cl_2N_3O \cdot HCl$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

**ASSAY**

• **PROCEDURE**

[NOTE—Use freshly prepared standard and sample solutions and inject within 2 h.]

**Solution A:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and *Solution A* (1:3)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** 0.5 mg/mL of anagrelide hydrochloride in acetonitrile. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add a small amount of 2 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to 80% of the final volume. Sonicate to dissolve and dilute with acetonitrile to volume.

**Standard solution:** 0.05 mg/mL of anagrelide hydrochloride in *Diluent*, from *Standard stock solution*

**Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask, add 3 drops of 2 N hydrochloric acid and 40 mL of acetonitrile. Sonicate to dissolve and dilute with acetonitrile to volume.

**Sample solution:** Transfer 5 mL of *Sample stock solution* to a 50-mL volumetric flask and dilute with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 4- $\mu$ m packing L11

**Flow rate:** 1.2 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NMT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{10}H_7Cl_2N_3O \cdot HCl$  in the portion of Anagrelide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Anagrelide Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS, Method II** (231): NMT 20 ppm

**Organic Impurities**

[NOTE—Use freshly prepared standard and sample solutions and inject within 2 h.]

• **PROCEDURE**

**Mobile phase:** Proceed as directed in the Assay.

**Diluent A:** Use the *Diluent* as described in the Assay.

**Diluent B:** Acetonitrile and water (1:3)

**Standard stock solution A:** 0.05 mg/mL of USP Anagrelide Related Compound A RS in *Diluent A*

**Standard stock solution B:** 0.05 mg/mL of anagrelide related compound B in acetonitrile. Transfer USP Anagrelide Related Compound B RS into a suitable volumetric flask, add acetonitrile equivalent to 50% of the final volume and a small amount of 2 N hydrochloric acid (3 drops per 200 mL of the final volume). Sonicate to dissolve, heat in the hot water bath if necessary, and dilute with acetonitrile to volume.

**System suitability solution:** 0.25 µg/mL of each of anagrelide related compound A and anagrelide related compound B in *Mobile phase*, from *Standard stock solution A* and *Standard stock solution B*

**Standard stock solution C:** 0.1 mg/mL of anagrelide hydrochloride in acetonitrile. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add acetonitrile equivalent to 80% of the final volume and a small amount of 0.12 N hydrochloric acid (1 mL per 100 mL of the final volume). Sonicate to dissolve and dilute with acetonitrile to volume.

**Standard solution:** 0.05 µg/mL of anagrelide hydrochloride in *Mobile phase*, from *Standard stock solution C*

**Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask. Add 45 mL of acetonitrile, sonicate, and swirl the flask until the preparation turns into a cloudy liquid. Add 1 drop of 0.12 N hydrochloric acid, swirl the flask until the liquid turns to clear, and dilute with acetonitrile to volume.

**Sample solution:** Transfer 5 mL of *Sample stock solution* into a 50-mL volumetric flask and dilute with *Diluent B* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-µm packing L11

**Refrigerated autosampler temperature:** 5°

**Flow rate:** 1.2 mL/min

**Injection size:** 50 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Resolution:** NLT 2.0 between anagrelide related compound B and anagrelide related compound A, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Anagrelide Hydrochloride, on the anhydrous basis, taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of anagrelide from the *Standard solution*

$C_S$  = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Anagrelide Hydrochloride (anhydrous) in the *Sample solution* (mg/mL)

$F$  = relative response factor for each individual impurity (see *Impurity Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any impurity peak less than 0.05%.]

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide related compound B <sup>a</sup>	0.40	0.43	0.3
Anagrelide related compound A <sup>b</sup>	0.55	0.37	0.15
Anagrelide	1.00	1.0	—
Anagrelide related compound C <sup>c</sup>	1.41	0.32	0.15
Anagrelide trichloro derivative <sup>d</sup>	2.44	1.0	0.15
Any unspecified impurity	—	1.0	0.1

<sup>a</sup> (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.

<sup>b</sup> Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.

<sup>c</sup> Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.

<sup>d</sup> 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** (921): Between 4.5% and 7.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cold place.
- **USP REFERENCE STANDARDS** (11)
  - USP Anagrelide Hydrochloride RS
  - USP Anagrelide Related Compound A RS
  - Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.  $C_{11}H_{14}Cl_2N_2O_2$  277.15
  - USP Anagrelide Related Compound B RS
  - (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.  $C_{10}H_9Cl_2N_3O_2$  274.10

**Anagrelide Capsules****DEFINITION**

Anagrelide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{10}H_7Cl_2N_3O$ .

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Solution A:** 1.0 g/L of sodium hexanesulfonate. Add 1.0 mL of phosphoric acid and filter.

**Mobile phase:** Acetonitrile and *Solution A* (7:13)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anagrelide Hydrochloride RS in acetonitrile. Initially add acetonitrile (about 80% of the volume of the flask) and a small quantity of 2 N hydrochloric acid (about 0.2 mL for every 100 mL of the final volume). Sonicate to dissolve, and dilute with acetonitrile to volume.

**Standard solution:** 0.01 mg/mL of anagrelide free base in *Diluent* from *Standard stock solution*

**Sample solution:** 0.01 mg/mL of anagrelide free base prepared from the contents of NLT 20 Capsules. Add *Diluent* (80% of the volume of the flask), sonicate for 10 min, and stir for 15 min. Further dilute with *Diluent* to volume, centrifuge for 15 min at 4000 rpm, and use the supernatant for analysis.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Column temperature:** 60°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>, based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of anagrelide from the *Sample solution*

$r_S$  = peak response of anagrelide from the *Standard solution*

$C_S$  = concentration of anagrelide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anagrelide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 15 min

**Mobile phase:** Proceed as directed in the *Assay*.

**Standard solution:** Transfer about 30.32 mg of USP Anagrelide Hydrochloride RS, equivalent to 25.00 mg of anagrelide, to a 100-mL volumetric flask. Add about 80 mL of acetonitrile and 3 drops of 2 N hydrochloric acid. Sonicate until dissolved. Dilute with acetonitrile to volume. Dilute this solution with *Medium* to obtain a final concentration of about (L/1000) mg/mL, where L is the Capsule label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 274 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Sample cooler temperature:** 5°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of anagrelide dissolved:

$$\text{Result} = (r_U/r_S) \times W_S(100 - W_C)/(25 \times 100) \times M_{r1}/M_{r2} \times V/L \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$W_S$  = weight of the USP Anagrelide Hydrochloride RS taken (mg)

$W_C$  = water content of the USP Anagrelide Hydrochloride RS (%)

$M_{r1}$  = molecular weight of anagrelide, 256.10

$M_{r2}$  = molecular weight of anagrelide hydrochloride, 292.56

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Capsule)

**Tolerances:** NLT 80% (Q) of the labeled amount of anagrelide is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE

**Buffer solution:** 6.8 g/L of monobasic potassium phosphate. Adjust with diluted phosphoric acid to a pH of 3.50 ± 0.05. Mix well and filter.

**Mobile phase:** Acetonitrile and *Buffer solution* (27:73)

**Diluent:** Acetonitrile:water (7:13)

**Related compound A stock solution:** 10 μg/mL of USP Anagrelide Related Compound A RS in *Diluent*

**Related compound C stock solution:** 10 μg/mL of USP Anagrelide Related Compound C RS in *Diluent*

**System suitability solution:** 0.2 μg/mL of each USP Anagrelide Related Compound A RS and USP Anagrelide Related Compound C RS and 0.02 mg/mL of USP Anagrelide Hydrochloride RS. Initially dissolve USP Anagrelide Hydrochloride RS in *Diluent* (about 80% of the volume of the flask), sonicate for 10 min, and stir for 15 min. Add appropriate amounts of *Related compound A stock solution* and *Related compound C stock solution*, and dilute with *Diluent* to volume.

**Standard stock solution:** 0.1 mg/mL of anagrelide free base by dissolving USP Anagrelide Hydrochloride RS in acetonitrile (about 80% of the volume of the flask). Add a small quantity of 2 N hydrochloric acid (about 0.2 mL for every 100 mL of the final volume), sonicate to dissolve, and dilute with acetonitrile to volume.

**Standard solution:** 0.10 μg/mL of anagrelide free base in *Diluent* from *Standard stock solution*

**Sample solution:** 0.02 mg/mL of anagrelide free base from NLT 20 Capsules. Initially add *Diluent* to about 80% of the volume of the flask, sonicate for 10 min, stir for about 15 min, and dilute with *Diluent* to volume. Centrifuge (about 4000 rpm) the solution for 15 min, and use the supernatant for analysis.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Column temperature:** 45°

**Flow rate:** 1.0 mL/min

**Injection size:** 30 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between anagrelide hydrochloride and anagrelide related compound C, and be-

tween anagrelide hydrochloride and anagrelide related compound A, *System suitability solution*  
**Column efficiency:** NLT 3000 theoretical plates, *Standard solution*  
**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the anagrelide peak, *Standard solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of anagrelide from the *Standard solution*

$C_U$  = nominal concentration of anagrelide in the *Sample solution* (mg/mL)

$C_S$  = concentration of anagrelide in the *Standard solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria:** The individual and total impurities meet the limits in *Impurity Table 1*. [NOTE—Anagrelide related compound A (RRT = 0.86) and anagrelide related compound C (RRT = 1.15) are process related and controlled in the drug substance.]

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide hydrochloride	1.0	—	—
Anagrelide related compound B <sup>a</sup>	0.3	0.34	1.0
Anagrelide trichloro derivative <sup>b</sup>	1.8–2.3	1.0	0.15
Any other individual impurity	—	—	0.2
Total Impurities	—	—	1.5

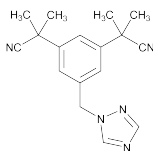
<sup>a</sup> [2-Amino-5,6-dichloroquinazoline-3(4*H*)-yl]acetic acid.

<sup>b</sup> 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>
  - USP Anagrelide Hydrochloride RS
  - USP Anagrelide Related Compound A RS
  - Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.  
C<sub>11</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> 277.15
  - USP Anagrelide Related Compound C RS
  - Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.

## Anastrozole



C<sub>17</sub>H<sub>19</sub>N<sub>5</sub> 293.37

1,3-Benzenediacetonitrile,  $\alpha,\alpha,\alpha',\alpha'$ -tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-.

$\alpha,\alpha,\alpha',\alpha'$ -Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-*m*-benzenediacetonitrile [120511-73-1].

» Anastrozole contains not less than 98.0 percent and not more than 102.0 percent of C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>, calculated on the anhydrous and solvent-free basis.

**Packaging and storage**—Preserve in well-closed containers. Store at room temperature.

#### USP Reference standards <11>—

USP Anastrozole RS

USP Anastrozole Related Compound A RS

2,2'-(5-methyl-1,3-phenylene)bis(2-methylpropanenitrile).

C<sub>15</sub>H<sub>18</sub>N<sub>2</sub> 226.32

#### Identification—

**A: Infrared Absorption** <197K>—

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method Ic* <921>: not more than 0.3%.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals**, *Method II* <231>: not more than 0.001%.

#### Related compounds—

*Solution A* and *Solution B*—Prepare as directed in the *Assay*.

**Peak identification solution**—Transfer accurately weighed quantities of USP Anastrozole RS and USP Anastrozole Related Compound A RS to a suitable volumetric flask, add a quantity of acetonitrile equivalent to about 40% of the volume of the flask to dissolve, and dilute with *Solution A* to volume to obtain a solution having known concentrations of about 0.5 mg of each per mL. Transfer 1 mL of this solution to a 50-mL volumetric flask, and dilute with *Solution A* to volume.

**Standard solution**—Dissolve an accurately weighed quantity of USP Anastrozole RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a solution having a known concentration of about 0.02 mg per mL.

**Test solution**—Transfer about 50 mg of Anastrozole to a 25-mL volumetric flask, and add about 10 mL of acetonitrile. Dissolve in and dilute with *Solution A* to volume.

**Blank solution**—Transfer 10 mL of acetonitrile to a 25-mL volumetric flask, and dilute with *Solution A* to volume.

**Chromatographic system** (see *Chromatography* <621>)—Prepare as directed in the *Assay*. Chromatograph the *Peak identification solution*, and record the peak responses as directed for *Procedure*: the relative retention times for anastrozole and anastrozole related compound A are listed in *Table 1*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor of the anastrozole peak is between 0.9 and 1.4; and the relative standard deviation for replicate injections of the anastrozole peak is not more than 5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Blank solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Adjust the peak areas for any interference from the *Blank solution*. Calculate the percentage of each anastrozole related compound in the portion of Anastrozole taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of anastrozole in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*; and  $r_U$  and  $r_S$  are the peak areas of anastrozole related compound obtained from the

Test solution and the Standard solution, respectively. The limits are given in Table 1. [NOTE—Any impurity of less than 0.05% is disregarded.]

Table 1

Name	Relative Retention	
	Time	Limit (%)
Anastrozole related compound B <sup>1</sup>	0.6	0.2
Anastrozole	1.0	—
Anastrozole related compound C <sup>2</sup>	2.0	0.2
Anastrozole related compound A <sup>3</sup>	4.0	—
Anastrozole related compound D <sup>4</sup>	4.3	0.1
Anastrozole related compound E <sup>5</sup>	5.4	0.1
Individual unspecified impurity	—	0.1
Total unspecified impurities	—	0.2
Total impurities	—	0.5

<sup>1</sup>2-(3-(1-Cyanoethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropanenitrile [C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>, 279.34].

<sup>2</sup>2,3-Bis(3-(1-cyano-1-methylethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropanenitrile [C<sub>30</sub>H<sub>31</sub>N<sub>9</sub>, 517.63].

<sup>3</sup>The relative retention time of anastrozole related compound A has been included for system suitability purposes only and is not intended for quantification.

<sup>4</sup>2,2'-(5-(Bromomethyl)-1,3-phenylene)bis(2-methylpropanenitrile) [C<sub>15</sub>H<sub>17</sub>BrN<sub>2</sub>, 305.21].

<sup>5</sup>2,2'-(5-(Dibromomethyl)-1,3-phenylene)bis(2-methylpropanenitrile) [C<sub>15</sub>H<sub>16</sub>Br<sub>2</sub>N<sub>2</sub>, 384.11].

### Assay—

**Solution A**—Prepare a mixture of water, methanol, acetonitrile, and trifluoroacetic acid (600:300:100:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Solution B**—Prepare a mixture of methanol, water, acetonitrile, and trifluoroacetic acid (450:400:150:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer a suitable quantity of USP Anastrozole RS to a suitable volumetric flask, and add a quantity of acetonitrile equivalent to about 40% of the volume of the flask. Dissolve in and dilute with *Solution A* to volume to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer about 25 mg of Anastrozole to a 50-mL volumetric flask, and add about 20 mL of acetonitrile. Dissolve in and dilute with *Solution A* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 3.2-mm × 10-cm column that contains 5-μm packing L42. The flow rate is about 0.75 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–10	100	0	isocratic
10–40	100→0	0→100	linear gradient
40–41	0→100	100→0	linear gradient
41–56	100	0	equilibration

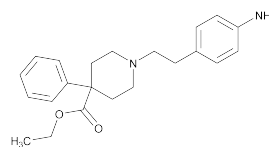
[NOTE—These gradient elution times are established on an HPLC system with a dwell time of approximately 0 minutes. The gradient elution times in the table can be adjusted by subtracting the dwell time to achieve the separation described.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor of the anastrozole peak is between 0.9 and 1.4; and the relative standard deviation for replicate injections of the anastrozole peak is not more than 1.5%.

**Procedure**—Inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of C<sub>17</sub>H<sub>19</sub>N<sub>5</sub> in the portion of Anastrozole taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of anastrozole in the *Standard preparation*;  $C_U$  is the concentration of anastrozole in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Anileridine



C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub> 352.47

4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester.

Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotate [144-14-9].

» Anileridine contains not less than 98.5 percent and not more than 101.0 percent of C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

### Identification—

**A:** Dissolve 40 mg in 2.3 mL of 0.1 N hydrochloric acid in a 100-mL volumetric flask, dilute with water to volume, and mix (*Stock solution*). Transfer 4.0 mL of this solution to a 100-mL volumetric flask, and add 25 mL of pH 7.0 *Buffer solution*. (Prepare the *Buffer solution* by dissolving 22.73 g of anhydrous dibasic sodium phosphate and 14.52 g of monobasic potassium phosphate in water to make 1000 mL. Dilute 25 mL of the buffer with water to 100 mL: the pH, determined potentiometrically, is 7.0 ± 0.05.) Dilute with water to volume, and mix (*Solution A*). Transfer 20.0 mL of the *Stock solution* to a 100-mL volumetric flask, add 25 mL of the pH 7.0 *Buffer solution*, dilute with water to volume, and mix (*Solution B*): the UV absorption spectrum of *Solution A* exhibits a maximum at 234 ± 1 nm; and the UV absorption spectrum of *Solution B* exhibits a maximum at 285 ± 2 nm. The ratio  $5A_{234} / A_{285}$  is about 8.8.

**B:** To 5 mL of a solution in 0.1 N hydrochloric acid (1 in 5000), add 2 mL of a solution of *p*-dimethylaminobenzaldehyde in alcohol (1 in 100): a yellow color develops immediately.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Dissolve 180 mg in a mixture of 1 mL of nitric acid and 40 mL of water: the solution shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.040%).

**Assay**—Dissolve about 350 mg of Anileridine, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 17.62 mg of C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>.



## Anileridine Injection

» Anileridine Injection is a sterile solution of Anileridine in Water for Injection, prepared with the aid of Phosphoric Acid. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of anileridine ( $C_{22}H_{28}N_2O_2$ ), as the phosphate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Anileridine Hydrochloride RS

USP Endotoxin RS

### Identification—

**A:** Dilute a volume of Injection, equivalent to about 1.25 mg of anileridine, with water to 5 mL, and add 2 mL of a 1 in 100 solution of *p*-dimethylaminobenzaldehyde in alcohol: a yellow color develops immediately.

**B:** A volume of Injection, diluted with water to a concentration of about 25 mg of anileridine in 1000 mL, exhibits absorbance maxima at  $234 \pm 1$  and  $285 \pm 2$  nm.

**Bacterial endotoxins** (85)—It contains not more than 7.2 USP Endotoxin Units per mg of anileridine.

**pH** (791): between 4.5 and 5.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Standard preparation**—[NOTE—Prepare on the day of the assay.] Dissolve an accurately weighed quantity of USP Anileridine Hydrochloride RS in 0.1 N hydrochloric acid, and quantitatively dilute with the same solvent to obtain a solution having a known concentration of about 250 µg per mL. (Each mg of anileridine hydrochloride is equivalent to 0.8286 mg of anileridine.)

**Assay preparation**—Transfer to a 500-mL volumetric flask an accurately measured volume of Injection, equivalent to about 100 mg of anileridine, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and 0.1 N hydrochloric acid to provide the blank to separate 200-mL volumetric flasks. To each flask add 25 mL of water, 5 mL of 1 N hydrochloric acid, and 5 mL of sodium nitrite solution (1 in 1000), and mix. Allow to stand for 2 minutes, then add to each flask 5 mL of ammonium sulfamate solution (1 in 200), and mix. Allow to stand for 3 minutes, then add 5 mL of N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), and mix. Allow to stand for 1 hour, dilute with water to volume, and mix. Determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 560 nm, with a suitable spectrophotometer, using the reagent blank to set the instrument. Calculate the quantity, in mg, of anileridine ( $C_{22}H_{28}N_2O_2$ ) in each mL of the Injection taken by the formula:

$$(352.48 / 425.40)(0.5C / V)(A_U / A_S)$$

in which 352.48 and 425.40 are the molecular weights of anileridine and anileridine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Anileridine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Anileridine Hydrochloride

$C_{22}H_{28}N_2O_2 \cdot 2HCl$  425.39

4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester, dihydrochloride.

Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipicotate dihydrochloride [126-12-5].

» Anileridine Hydrochloride contains not less than 96.0 percent and not more than 102.0 percent of  $C_{22}H_{28}N_2O_2 \cdot 2HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Anileridine Hydrochloride RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** Dissolve 50 mg in water in a 100-mL volumetric flask, dilute with water to volume, and mix (*Stock solution*). Transfer 4.0 mL of this solution to a 100-mL volumetric flask, and add 25 mL of *pH 7.0 Buffer solution*. (Prepare the *pH 7.0 Buffer solution* by dissolving 22.73 g of anhydrous dibasic sodium phosphate and 14.52 g of monobasic potassium phosphate in water to make 1000.0 mL. Dilute 25 mL of the buffer with water to 100 mL: the pH, determined potentiometrically, is  $7.0 \pm 0.05$ .) Dilute with water to volume, and mix (*Solution A*). Transfer 20.0 mL of the *Stock solution* to a 100-mL volumetric flask, add 25 mL of the *pH 7.0 Buffer solution*, dilute with water to volume, and mix (*Solution B*): the UV absorption spectrum of *Solution A* exhibits a maximum at  $234 \pm 1$  nm, and the UV absorption spectrum of *Solution B* exhibits a maximum at  $285 \pm 2$  nm.

**C:** To 5 mL of a solution (1 in 5000) add 2 mL of a 1 in 100 solution of *p*-dimethylaminobenzaldehyde in alcohol: a yellow color develops immediately.

**D:** A solution (1 in 100) responds to the tests for *Chloride* (191).

**pH** (791): between 2.5 and 3.0, in a solution (1 in 20).

**Loss on drying** (731)—Dry it at a pressure below 5 mm of mercury at  $100^\circ$  for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride content**—Dissolve about 200 mg, accurately weighed, in 50 mL of water in a glass-stoppered flask. Add 25.0 mL of 0.1 N silver nitrate VS, then add 5 mL of 2 N nitric acid and 5 mL of nitrobenzene, shake vigorously, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl is between 16.0% and 17.2%.

**Assay**—Dissolve about 200 mg of Anileridine Hydrochloride, accurately weighed, in 10 mL of glacial acetic acid by heating on a steam bath. Cool immediately in a cold water bath, add 5 mL of mercuric acetate TS, 20 mL of acetone, and 0.5 mL of indicator solution (70 mg of  $\alpha$ -naphtholbenzein, 10 mg of crystal violet, and 40 mg of quinaldine red in 100 mL of glacial acetic acid), and titrate with 0.1 N perchloric acid VS to a gray-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.27 mg of  $C_{22}H_{28}N_2O_2 \cdot 2HCl$ .

## Anileridine Hydrochloride Tablets

» Anileridine Hydrochloride Tablets contain an amount of anileridine hydrochloride ( $C_{22}H_{28}N_2O_2 \cdot 2HCl$ ) equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of anileridine ( $C_{22}H_{28}N_2O_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Anileridine Hydrochloride RS

### Identification—

**A:** Place a quantity of finely powdered Tablets, equivalent to about 50 mg of anileridine, in a 250-mL volumetric flask, add 100 mL of water, and heat on a steam bath. Cool, dilute with water to volume, mix, and filter: five mL of the filtrate responds to *Identification* test C under *Anileridine Hydrochloride*.

**B:** Transfer to a 100-mL volumetric flask a quantity of finely powdered Tablets, equivalent to about 50 mg of anileridine. Add about 30 mL of water, and heat on a steam bath. Cool, dilute with water to volume, mix, and filter (*Stock solution*): the filtrate responds to *Identification* test B under *Anileridine Hydrochloride*, beginning with "Transfer 4.0 mL of this solution."

### Dissolution (711)—

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of anileridine ( $C_{22}H_{28}N_2O_2$ ) dissolved, employing the procedure set forth in the *Assay*, using a filtered portion of the solution under test as the *Assay preparation* in comparison with a Standard solution having a known concentration of USP Anileridine Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 65% (Q) of the labeled amount of  $C_{22}H_{28}N_2O_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

**Standard preparation**—Prepare as directed in the *Assay* under *Anileridine Injection*.

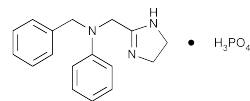
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of anileridine, to a 250-mL volumetric flask. Add 25 mL of 1 N hydrochloric acid and 100 mL of water, and heat on a water bath. Cool, dilute with water to volume, and mix. Filter the solution, discarding the first 25 mL of the filtrate.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Anileridine Injection*. Calculate the quantity, in mg, of  $C_{22}H_{28}N_2O_2$  in the portion of Tablets taken by the formula:

$$(352.48/425.40)(0.25C)(A_U / A_S)$$

in which 352.48 and 425.40 are the molecular weights of anileridine and anileridine hydrochloride, respectively; C is the concentration, in  $\mu\text{g}$  per mL, of USP Anileridine Hydrochloride RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Antazoline Phosphate



$C_{17}H_{19}N_3 \cdot H_3PO_4$  363.35

1-*H*-imidazole-2-methanamine, 4,5-dihydro-*N*-phenyl-*N*-(phenylmethyl)-, phosphate (1:1).

2-[(*N*-Benzylanilino)methyl]-2-imidazoline phosphate (1:1) [154-68-7].

» Antazoline Phosphate contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{19}N_3 \cdot H_3PO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Antazoline Phosphate RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The  $R_f$  value of the principal spot in the chromatogram of the *Identification* corresponds to that of *Standard preparation A* as obtained in the test for *Chromatographic purity*.

**Melting range, Class Ia** (741): between 194° and 198°, with decomposition.

**pH** (791): between 4.0 and 5.0, in a solution (1 in 50).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

### Chromatographic purity—

**Standard solutions**—Dissolve USP Antazoline Phosphate RS in methanol, and mix to obtain a solution having a known concentration of 0.10 mg per mL. Quantitatively dilute with methanol to obtain 5 *Standard solutions* having the following compositions:

Standard preparation	Dilution	Concentration ( $\mu\text{g}$ RS per mL)	Percentage (% for comparison with test specimen)
A	(1 in 2)	50	0.5
B	(2 in 5)	40	0.4
C	(3 in 10)	30	0.3
D	(1 in 5)	20	0.2
E	(1 in 10)	10	0.1

**Test solution**—Dissolve an accurately weighed quantity of Antazoline Phosphate in methanol to obtain a solution containing 10 mg per mL.

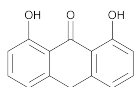
**Identification solution**—Dilute a portion of the *Test solution* quantitatively with methanol to obtain a solution containing 50  $\mu\text{g}$  per mL.

**Procedure**—Apply separately 10  $\mu\text{L}$  of the *Test solution*, 10  $\mu\text{L}$  of the *Identification solution*, and 10  $\mu\text{L}$  of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, methanol, and diethylamine (17:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromato-

gram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*. [NOTE—Disregard any spots observed at the origins of the chromatograms.] No secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test solution* corresponds to not more than 1.0%.

**Assay**—Dissolve about 750 mg of Antazoline Phosphate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a saturated solution of lithium chloride in glacial acetic acid (see *Titrimetry* <541>). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.34 mg of  $C_{17}H_{19}N_3 \cdot H_3PO_4$ .

## Anthralin



$C_{14}H_{10}O_3$

9(10*H*)-Anthracenone, 1,8-dihydroxy-;  
1,8-Dihydroxy-9-anthrone [1143-38-0].

226.23

### DEFINITION

Anthralin contains NLT 97.0% and NMT 102.0% of anthralin ( $C_{14}H_{10}O_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B. ULTRAVIOLET ABSORPTION** <197U>  
Sample solution: 10 µg/mL in chloroform  
Acceptance criteria: Meets the requirements

### ASSAY

#### PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 5 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** 0.25 mg/mL of Anthralin in dichloromethane

**Sample solution:** Transfer 5 mL each of *Sample stock solution* and *Internal standard solution* into a 25-mL volumetric flask, dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 354 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*

[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution*

**Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anthralin ( $C_{14}H_{10}O_3$ ) in the portion of Anthralin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*

$R_S$  = peak response ratio of anthralin to *o*-nitroaniline from the *Standard solution*

$C_S$  = concentration of USP Anthralin RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Anthralin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

- **CHLORIDE AND SULFATE**, *Chloride* <221>

Sample: 1 g

**Analysis:** To 15 mL of water add the *Sample*, mix, and filter. Acidify 5 mL of the filtrate with nitric acid, and add a few drops of silver nitrate TS.

**Acceptance criteria:** No more opalescence is produced immediately than is present in a 5-mL portion of the filtrate to which nothing has been added.

- **CHLORIDE AND SULFATE**, *Sulfate* <221>

Sample: 5 mL of the untreated filtrate obtained in the test for *Chloride*

**Analysis:** To the *Sample* add 3 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.

**Acceptance criteria:** No more turbidity is produced than is present in a 5-mL portion of the filtrate to which nothing has been added.

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* <741>: 178°–181°

- **LOSS ON DRYING** <731>

**Analysis:** Dry a sample over silica gel for 4 h.

**Acceptance criteria:** NMT 0.5%

- **ACIDITY OR ALKALINITY**

**Analysis:** Suspend a sample in water, and filter.

**Acceptance criteria:** The filtrate is neutral to litmus.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place. Protect from light.

- **USP REFERENCE STANDARDS** <11>  
USP Anthralin RS

## Anthralin Cream

### DEFINITION

Anthralin Cream is Anthralin in an aqueous (oil-in-water) or oily (water-in-oil) cream vehicle. Cream labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ), and Cream labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ).

### ASSAY

#### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Weigh 5 g of Cream into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Cream. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.

**Sample solution:** Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 354 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*

[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution*

**Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anthralin ( $C_{14}H_{10}O_3$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*

$R_S$  = peak response ratio of anthralin to *o*-nitroaniline peak from the *Standard solution*

$C_S$  = concentration of USP Anthralin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of anthralin in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–115.0% for Cream labeled to contain more than 0.1% of anthralin; 90.0%–130.0% for Cream labeled to contain 0.1% or less of anthralin

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.

• **LABELING:** Label it to indicate whether the cream vehicle is aqueous or oily.

• **USP REFERENCE STANDARDS** <11>  
USP Anthralin RS

## Anthralin Ointment

### DEFINITION

Anthralin Ointment is Anthralin in a petrolatum or other oleaginous vehicle. Ointment labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ), and Ointment labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ).

### ASSAY

#### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Weigh 5 g of Ointment into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Ointment. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.

**Sample solution:** Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 354 nm**Column:** 4.6-mm × 25-cm; packing L3**Flow rate:** 2 mL/min**Injection volume:** 10 µL**System suitability****Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*

[NOTE—The relative retention times for anthralin, danthron, dianthrone, and o-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

**Suitability requirements****Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution***Tailing factor:** NMT 1.5, *System suitability solution***Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution***Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of anthralin (C<sub>14</sub>H<sub>10</sub>O<sub>3</sub>) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 $R_U$  = peak response ratio of anthralin to o-nitroaniline from the *Sample solution* $R_S$  = peak response ratio of anthralin to o-nitroaniline from the *Standard solution* $C_S$  = concentration of USP Anthralin RS in the *Standard solution* (µg/mL) $C_U$  = nominal concentration of anthralin in the *Sample solution* (µg/mL)**Acceptance criteria:** 90.0%–115.0% for Ointment labeled to contain more than 0.1% of anthralin; 90.0%–130.0% for Ointment labeled to contain 0.1% or less of anthralin**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.
- **USP REFERENCE STANDARDS (11)**  
USP Anthralin RS

**Anthrax Vaccine Adsorbed**

» Anthrax Vaccine Adsorbed is a sterile, milky-white suspension made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis*. The final product contains no dead or live bacteria. The production cultures are grown in a chemically defined protein-free medium containing amino acids, vitamins, inorganic salts, and sugars. The sterile filtrate is adsorbed on sterile aluminum hydroxide, concentrated 10-fold, and resuspended in sterile physiological saline containing formaldehyde with benzethonium chloride as a preservative. Sublots may be combined to produce final lots. The product meets potency requirements when tested against the U.S. Reference Standard Anthrax Vaccine, in accordance with approved procedures (guinea pig intracutaneous challenge models).

**Packaging and storage**—Preserve in multiple-dose tight Type I glass containers, and store at a temperature between 2° and 8°. Do not freeze.

**Expiration date**—The expiration date is 18 months from the date of manufacture.

**Labeling**—Label it to state that it is to be well shaken before use and that it is not to be frozen.

**FILTRATE—****Identification—**

**Trichloroacetic acid solution**—Prepare a solution of trichloroacetic acid (see *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*) in water containing 100 g trichloroacetic acid per 100 mL of the solution.

**Sample buffer**—Prepare a solution containing 141 mM tris(hydroxymethyl)aminomethane, 106 mM tris(hydroxymethyl)aminomethane hydrochloride, 0.51 mM edetate disodium, 2% (w/v) dodecyl lithium sulfate, 10% (v/v) glycerol, 0.22 mM Coomassie blue G-250, and 0.175 mM phenolsulfonphthalein. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.5.

**Running buffer**—Prepare a solution containing 25 mM tris(hydroxymethyl)aminomethane, 192 mM glycine, and 0.1% (w/v) dodecyl sodium sulfate (see *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*) in water. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.5.

**Transblotting buffer**—Prepare a solution containing 12.5 mM tris(hydroxymethyl)aminomethane, 96 mM glycine, and 10% (v/v) methanol. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.0.

**Blocking buffer**—Prepare a solution containing 10 mM monobasic sodium phosphate, 150 mM sodium chloride, 5% (w/v) nonfat dry milk, and 0.05% (w/v) Polysorbate 20. Adjust with sodium hydroxide to a pH of 7.4.

**Primary antibody solutions**—Prepare suitable monoclonal antibodies raised against the Protective Antigen (PA), the Lethal Factor (LF), and the Edema Factor (EF), respectively, of *Bacillus anthracis* in murine ascites cells, harvested, and used without further purification. Immediately before use, dilute each of the murine ascites fluids containing the monoclonal antibodies 1:1000 (v/v) with the *Blocking buffer*.

**Secondary antibody solution**—Immediately before use, dissolve according to the manufacturer's instructions, if necessary, and dilute the stock horseradish peroxidase conjugated to goat anti-mouse IgG solution 1:1000 with *Blocking buffer*.

**Chromogenic visualization solution**—Prepare a 150 mg per mL solution of 4-chloro-1-naphthol in water.

**Test solution**—Use Anthrax Vaccine Filtrate as is.

**Procedure**—In a suitable centrifuge tube transfer 30/c mL of the *Test solution*, where c is the total protein concentration, in µg per mL, of the solution as determined in the test for *Total protein*. Add 16.5/c mL of *Trichloroacetic acid solution*, and incubate for at least 10 minutes. Centrifuge at 9000g for about 10 minutes, decant off the supernatant, and hold the tube inverted to drain on a filter paper. Dissolve the pellet in about 60 µL of *Sample buffer*, and transfer the solution to a polypropylene microfuge tube that has a lid. Close the lid tightly, secure with a lid-lock, and heat at 100° for 5 minutes. Allow the solution to cool to room temperature, and centrifuge at 10,000g for 15 seconds to collect the liquids. In a suitable device for polyacrylamide-gel electrophoresis (see *Electrophoresis* (726) and the section *Polyacrylamide Gel Electrophoresis* under *Biotechnology-Derived Articles—Tests* (1047)) add appropriate volumes of the *Running buffer* in the upper and the lower buffer chambers. Attach a 4%–20% gradient tris-glycine polyacrylamide slab gel sandwiched between two glass plates, such that the wells for sample application are exposed to the *Running buffer* in the upper buffer chamber. Apply about 20-µL aliquots of the treated *Test solution* in three alternate lanes. [NOTE—Do not apply any solution in the outside lanes.] Connect the lower buffer chamber electrode to the positive ter-

minal and the upper buffer chamber electrode to the negative terminal of a suitable power supply unit, and carry out the electrophoresis at a constant current of about 40 mA. When the dye-front is about 1 cm from the bottom of the gel (about 40 minutes), stop the current, and remove the gel from the gel assembly. [NOTE—Do not touch the gel with bare hand. Use gloves.]

Place 3–4 filter papers, cut to the size of the gel and soaked in the *Transblotting buffer*, on the anode plate of a suitable semidry electroblotter. Cut a nitrocellulose membrane to the same size as the gel plus 1–2 mm on each side, and “wet” the membrane by immersing it into the *Transblotting buffer* for about 15 seconds, such that there is no air-bubble between the buffer and the membrane. Place the “wet” membrane immediately on the stack of filter papers, and remove all air bubbles between the membrane and filter paper by rolling a pipet, or equivalent, gently over the surface of the membrane. Place a few drops of the *Transblotting buffer* on the membrane, and then carefully place the gel on it. Gently roll a pipet, or equivalent, over the surface of the gel to ensure intimate contact between the gel and the membrane, making sure that there are no air bubbles in between. Place a filter paper cut to the size of the gel and soaked in the *Transblotting buffer*, such that there is no air-bubble between the filter paper and the gel. Place 2–3 additional filter papers, prepared in a similar manner, on the top, and complete the transfer stack by placing the cathode plate on the top. Apply a current of about 250 mA, and continue transfer for 90 minutes.

Remove the membrane, and wash it quickly by immersing into water for 15 seconds. [NOTE—Do not touch the membrane with bare hand. Use gloves.] Cut the membrane into three strips such that each strip contains a lane containing the *Test solution*, and mark the strips as PA, LF, and EF at the top. Place each strip in a heat-sealable bag, add 5 mL of *Blocking buffer*, and seal the bag. Incubate for 30 minutes with constant agitation. Open each bag, and pour out the *Blocking buffer*. Add 9 mL of the diluted *Primary antibody solution* against PA to the bag containing the strip marked PA. Similarly, add 9 mL of the diluted *Primary antibody solution* against LF and EF to the bags containing strips labeled LF and EF, respectively. Seal the bags, and incubate under agitation for 2 hours at room temperature or overnight at 2° to 8°. Remove the strips from the plastic bags, and place in separate plastic boxes. Add sufficient *Blocking buffer* so that each strip is completely immersed. Agitate for at least 30 minutes at room temperature with two changes of *Blocking buffer*. Remove the strips, and place each strip in a new heat-sealable plastic bag. Add 9 mL of the *Secondary antibody solution* to each plastic bag. Seal the bags, and incubate for 1 hour at room temperature under agitation. Remove the strips from the plastic bags, and place in separate plastic boxes. Add sufficient *Blocking buffer* so that each strip is completely immersed. Agitate for at least 30 minutes at room temperature with two changes of the *Blocking buffer*. Transfer each strip into a new heat-sealable plastic bag, add 9 mL *Chromogenic visualization solution*, 10  $\mu$ L of 30% (v/v) hydrogen peroxide, and seal the bags. Incubate for about 30 minutes under agitation. Transfer the strips into separate plastic boxes, and remove the excess 4-chloro-1-naphthol by incubating with water under agitation for 10 minutes. Visual observation indicates a strong positive band on the strip labeled PA (Protective Antigen), a faintly detectable band on the strip labeled LF (Lethal Factor), and no detectable band on the strip labeled EF (Edema Factor).

### 83 kDa protein—

*Trichloroacetic acid solution*, *Sample buffer*, *Running buffer*, and *Test solution*—Prepare as directed under *Identification*.

*Staining solution*—Prepare a solution of Coomassie blue G-250 having a concentration of 1.25 g per L in a mixture of water, methanol, and acetic acid (5:4:1, v/v).

*Protein molecular weight standard solution*—Reconstitute a vial of protein molecular weight standard mixture contain-

ing proteins of molecular weights at least in the range of 14 to 200 kDa, according to manufacturer's instruction. Dilute the solution with *Sample buffer* such that the concentration of each protein in the solution is about 0.5  $\mu$ g per  $\mu$ L.

*Procedure*—In a suitable centrifuge tube transfer 10/*c* mL of the *Test solution*, where *c* is the total protein concentration, in  $\mu$ g per mL, of the solution as determined by the test for *Total protein* (see below). Add 5.5/*c* mL of *Trichloroacetic acid solution*, and incubate for at least 10 minutes. Centrifuge at 9000*g* for about 10 minutes, decant off the supernatant, and hold the tube inverted to drain on a filter paper. Dissolve the pellet in 20  $\mu$ L of *Sample buffer*, and transfer the solution to a polypropylene microfuge tube with a lid. Transfer 20  $\mu$ L of *Protein molecular weight standard solution* to another polypropylene microfuge tube with a lid. Close the lids tightly, secure with lid-locks, and heat both solutions at 100° for 5 minutes. Allow the solutions to cool to room temperature, and centrifuge at 10,000*g* for 15 seconds to collect the liquids. Apply the solutions to two consecutive lanes of a 4%–20% gradient tris-glycine polyacrylamide slab gel [NOTE—Do not apply any solution in the outside lanes.], and electrophorese as directed under *Identification* (see *Electrophoresis* (726) and the section *Polyacrylamide Gel Electrophoresis* under *Biotechnology-Derived Articles*—*Tests* (1047)). When the dye-front is about 1 cm from the bottom of the gel (about 40 minutes), stop the current, and remove the gel from the gel assembly. Soak the gel in a suitable volume of the *Staining solution* for at least 1 hour, such that the gel is completely immersed in the *Staining solution* during staining. [NOTE—Do not touch the gel with bare hand. Use disposable gloves.] Destain the gel with a large volume of water under constant agitation with repeated changes of water until the background of the gel is completely color free. Using the molecular weights of the proteins in *Protein molecular weight standard solution*, identify the band corresponding to the Protective Antigen (MW about 83 kDa) in the *Test solution* lane. [NOTE—This band is also the single most predominant band in the lane of the *Test solution*.] Scan the gel, and determine the relative amount (by peak area) of the 83-kDa band by densitometry in the lane of the *Test solution*. The content of 83 kDa band is not less than 35% of the total peak area.

### Total protein—

*Standard solution A*—Prepare a solution of albumin bovine serum (see *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*) in water to obtain a known concentration of about 2.0 mg per mL.

*Standard solutions B, C, D, and E*—Dilute *Standard solution A* with water to obtain solutions having protein concentrations of 4, 8, 16, and 24  $\mu$ g per mL, respectively.

*Test solution*—Use Anthrax Vaccine Filtrate as is.

*Procedure* (See *Biotechnology-Derived Articles*—*Tests* (1047), *Total Protein Assay, Method 3*)—To a series of test tubes transfer 800  $\mu$ L each of *Standard solutions B, C, D, and E* and the *Test solution*. Also transfer 800  $\mu$ L of water to be used as the blank. Add 200  $\mu$ L of Coomassie blue G-250 dye solution (see *Reagent Specifications* in the section *Reagents, Indicators, and Solutions*) to each tube, and mix without foaming. Determine absorbances of the solutions at 595 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)), using the blank to set the instrument to zero. [NOTE—Do not use quartz (silica) spectrophotometer cells; the dye binds to silica.] Construct a standard curve by plotting the absorbances versus protein concentrations, in  $\mu$ g per mL, of *Standard solutions B, C, D, and E* and by drawing a best-fit straight line using the linear regression method. From the standard curve, determine the total protein concentration of the *Test solution* using the absorbance value. The protein concentration is between 5 and 20  $\mu$ g per mL.

**FINAL PRODUCT—****Aluminum—**

**Standard solutions**—Prepare as directed for *Standard Preparations* under *Aluminum* (206), except to prepare solutions containing 10, 20, 30, 40, and 50 µg per mL of aluminum.

**Test solution**—Mix Anthrax Vaccine Adsorbed, Final Product well, and transfer 0.2 mL to a 10-mL volumetric flask. Add 0.5 mL of concentrated sulfuric acid and 0.5 mL of concentrated nitric acid, and mix gently. Incubate at room temperature for 30 minutes or until the solution becomes essentially clear. Dilute with water to volume.

**Procedure**—Proceed as directed for *Procedure* under *Aluminum* (206). Plot the absorbances versus the content of aluminum, in µg per mL, for the *Standard solutions*, and draw a best-fit straight line through the points using a linear regression model. Calculate the amount of aluminum in Anthrax Vaccine Adsorbed, in mg per mL. The aluminum concentration is between 0.8 and 1.5 mg per mL.

**Safety**—It meets the requirements when tested as directed in the section *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88).

**Sterility** (71)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* method under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.5 and 8.5.

**Sodium chloride—**

**Standard solutions A and B**—Prepare two solutions of sodium chloride in water having concentrations of 0.2 mM and 2.0 mM, respectively.

**Test solution**—Transfer 0.5 mL of Anthrax Vaccine Adsorbed, Final Product to a 50-mL volumetric flask. Dilute with water to volume.

**Procedure**—Determine the voltage readings of *Standard solutions A* and *B* and the *Test solution* using an ion-specific electrode specific for the chloride ion electrically coupled with a standard silver-silver chloride reference electrode. Plot the voltage readings versus concentration of chloride, in mg per mL, for *Standard solutions A* and *B*, and draw a straight line joining the points. Calculate the concentration of chloride ion in the *Test solution* from the voltage reading. Assuming that the chloride ion comes entirely from sodium chloride, calculate the concentrations of sodium chloride in the *Test solution*. The concentration of sodium chloride in Anthrax Vaccine Adsorbed is between 0.75% and 0.95% (w/v).

**Formaldehyde—**

**Potassium ferricyanide solution**—Dissolve 2.5 g of potassium ferricyanide in about 100 mL of water, and mix.

**Phenylhydrazine hydrochloride solution**—Dissolve 4 g of phenylhydrazine hydrochloride in 100 mL of absolute alcohol, add 2 mL of water, and mix.

**Standard stock solution**—To prepare a stock solution, proceed as directed in the *Assay* under *Formaldehyde Solution* to determine the concentration of formaldehyde in percent (w/v).

**Standard solutions**—Dilute the *Standard stock solution* in water to obtain solutions having concentrations of 0.005%, 0.01%, and 0.02% (w/v).

**Test solution**—Use Anthrax Vaccine Adsorbed, Final Product as is.

**Procedure**—To suitable glass centrifuge tubes transfer 1.0 mL each of water, the *Standard solutions*, and the *Test solution*. To each tube add 1.0 mL of *Potassium ferricyanide solution*, 4.0 mL of 18% (w/v) hydrochloric acid and 2.0 mL of *Phenylhydrazine hydrochloride solution*. Mix after each addition. Incubate for 50 to 60 minutes at room temperature. Centrifuge the solutions at 10,000g for at least 10 minutes, and measure absorbances of the supernatants at 540 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). Plot the absorbances versus concentrations of formaldehyde, in mg per mL, in the *Standard*

*solutions*, and draw the best-fit straight line through the points. Calculate the amount of formaldehyde in the sample in percent (w/v). The concentration of formaldehyde in Anthrax Vaccine Adsorbed is less than 0.02% (w/v).

**Benzethonium chloride—**

**Citrate buffer**—Dissolve 25 g of citric acid monohydrate in about 60 mL of water, and adjust with a solution of sodium hydroxide to a pH of 4.5. Transfer the solution to a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Dye solution**—Dissolve 50 mg of 2',4',5',7'-tetrabromofluorescein in about 100 mL water, and mix. Dilute 1 mL of this solution to 100 mL with water.

**Docusate sodium solution**—Dissolve 50 mg of docusate sodium in 1 L of water.

**Standard solution A**—Transfer about 0.5 g, accurately weighed, of benzethonium chloride to a 100-mL volumetric flask, dissolve in about 60 mL water, dilute with water to volume, and mix.

**Standard solutions B, C, D, and E**—Dilute *Standard solution A* with water to obtain solutions having concentrations of 0.001%, 0.002%, 0.003%, and 0.004% (w/v), respectively.

**Test solution**—Use Anthrax Vaccine Adsorbed, Final Product as is.

**Procedure**—Transfer 4.0 mL each of *Standard solutions B, C, D, and E* and the *Test solution* to suitable glass centrifuge tubes. Add 1.0 mL *Citrate buffer* and 0.4 mL of the *Dye solution* to each tube, and mix. Add 4.0 mL of 1,1,2,2-tetrachloroethane to each tube, and vigorously mix on a vortex mixer for 1 minute. Centrifuge at about 1000g for at least 15 minutes to separate the organic layer from the aqueous layer. Transfer 2.0 mL of the organic layer from the tubes to another set of glass tubes. Add 4.0 mL of water and 0.5 mL of *Citrate buffer* to each tube, and mix on a vortex mixer for approximately 1 minute. Titrate the benzethonium chloride-dye complex in each tube with the *Docusate sodium solution* (see *Titrimetry* (541)) to the colorimetric endpoint indicated by the disappearance of the pink color of the organic layer. [NOTE—Vigorously mix the solution on a vortex mixer after each addition of the *Docusate sodium solution*.] Plot the volumes of *Docusate sodium solution* required versus the concentrations of benzethonium chloride in *Standard solutions B, C, D, and E*, and draw a best-fit straight line through the points. Determine the concentration of benzethonium chloride in the *Test solution* from the volume of *Docusate sodium solution* required to titrate the *Test solution*. The concentration of benzethonium chloride in Anthrax Vaccine Adsorbed is between 0.0015% and 0.0030% (w/v).

**Relative potency—**

**Standard solutions**—Dilute approved U.S. Reference Standard Anthrax Vaccine 1:1.6, 1:4, 1:10, and 1:25 aseptically with a sterile 0.9% sodium chloride solution.

**Test solutions**—Dilute Anthrax Vaccine Adsorbed, Final Product 1:1.6, 1:4, 1:10, and 1:25 aseptically with a sterile 0.9% sodium chloride solution.

**Procedure**—Assign each dilution to a set of 12 randomly selected guinea pigs, strain Mdh:S(RA), 6 males and 6 females, each weighing 315 to 385 g on the day of vaccination. Inject the animals subcutaneously in the ventral abdomen with 0.5 mL of the assigned dilutions. On the 14th day post-vaccination, challenge the animals with approximately 1000 spores of *Bacillus anthracis* Vollum 1B, and record the deaths daily for a 10-day observation period. Record the numbers of surviving animals for each of the *Standard solutions* and the *Test solutions* at the end of the test. Perform calculations by estimating best-fit lines for the *Standard solutions* and the *Test solutions* using a logistic regression model that utilizes the number of animals that survived at the end of the test and the time to death for the animals that died. Evaluate statistically the lines corresponding to the *Standard solutions* and the *Test solutions* for parallelism. Determine the common slope, and draw the parallel lines using the common slope. The relative potency of Anthrax Vaccine Ad-

sorbed with respect to the corresponding Approved U.S. Reference Standard Anthrax Vaccine is the antilog of the horizontal distance between the two parallel lines. The relative potency of Anthrax Vaccine Adsorbed is acceptable if it is between 0.53 and 1.79, both values inclusive.

### Anticoagulant Citrate Dextrose Solution

» Anticoagulant Citrate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, and Dextrose in Water for Injection. It contains in each 1000 mL:

	Solution A	Solution B
Total Citrate, expressed as citric acid, anhydrous ( $C_6H_8O_7$ )		
not less than . . . . .	20.59 g	12.37 g
not more than . . . . .	22.75 g	13.67 g
Dextrose ( $C_6H_{12}O_6 \cdot H_2O$ )		
not less than . . . . .	23.28 g	13.96 g
not more than . . . . .	25.73 g	15.44 g
Sodium (Na)		
not less than . . . . .	4.90 g	2.94 g
not more than . . . . .	5.42 g	3.25 g

It contains no antimicrobial agents.  
Prepare Anticoagulant Citrate Dextrose Solution as follows:

	Solution A	Solution B
Citric Acid (anhydrous) . .	7.3 g	4.4 g
Sodium Citrate (dihydrate)	22.0 g	13.2 g
Dextrose (monohydrate)	24.5 g	14.7 g
Water for Injection, a sufficient quantity to make	1000mL	1000mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 8 g and 4.8 g of monohydrated citric acid may be used instead of the indicated, respective amounts of anhydrous citric acid; 19.3 g and 11.6 g of anhydrous sodium citrate may be used instead of the indicated, respective amounts of dihydrated sodium citrate; and 22.3 g and 13.4 g of anhydrous dextrose may be used instead of the indicated, respective amounts of monohydrated dextrose.

**Packaging and storage**—Preserve in single-dose containers, of colorless, transparent, Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* <161>).

**Labeling**—Label it to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.

**USP Reference standards** <11>—

USP Citric Acid RS

USP Endotoxin RS

**Identification**—It responds to the *Identification* test under *Dextrose*, and, when concentrated to one-half its volume, responds to the tests for *Citrate* <191> and for *Sodium* <191>.

**Bacterial endotoxins** <85>—It contains not more than 5.56 USP Endotoxin Units per mL.

**pH** <791>: between 4.5 and 5.5.

**Chloride** <221>—A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay for total citrate**—

*Mobile Phase, Standard Preparation 1, and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* <345>.

*Assay preparation*—Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under general chapter <345>.

*Procedure*—Proceed as directed for *Procedure* under general chapter <345>, and calculate the quantity, in mg, of anhydrous citric acid ( $C_6H_8O_7$ ) in the volume of Solution taken by the formula:

$$0.001(192.12/189.10)C_5 D(r_U / r_S)$$

in which 192.12 is the molecular weight of anhydrous citric acid; 189.10 is the molecular weight of citrate ( $C_6H_5O_7$ );  $C_5$  is the concentration, in  $\mu\text{g}$  per mL, of citrate in *Standard Preparation 1*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the citrate peak areas obtained from the *Assay preparation* and *Standard Preparation 1*, respectively.

**Assay for sodium**—Proceed as directed in the *Assay for sodium under Anticoagulant Citrate Phosphate Dextrose Adenine Solution*.

**Assay for dextrose**—Determine the angular rotation of Solution in a suitable polarimeter tube (see *Optical Rotation* <781>). Where the Solution is labeled to contain anhydrous dextrose, calculate the percentage (g per 100 mL) of  $C_6H_{12}O_6$  in the portion of Solution taken by the formula:

$$(100/52.9)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees. Where the Solution is labeled to contain dextrose monohydrate, calculate the percentage (g per 100 mL)  $C_6H_{12}O_6 \cdot H_2O$  in the portion of Solution taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.



## Anticoagulant Citrate Phosphate Dextrose Solution

» Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, and Dextrose in Water for Injection. It contains, in each 1000 mL, not less than 2.11 g and not more than 2.33 g of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ); not less than 24.22 g and not more than 26.78 g of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ); not less than 19.16 g and not more than 21.18 g of total citrate, expressed as citric acid, anhydrous ( $\text{C}_6\text{H}_8\text{O}_7$ ); and not less than 6.21 g and not more than 6.86 g of Sodium (Na). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Solution as follows:

Citric Acid (anhydrous) . . . . .	2.99 g
Sodium Citrate (dihydrate) . . . . .	26.3 g
Monobasic Sodium Phosphate (monohydrate; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ) . . . . .	2.22 g
Dextrose (monohydrate) . . . . .	25.5 g
Water for Injection, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 23.2 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

**Packaging and storage**—Preserve in single-dose containers, of colorless, transparent, Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* <161>).

**Labeling**—Label it to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.

**USP Reference standards** <11>—

USP Citric Acid RS

USP Endotoxin RS

**Identification**—It responds to the *Identification* test under *Dextrose*, and to the tests for *Phosphate* <191>, and, when concentrated to one-half its volume, responds to the tests for *Citrate* <191> and for *Sodium* <191>.

**Bacterial endotoxins** <85>—It contains not more than 5.56 USP Endotoxin Units per mL.

**pH** <791>: between 5.0 and 6.0.

**Chloride** <221>—A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay for total citrate and total phosphate**—

*Mobile Phase, Standard Preparation 2, and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* <345>.

*Assay preparation for total citrate assay*—Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under general chapter <345>.

*Assay preparation for total phosphate assay*—Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Phosphate Assay* under general chapter <345>.

*Procedure*—Proceed as directed for *Procedure* under general chapter <345>, and calculate the quantity, in mg, of anhydrous citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ) in the volume of Solution taken by the formula:

$$0.001(192.12/189.10)C_S D(r_U / r_S)$$

in which 192.12 is the molecular weight of anhydrous citric acid; 189.10 is the molecular weight of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ );  $C_S$  is the concentration, in  $\mu\text{g}$  per mL, of citrate in *Standard Preparation 2*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the citrate peak areas obtained from the *Assay preparation for total citrate assay* and *Standard Preparation 2*, respectively.

Calculate the quantity, in mg, of phosphate, expressed as monobasic sodium phosphate monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ), in the volume of Solution taken by the formula:

$$0.001(137.99/94.97)C_S D(r_U / r_S)$$

in which 137.99 is the molecular weight of monobasic sodium phosphate monohydrate; 94.97 is the molecular weight of phosphate ( $\text{PO}_4$ );  $C_S$  is the concentration, in  $\mu\text{g}$  per mL, of phosphate in *Standard Preparation 2*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the phosphate peak areas obtained from the *Assay preparation for total phosphate assay* and *Standard Preparation 2*, respectively.

**Assay for dextrose**—Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5 to 4 minutes. Boil the solution for 2 minutes, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at  $110^\circ$  to constant weight. Perform a blank determination, and correct the weight of the precipitate from the sample for any precipitate obtained in the blank. Each mg of cuprous oxide precipitate obtained from the substance under assay is equivalent to 0.496 mg of  $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ .

**Assay for sodium**—Proceed as directed in the *Assay for sodium* under *Anticoagulant Citrate Phosphate Dextrose Adenine Solution*.

## Anticoagulant Citrate Phosphate Dextrose Adenine Solution

» Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, Dextrose, and Adenine in Water for Injection. It contains, in each 1000 mL, not less than 2.11 g and not more than 2.33 g of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ); not less than 30.30 g and not more than 33.50 g of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ); not less than 19.16 g and not more than 21.18 g of total citrate, expressed as citric acid, anhydrous ( $\text{C}_6\text{H}_8\text{O}_7$ ); not less than 6.21 g and not more than 6.86 g of sodium (Na); and not less than 0.247 g and not more than 0.303 g of adenine ( $\text{C}_5\text{H}_5\text{N}_5$ ). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Adenine Solution as follows:

Citric Acid (anhydrous) . . . . .	2.99 g
Sodium Citrate (dihydrate) . . . . .	26.3 g
Monobasic Sodium Phosphate (monohydrate; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ) . . . . .	2.22 g
Dextrose (monohydrate) . . . . .	31.9 g
Adenine ( $\text{C}_5\text{H}_5\text{N}_5$ ) . . . . .	0.275 g
Water for Injection, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 29.0 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

**Packaging and storage**—Preserve in single-dose containers, of colorless, transparent, Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* (161)).

**Labeling**—Label it to indicate the number of mL of solution required per 100 mL of whole blood or the number of mL of solution required per volume of whole blood to be collected.

**USP Reference standards** (11)—

USP Adenine RS  
USP Citric Acid RS  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 5.56 USP Endotoxin Units per mL.

**pH** (791): between 5.0 and 6.0.

**Chloride** (221)—A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for total citrate and total phosphate**—

*Mobile Phase, Standard Preparation 2, and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* (345).

*Assay preparation for total citrate assay*—Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under general chapter (345).

*Assay preparation for total phosphate assay*—Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Phosphate Assay* under general chapter (345).

*Procedure*—Proceed as directed for *Procedure* under general chapter (345), and calculate the quantity, in mg, of anhydrous citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ) in the volume of Solution taken by the formula:

$$0.001(192.12/189.10)C_5 D(r_U / r_S)$$

in which 192.12 is the molecular weight of anhydrous citric acid; 189.10 is the molecular weight of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ );  $C_5$  is the concentration, in  $\mu\text{g}$  per mL, of citrate in *Standard Preparation 2*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the citrate peak areas obtained from the *Assay preparation for total citrate assay* and *Standard Preparation 2*, respectively.

Calculate the quantity, in mg, of phosphate, expressed as monobasic sodium phosphate monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ), in the volume of Solution taken by the formula:

$$0.001(137.99/94.97)C_5 D(r_U / r_S)$$

in which 137.99 is the molecular weight of monobasic sodium phosphate monohydrate; 94.97 is the molecular weight of phosphate ( $\text{PO}_4$ );  $C_5$  is the concentration, in  $\mu\text{g}$  per mL, of phosphate in *Standard Preparation 2*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the phosphate peak areas obtained from the *Assay preparation for total phosphate assay* and *Standard Preparation 2*, respectively.

**Assay for sodium**—

*Lithium diluent solution*—Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, then add water to volume, and mix. This solution contains 15 mEq of lithium per L.

*Standard preparation*—Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq of sodium per L. Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with *Lithium diluent solution* to volume, and mix.

*Assay preparation*—Pipet 25 mL of Solution into a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with *Lithium diluent solution* to volume, and mix.

*Procedure*—Using a suitable flame photometer, adjusted to read zero with *Lithium diluent solution*, concomitantly determine the sodium flame emission readings for the *Standard preparation* and the *Assay preparation* at the wavelength of maximum emission at about 589 nm. Calculate the quantity, in g, of Na in 1000 mL of Anticoagulant Citrate Phosphate Dextrose Adenine Solution taken by the formula:

$$2(8.18)(22.99/58.44)(R_U/R_S)$$

in which 8.18 is the weight, in g, of sodium chloride taken to make the *Standard preparation*; 22.99 is the atomic

weight of sodium; 58.44 is the molecular weight of sodium chloride; and  $R_U$  and  $R_S$  are the sodium emission readings obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextrose**—Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5 to 4 minutes. Boil the solution for 2 minutes, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight. Perform a blank determination, and make any necessary correction. Each mg of cuprous oxide precipitate obtained is equivalent to 0.496 mg of  $C_6H_{12}O_6 \cdot H_2O$ .

#### Assay for adenine—

**Mobile phase**—Dissolve 3.45 g of ammonium dihydrogen phosphate in 950 mL of water in a 1000-mL volumetric flask, add 10 mL of glacial acetic acid, dilute with water to volume, mix, pass through a membrane filter having a 1- $\mu$ m or finer porosity, and degas.

**Standard preparations**—Dissolve accurately weighed quantities of USP Adenine RS in dilute hydrochloric acid (1 in 120) in three separate volumetric flasks, dilute with the dilute hydrochloric acid solution to volume, and mix to obtain Standard preparations having known concentrations of about 0.25, 0.275, and 0.30 mg of adenine per mL, respectively. Protect from light.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm stainless steel column that contains packing L9. The flow rate is about 2.0 mL per minute. Prepare a solution containing USP Adenine RS and purine, each at about 0.275 mg per mL, in dilute hydrochloric acid (1 in 120), and chromatograph not less than four injections (about 20  $\mu$ L) of this solution: the relative standard deviation of the peak response of adenine is not more than 2.5%, the relative standard deviation of the retention time of adenine is not more than 2.0%, and the resolution of adenine and purine is not less than 3.0.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the Solution and the *Standard preparations*, record the chromatograms, and measure the responses for the major peaks. Plot the responses against the concentrations, in mg of USP Adenine RS per mL of the *Standard preparations*. Calculate the quantity, in mg, of  $C_5H_5N_5$  in each mL of the Solution taken as the value read directly from the Standard curve corresponding to the response obtained from the portion of the Anticoagulant Citrate Phosphate Dextrose Adenine Solution chromatographed.

### Anticoagulant Sodium Citrate Solution

» Anticoagulant Sodium Citrate Solution is a sterile solution of Sodium Citrate in Water for Injection. It contains, in each 100 mL, not less than 3.80 g and not more than 4.20 g of  $C_6H_5Na_3O_7 \cdot 2H_2O$ . It contains no antimicrobial agents.

Sodium Citrate (dihydrate) . . . . .	40 g
Water for Injection, a sufficient quantity to make . . . . .	1000 mL

NOTE—Anhydrous sodium citrate (35.1 g) may be used instead of the dihydrate.

Dissolve the Sodium Citrate in sufficient Water for Injection to make 1000 mL, and filter until clear. Place the solution in suitable containers, and sterilize.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

#### USP Reference standards <11>—

USP Citric Acid RS

USP Endotoxin RS

**Identification**—When evaporated to a concentration of 1 in 20, it responds to the tests for *Sodium* <191> and for *Citrate* <191>.

**Bacterial endotoxins** <85>—It contains not more than 5.56 USP Endotoxin Units per mL.

**pH** <791>: between 6.4 and 7.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

#### Assay—

**Mobile Phase, Standard Preparation 1, and Chromatographic System**—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* <345>.

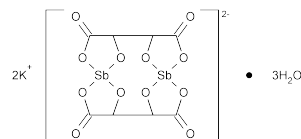
**Assay preparation**—Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under general chapter <345>.

**Procedure**—Proceed as directed for *Procedure* under general chapter <345>, and calculate the quantity, in mg, of sodium citrate dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ ) in the volume of Solution taken by the formula:

$$0.001(294.10/189.10)C_S D(r_U / r_S)$$

in which 294.10 is the molecular weight of sodium citrate dihydrate; 189.10 is the molecular weight of citrate ( $C_6H_5O_7$ );  $C_S$  is the concentration, in  $\mu$ g per mL, of citrate in *Standard Preparation 1*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the citrate peak areas obtained from the *Assay preparation* and *Standard Preparation 1*, respectively.

### Antimony Potassium Tartrate



$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$  667.87

Antimonate(2-), bis[ $\mu$ -[2,3-dihydroxybutanedioato(4-)- $O^1$ ,  $O^2$ : $O^3$ ,  $O^4$ ]]-di-, dipotassium, trihydrate, stereoisomer.

Dipotassium bis[ $\mu$ -[L-(+)-tartrato(4-)]diantimonate(2-) trihydrate [28300-74-5].

Anhydrous 613.82 [11071-15-1].

» Antimony Potassium Tartrate contains not less than 99.0 percent and not more than 103.0 percent of  $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ .

**Packaging and storage**—Preserve in well-closed containers.

**Completeness of solution** <641>: meets the requirements, using a 750-mg specimen and water as the solvent.

**Identification**—

**A:** When heated to redness, it chars, emits an odor resembling that of burning sugar, and leaves a blackened residue. This residue has an alkaline reaction, and when a small fragment of it is held in a nonluminous flame, the flame is tinted violet.

**B:** In a solution (1 in 20), acidified with hydrochloric acid, hydrogen sulfide TS produces an orange-red precipitate, which is soluble in ammonium sulfide TS and in 1 N sodium hydroxide.

**C:** It responds to the test for *Tartrate* <191>.

**Acidity or alkalinity**—Dissolve 1.0 g in 50 mL of carbon dioxide-free water, and titrate with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5: not more than 2.0 mL is required.

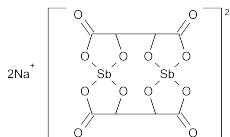
**Loss on drying** <731>—Dry it at 105° to constant weight: it loses not more than 2.7% of its weight.

**Arsenic**—Dissolve 100 mg in 5 mL of hydrochloric acid. Add 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid. Mix, transfer to a color-comparison tube, and allow to stand for 30 minutes. Viewed downward over a white surface, the color of the solution appears no deeper than that of a blank to which has been added 15 µg of arsenic (0.015%).

**Lead** <251>: not more than 0.002%.

**Assay**—Dissolve about 500 mg of Antimony Potassium Tartrate, accurately weighed, in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue color. Each mL of 0.1 N iodine is equivalent to 16.70 mg of  $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ .

## Antimony Sodium Tartrate



$C_8H_4Na_2O_{12}Sb_2$  581.61  
Antimonate(2-), bis[ $\mu$ -[2,3-dihydroxybutanedioato(4-)-O<sup>1</sup>, O<sup>2</sup>:O<sup>3</sup>, O<sup>4</sup>]]di-, disodium, stereoisomer.  
Disodium bis[ $\mu$ -[L-(+)-tartrato(4-)]diantimonate(2-)  
[34521-09-0].

» Antimony Sodium Tartrate contains not less than 98.0 percent and not more than 101.0 percent of  $C_8H_4Na_2O_{12}Sb_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—It responds to the tests for *Antimony* <191>, for *Sodium* <191>, and for *Tartrate* <191>.

**Acidity or alkalinity**—Dissolve 1.0 g in 50 mL of carbon dioxide-free water, and titrate with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5: not more than 2.0 mL is required.

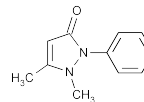
**Loss on drying** <731>—Dry it at 105° to constant weight: it loses not more than 6.0% of its weight.

**Arsenic, Method II** <211>: 8 ppm.

**Lead** <251>: not more than 0.002%.

**Assay**—Dissolve about 500 mg of Antimony Sodium Tartrate, accurately weighed, in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue color. Each mL of 0.1 N iodine is equivalent to 14.54 mg of  $C_8H_4Na_2O_{12}Sb_2$ .

## Antipyrine



$C_{11}H_{12}N_2O$  188.23

1,2-Dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one.  
2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one [60-80-0].

» Antipyrine contains not less than 99.0 percent and not more than 100.5 percent of  $C_{11}H_{12}N_2O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Antipyrine RS

**Completeness and color of solution**—It is completely soluble in its own weight of cold water, the solution being colorless or not more than slightly yellow when viewed transversely in a tube having a diameter of about 20 mm.

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 20 µg per mL.

*Medium:* methanol.

Absorptivities at 266 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Add tannic acid TS to a solution of it: a white precipitate is formed.

**Melting range** <741>: between 110° and 112.5°.

**Loss on drying** <731>—Dry it at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.15%.

**Heavy metals** <231>—Dissolve 1 g in 2 mL of 1 N acetic acid, and add water to make 25 mL: the limit is 0.002%.

**Ordinary impurities** <466>—

*Test solution:* chloroform.

*Standard solution:* chloroform.

*Eluant:* a mixture of chloroform, acetone, butyl alcohol, and formic acid (60:15:15:15).

*Visualization:* 1.

**Assay**—Transfer about 150 mg of Antipyrine, accurately weighed, to a 250-mL iodine flask, and dissolve in 25 mL of water. Add 2 g of sodium acetate, 1 mL of diluted acetic acid, and 20.0 mL of 0.1 N iodine VS, mix, and allow to stand in a cool, dark place for 20 minutes. Add 25 mL of alcohol to dissolve the precipitate, and titrate the excess iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Each mL of 0.1 N iodine is equivalent to 9.412 mg of  $C_{11}H_{12}N_2O$ .

## Antipyrine and Benzocaine Otic Solution

» Antipyrine and Benzocaine Otic Solution is a solution of Antipyrine and Benzocaine in Glycerin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of antipyrine ( $C_{11}H_{12}N_2O$ ) and benzocaine ( $C_9H_{11}NO_2$ ). [NOTE—In the preparation of this Otic Solution, use Glycerin that has a low water content, in order that the Otic Solution may comply with the *Water* limit. This may be ensured by using Glycerin having a specific gravity of not less than 1.2607, corresponding to a concentration of 99.5 percent.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Antipyrine RS  
USP Benzocaine RS

### Identification—

**A:** Transfer 5 mL to a separator containing 25 mL of water, and extract the solution with two 25-mL portions of a mixture of equal volumes of ether and solvent hexane. Combine the extracts, and retain the water solution for *Identification* test **B**. Extract the ether-hexane solution with 50 mL of water, and discard the water layer. Evaporate the ether-hexane solution to dryness, dry the residue in vacuum at 40° to 50° for 1 hour, and dissolve the residue in 1 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima at the same wavelengths as that of a similar solution of USP Benzocaine RS, concomitantly measured.

**B:** Add 5 mL of 1 N sodium hydroxide solution to the water solution retained from *Identification* test **A**, and extract with two 25-mL portions of chloroform. Evaporate the combined extracts to dryness, dry the residue in vacuum at 40° to 50° for 1 hour, and dissolve the residue in 3 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima at the same wavelengths as that of a similar solution of USP Antipyrine RS, concomitantly measured.

**C:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water, Method I** (921): not more than 1.0% is found.

### Assay—

**Ammonium acetate solution**—Dissolve 7.7 g of ammonium acetate in water, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Ammonium acetate solution* and acetonitrile (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 15 mg of USP Benzocaine RS, accurately weighed, to a 100-mL volumetric flask, add 15J mg of USP Antipyrine RS, accurately weighed, J being the ratio of the labeled amount, in mg, of antipyrine to the labeled amount, in mg, of benzocaine per mL of Otic Solution. Add 50 mL of methanol, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Otic Solution, equivalent to about 15 mg of benzocaine, to a 100-mL volumetric flask. Dissolve in 50 mL of methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 15-cm column that contains 5-μm packing L15. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the benzocaine peak is not more than 2.5, the column efficiency for the benzocaine peaks is not less than 1500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.35 for antipyrine, and 1.0 for benzocaine. Calculate the quantity, in mg, of benzocaine ( $C_9H_{11}NO_2$ ) in each mL of the Otic Solution taken by the formula:

$$1000(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Benzocaine RS in the *Standard preparation*; V is the volume, in mL, of Otic Solution taken; and  $r_U$  and  $r_S$  are the peak responses due to benzocaine in the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of antipyrine ( $C_{11}H_{12}N_2O$ ) in each mL of the Otic Solution taken by the same formula, changing the terms to refer to antipyrine instead of benzocaine.

## Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution

» Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution is a solution of Antipyrine, Benzocaine, and Phenylephrine Hydrochloride in a suitable nonaqueous solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of antipyrine ( $C_{11}H_{12}N_2O$ ), benzocaine ( $C_9H_{11}NO_2$ ), and phenylephrine hydrochloride ( $C_9H_{13}NO_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Antipyrine RS  
USP Benzocaine RS  
USP Phenylephrine Hydrochloride RS

**Identification**—The retention times of the major peaks in the chromatograms of the *Assay preparations* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Assay—

**Mobile phase**—Mix 480 mL of acetonitrile, 3520 mL of a 0.005 M solution of sodium 1-heptanesulfonate in water, and 4 mL of phosphoric acid.

**Standard preparation**—Accurately weigh about 25 mg of USP Antipyrine RS, about 25 mg of USP Benzocaine RS, and about 25 mg of USP Phenylephrine Hydrochloride RS into a 250-mL volumetric flask. Add 5 mL of a 0.5 mg per mL solution of p-aminobenzoic acid in *Mobile phase*. Add 150 mL of *Mobile phase*, and mix to effect solution, sonicating if necessary. Dilute with *Mobile phase* to volume, and mix.

**Assay preparation A**—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of antipyrine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL

volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation B**—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of benzocaine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation P**—Transfer an accurately measured volume of Otic Solution, equivalent to about 5 mg of phenylephrine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.19 for *p*-aminobenzoic acid, 0.26 for phenylephrine, 0.64 for antipyrine, and 1.0 for benzocaine; the resolution, *R*, between phenylephrine and aminobenzoic acid is not less than 1.5, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20 or 25 µL) of the *Standard preparation* and each of the *Assay preparations* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of antipyrine (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL of USP Antipyrine RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the antipyrine peak responses obtained from *Assay preparation A* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Benzocaine RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the benzocaine peak responses obtained from *Assay preparation B* and the *Standard preparation*, respectively. Calculate the quantity, in µg of phenylephrine hydrochloride (C<sub>9</sub>H<sub>13</sub>NO<sub>2</sub> · HCl) in each mL of the Otic Solution taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the phenylephrine peak responses obtained from *Assay preparation P* and the *Standard preparation*, respectively.

## Antithrombin III Human

### DEFINITION

Antithrombin III Human is a glycoprotein, which is the major inhibitor of thrombin and other activated clotting factors, including factors IX, X, XI, and XII, and the cofactor through which heparin exerts its effect. It is obtained from human plasma of healthy donors who must, as far as can be ascertained, be free from detectable agents of infection transmissible by transfusion of blood or blood derivatives. The method of manufacturing includes steps that have been shown to remove or inactivate known agents of infection. If substances are used for inactivation

of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to an acceptable level and that any residues are such as not to compromise the safety of the preparation for patients. The antithrombin III concentrate is passed through a bacteria-retentive filter, filled aseptically into its final, sterile containers, and immediately frozen. It is then freeze-dried, and the containers are closed under vacuum. No antimicrobial preservative is added at any stage of production. Antithrombin III Human complies with the requirements for *Biologics* <1041>. When reconstituted in the recommended volume of diluent, the potency is NLT 25 USP Antithrombin III Units/mL. [NOTE—One USP Antithrombin III Unit is the amount of antithrombin III that forms a complex with one unit of thrombin at 25° in the presence of heparin at a pH of 8.4.]

### IDENTIFICATION

- Meets the requirements of the *Assay*

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve Tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

**Solution B:** 0.05% (w/v) of albumin human in *Solution A*

**Solution C:** 10 mg/mL of polybrene in *Solution B*

**Solution D:** 15 USP Heparin Units/mL of USP Heparin Sodium for Assays RS in *Solution B*

**Solution E:** Reconstitute thrombin bovine, and dilute with *Solution B* to obtain a solution having a concentration of 2.0 Thrombin Units/mL.

**Solution F:** Prepare a solution of chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for factor II<sub>a</sub> in water to obtain a solution having a concentration of about 5.0 mM, and dilute the solution further with *Solution C* to 1.0 mM.

**Stopping solution:** 20% (v/v) of acetic acid in water

**Standard solution A:** USP Antithrombin III Human RS in *Solution D* to obtain a solution containing 1.0 USP Antithrombin III Unit

**Standard solutions B, C, D, and E:** Dilute *Standard solution A* with *Solution D* 60-, 120-, 180-, and 300-fold.

**Sample solution A:** Dissolve a quantity of Antithrombin III Human in *Solution D* to obtain a solution having the same concentration as *Standard solution A*.

**Sample solutions B, C, D, and E:** Dilute *Sample solution A* with *Solution D* 60-, 120-, 180-, and 300-fold.

**Analysis:** Pipet 400 µL each of *Standard solutions B, C, D, and E* and *Sample solutions B, C, D, and E* into suitable tubes placed in a water bath set at 37°. Add 200 µL of *Solution E*, prewarmed at 37° to each tube, mix, and incubate for 1 min. Add 200 µL of *Solution F*, prewarmed at 37° to each tube, mix, and incubate for 60 s. Stop the reaction by adding 200 µL *Stopping solution*. To prepare a blank, add the reagents in reverse order, starting with 200 µL of *Stopping solution*, followed by the addition of 200 µL of *Solution F*, then adding 200 µL of *Solution E*, and ending with 400 µL of *Solution D*. Record the absorbance at 405 nm against the blank.

For *Standard solutions* and *Sample solutions*, calculate the regression of the absorbance against log concentrations, and calculate the activity of Antithrombin III Human in USP Antithrombin III Units, using a suitable statistical method for parallel-line assays. The four independent relative activity estimates are then combined to obtain the final mean, and the confidence limits are calculated.

**Acceptance criteria:** 80%–120% of the potency stated on the label. The specific activity is NLT 6.0 USP Anti-thrombin III Units/mg of total protein. The confidence interval ( $P = 0.95$ ) is between 90% and 110% of the estimated potency.

## IMPURITIES

### Organic Impurities

#### • PROCEDURE: HEPARIN CONTENT

**Solution A:** Dissolve Tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% polyethylene glycol 6000 to obtain a solution having concentrations of 0.050 M, 0.0075 M, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

**Solution B:** Solution of chromogenic substrate for amidolytic test for factor  $X_a$  in water to obtain a solution of concentration of 2.5 mM

**Solution C:** Dissolve Factor  $X_a$  in *Solution A* to obtain a solution containing 20 nanokatalytic units (nkats).

**Solution D:** 20% (v/v) of acetic acid in water

**Standard solution:** Dissolve USP Antithrombin III Human RS in *Solution A* to obtain a solution containing 1.0 USP Antithrombin III Unit.

**Sample solution:** Dissolve Antithrombin III Human in *Solution A* to obtain a solution containing 1.0 USP Antithrombin III Unit.

**Analysis:** Pipet 250  $\mu$ L each of *Solution A*, the *Standard solution*, and the *Sample solution* to suitable tubes placed in a water bath set at 37°. Add 250  $\mu$ L of *Solution C* prewarmed at 37° to each tube, and incubate for 2 min. Add 250  $\mu$ L of *Solution B* prewarmed at 37° to each tube, mix, and incubate for 120 s. Stop the reaction by adding 250  $\mu$ L of *Solution D*. Record the absorbance at 405 nm, using *Solution A* as the blank. Calculate the USP Heparin Unit per USP Antithrombin III Unit:

$$\text{Result} = P_R (A_F - A_U) / (A_F - A_S)$$

$P_R$  = heparin content of USP Antithrombin III Human RS in USP Heparin Unit per USP Antithrombin III Unit

$A_F$  = absorbance value from *Solution A*

$A_U$  = absorbance value from the *Sample solution*

$A_S$  = absorbance value from the *Standard solution*

**Acceptance criteria:** NMT 0.1 USP Heparin Unit per USP Antithrombin III Unit

## SPECIFIC TESTS

- **STERILITY TESTS** <71>: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*
- **WATER DETERMINATION, Method I** <921>: NMT 3.0%
- **PYROGEN TEST** <151>: Inject 50 USP Antithrombin III Units per kg of the rabbit's weight, calculated from the activity stated on the label: meets the requirements.
- **GENERAL SAFETY:** Meets the requirements for biologics as set forth under *Biological Reactivity Tests, In Vivo* <88>, *Safety Tests—Biologicals*
- **OSMOLALITY AND OSMOLARITY, Osmolality** <785>: Reconstitute with the diluent according to the manufacturer's instruction: NLT 240 mOsmol/kg for the solution.
- **pH** <791>: Reconstitute with the diluent according to the manufacturer's instruction: 6.0–7.5.
- **MOLECULAR WEIGHT DISTRIBUTION**

**Mobile phase:** Solution containing 0.1 M sodium phosphate, 0.15 M sodium chloride, and 0.05% sodium azide, having a pH of 6.5. Degas and filter.

**Solution A:** 4–5 mg/mL of thyroglobulin in *Mobile phase*

**Sample solution:** 8–10 mg/mL of Antithrombin III Human

**System suitability solution:** Dilute USP Albumin Human RS, if necessary, with water to obtain a solution containing 5%.

## Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 7.5-  $\times$  75-mm guard column and a 7.5-  $\times$  300-mm analytical column, both containing packing L59

**Temperature:** Ambient

**Flow rate:** 0.5 mL/min maintained constant to  $\pm 1\%$

**Injection size:** 10  $\mu$ L

## System suitability

**Sample:** *System suitability solution*

## Suitability requirements

**Column efficiency:** Greater than 1500 theoretical plates

**Tailing factor:** 0.5–2.5

## Analysis

**Samples:** *Solution A* and *Sample solution*

**Acceptance criteria:** Note the retention times of the major peak in the *Solution A* chromatogram. The relative peak area of the high-molecular weight peak eluting at about the same retention time as the major peak in the *Solution A* chromatogram, or earlier, is NMT 13%.

## • TOTAL PROTEIN CONTENT

**Solution A:** 1000 mg/mL of trichloroacetic acid in water

**Sample solution:** 7.5 mg/mL of Antithrombin III Human in 0.15 M sodium chloride solution

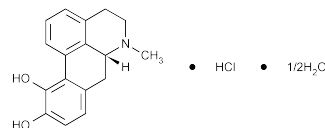
**Blank:** 0.15 M solution of sodium chloride

**Analysis:** To each of 2.0 mL of the *Sample solution* and the *Blank* in suitable centrifuge tubes, add 1.5 mL of *Solution A*. Mix, allow to stand for at least 10 min, centrifuge for 5 min, and decant the supernatant. Resuspend the precipitates in 1.5 mL of *Solution A*, centrifuge for 5 min, decant the supernatant, and hold the tubes inverted on a filter paper to drain. Quantitatively transfer the residues with a minimum quantity of water to a micro-Kjeldahl flask, and determine the nitrogen content using *Method II* (see *Nitrogen Determination* <461>). Multiply the result, corrected for the *Blank*, by 6.25 to calculate the quantity of protein.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Use a Type I glass container with an appropriate stopper and seal. Store protected from light between 2° and 8°, excursions permitted up to 25°.
- **LABELING:** The labeling should state the content of antithrombin III in USP Antithrombin III Units. The diluent and the volume to be used to reconstitute the preparation are indicated.
- **USP REFERENCE STANDARDS** <11>
  - USP Albumin Human RS
  - USP Antithrombin III Human RS
  - USP Heparin Sodium for Assays RS

## Apomorphine Hydrochloride



$C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$  312.79

4*H*-Dibenzo[*de,g*]quinoline-10,11-diol, 5,6,6a,7-tetrahydro-6-methyl-, hydrochloride, hemihydrate, (*R*)-.

6aβ-Aporphine-10,11-diol hydrochloride hemihydrate [41372-20-7].

Anhydrous 303.79 [314-19-2].

» Apomorphine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of  $C_{17}H_{17}NO_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apomorphine Hydrochloride RS

**Color of solution**—Place 100 mg in a suitable test tube, add 10 mL of cold, oxygen-free water, and agitate gently until dissolved: the color of the resulting solution, observed promptly after the Apomorphine Hydrochloride has dissolved, is not more intense than that of a color standard prepared as follows. Dissolve 5 mg of Apomorphine Hydrochloride in 100.0 mL of water. Transfer 1.0 mL of this solution to a test tube of the same size as that used for the test solution, dilute with 6 mL of water, add 1 mL of sodium bicarbonate solution (1 in 20), and then add 0.50 mL of iodine TS. Allow to stand for 30 seconds, add 0.60 mL of sodium thiosulfate solution (1 in 40), and dilute with water to 10 mL.

**Identification**—

**A: Infrared Absorption** (197K).

**B:** To 5 mL of a solution (1 in 100) add a slight excess of sodium bicarbonate solution (1 in 20): a white or greenish-white precipitate is formed. Add 3 drops of iodine TS, and shake vigorously: an emerald-green color is produced. Add 5 mL of ether and, after vigorous shaking, allow the layers to separate: the ether is colored deep ruby-red while the water layer retains its green color.

**C:** Dissolve it in nitric acid: a dark purple solution is produced.

**D:** To a solution of it add silver nitrate TS: a white precipitate, which is insoluble in nitric acid, is formed. This precipitate soon darkens by reduction to metallic silver, the reduction being accelerated by the addition of 6 N ammonium hydroxide.

**Specific rotation** (781S): between  $-60.5^\circ$  and  $-63.0^\circ$ .

*Test solution:* 15 mg per mL, in dimethylsulfoxide.

**Loss on drying** (731)—Dry it at  $105^\circ$  for 2 hours: it loses between 2.0% and 3.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Decomposition products**—Shake 100 mg with 5 mL of ether: the latter acquires not more than a pale reddish color.

**Ordinary impurities** (466)—

*Solvent for Test Solution:* methanol.

*Solvent for Standard Solution:* methanol.

*Eluant:* a mixture of 1-butanol, water, and formic acid (7:2:1).

*Visualization:* a freshly prepared mixture of 10% ferric chloride solution and 5% potassium ferricyanide solution (2:1). Allow the chromatograms to develop until the solvent front has moved about 8 cm. [NOTE—The development time is 1.5 to 2 hours.] Allow the plates to dry at room temperature for 1 hour prior to spraying.

**Assay**—Dissolve about 300 mg of Apomorphine Hydrochloride, accurately weighed, in 100 mL of glacial acetic acid with the aid of heat provided by a steam bath. Add 0.1 mL of acetic anhydride to the hot solution, and stir for 5 minutes. Cool to room temperature, add 5 mL of mercuric acetate TS and 0.25 mL of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.38 mg of  $C_{17}H_{17}NO_2 \cdot HCl$ .

## Apomorphine Hydrochloride Tablets

» Apomorphine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apomorphine Hydrochloride RS

**Color of solution**—Dissolve a quantity of powdered Tablets, equivalent to 5 mg of apomorphine hydrochloride, in water to make 100.0 mL. Transfer 1.0 mL of the solution to a test tube, dilute with 6 mL of water, and, if necessary, filter through a small pledget of cotton. Add 1 mL of sodium bicarbonate solution (1 in 20), then add 0.50 mL of iodine TS. Allow to stand for 30 seconds, then add 0.60 mL of sodium thiosulfate solution (1 in 40), and dilute with water to 10 mL. This solution represents the color standard.

Place a quantity of powdered Tablets, equivalent to about 50 mg of apomorphine hydrochloride, in a test tube of suitably small size, add 10.0 mL of cold, oxygen-free water, insert the stopper in the test tube, and agitate gently until no more dissolves; if necessary, filter immediately through a small pledget of cotton. The color of the solution, observed promptly after preparation, is not more intense than that of the color standard. Use closely matched test tubes for the comparison.

**Identification**—To 5 mL of a filtered solution of Tablets, containing about 10 mg of apomorphine hydrochloride, add a slight excess of sodium bicarbonate solution (1 in 20): a white or greenish-white precipitate is formed. Add 3 drops of iodine TS, and shake vigorously: an emerald-green color is produced. Add 5 mL of ether, and, after vigorous shaking, allow the layers to separate: the ether is colored deep ruby-red while the water layer retains its green color.

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Place 1 Tablet in a 500-mL volumetric flask containing 100 mL of 0.1 N hydrochloric acid, and shake for 15 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with 0.1 N hydrochloric acid to provide a solution containing approximately 12  $\mu$ g of apomorphine hydrochloride per mL. Concurrently determine the absorbances of this solution and of a solution of USP Apomorphine Hydrochloride RS in the same medium having a known concentration of about 12  $\mu$ g of anhydrous apomorphine hydrochloride per mL, in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$  in the Tablet taken by the formula:

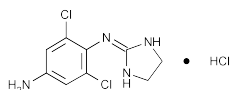
$$(312.80 / 303.79)(TC / D)(A_U / A_S)$$

in which 312.80 and 303.79 are the molecular weights of apomorphine hydrochloride hemihydrate and anhydrous apomorphine hydrochloride, respectively;  $T$  is the labeled quantity, in mg, of apomorphine hydrochloride in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of anhydrous apomorphine hydrochloride in the Standard solution;  $D$  is the concentration, in  $\mu$ g per mL, of apomorphine hydrochloride in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.



**Assay**—Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 50 mg of apomorphine hydrochloride, in 25 mL of water in a separator, add 500 mg of sodium bicarbonate, and completely extract with successive small portions of ether. Combine the ether extracts in a separator, and wash them with three 5-mL portions of water. Shake the combined water washings with 10 mL of ether, and add this ether to the combined ether extracts. Extract the ether solutions with 20.0 mL of 0.02 N sulfuric acid VS, and wash with three 5-mL portions of water. Combine the acid extract and washings in a beaker, and warm on a steam bath to expel any residual ether. Cool, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.02 N sulfuric acid is equivalent to 6.256 mg of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ .

## Apraclonidine Hydrochloride



$C_9H_{10}Cl_2N_4 \cdot HCl$  281.57

1,4-Benzenediamine, 2,6-dichloro-N<sup>1</sup>-2-imidazolidinylidene-, monohydrochloride.

2-[(4-Amino-2,6-dichlorophenyl)imino]imidazolidine monohydrochloride [73218-79-8].

» Apraclonidine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_9H_{10}Cl_2N_4 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apraclonidine Hydrochloride RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** It responds to the tests for *Chloride* (191).

**pH** (791): between 5.0 and 6.6 in a solution (1 in 100).

**Loss on drying** (731)—Dry it in vacuum at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

### Chromatographic purity—

**Phosphate buffer**—Transfer 6.8 mL of phosphoric acid to a 2000-mL volumetric flask, add about 1900 mL of water, and mix. Adjust with sodium hydroxide solution (1 in 2) to a pH of 3.0, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, *Phosphate buffer*, and methanol (56:40:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Mobile phase* containing about 0.8 mg of USP Apraclonidine Hydrochloride RS per mL.

**Test solution**—Transfer about 20 mg of Apraclonidine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the

*System suitability solution*, and record the peak responses as directed for *Procedure*: the tailing factor for the apraclonidine peak is not more than 2.2, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. [NOTE—Allow about five times the elution time of apraclonidine before making the next injection.] Calculate the percentage of each peak, other than the solvent peak and the apraclonidine peak, in the specimen of Apraclonidine Hydrochloride taken by the same formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of each peak other than the principal peak, and  $r_t$  is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% for any individual impurity and not more than 2.0% total impurities are found.

**Assay**—Dissolve about 125 mg of Apraclonidine Hydrochloride, accurately weighed, in 40 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically from the second inflection point, using a calomel-glass electrode system (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.08 mg of  $C_9H_{10}Cl_2N_4 \cdot HCl$ .

## Apraclonidine Ophthalmic Solution

» Apraclonidine Ophthalmic Solution is a sterile, aqueous solution of Apraclonidine Hydrochloride. It contains an amount of apraclonidine hydrochloride ( $C_9H_{10}Cl_2N_4 \cdot HCl$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of apraclonidine ( $C_9H_{10}Cl_2N_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apraclonidine Hydrochloride RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Apply 2 µL of Apraclonidine Ophthalmic Solution and 2 µL of a Standard solution of USP Apraclonidine Hydrochloride RS in methanol containing about 11.5 mg per mL to a suitable high performance thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.2-mm layer of chromatographic silica gel mixture, or equivalent. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (74:22:4) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light. [NOTE—The apraclonidine spot should appear as a blue spot.] Spray the plate with fluorecamine solution, prepared by dissolving about 25 mg of fluorecamine in 25 mL of acetone. [NOTE—Avoid prolonged or repeated breathing of the aerosol from the fluorecamine spray. Also avoid prolonged or repeated contact with skin. Fluorecamine solu-

tion should be sprayed only in a hood.] Examine the plate under normal light and long-wavelength UV light. [NOTE—The apraclonidine spot should appear as a yellow spot under normal light and as a white spot under long-wavelength UV light.] The  $R_f$  value and appearance of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.4 and 7.8.

#### Assay—

**Phosphate buffer**—Prepare as directed in the test for *Chromatographic purity under Apraclonidine Hydrochloride*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Phosphate buffer*, acetonitrile, and methanol (68:30:2). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Apraclonidine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a *Stock standard solution* having a known concentration of about 0.23 mg per mL. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 11.5 µg of USP Apraclonidine Hydrochloride RS per mL (equivalent to about 10 µg of apraclonidine per mL).

**Resolution solution**—Transfer about 1 mL of propiophenone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Stock standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of apraclonidine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for apraclonidine and 1.0 for propiophenone; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; the resolution,  $R$ , between the analyte and propiophenone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of apraclonidine ( $C_9H_{10}Cl_2N_4$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$(245.11 / 281.57)(2C / V)(r_U / r_S)$$

in which 245.11 and 281.57 are the molecular weights of apraclonidine and apraclonidine hydrochloride, respectively;  $C$  is the concentration, in µg per mL, of USP Apraclonidine Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the apraclonidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aprotinin

RPDFCLEPPY TGPCKARIIR YFYNAKAGLC QTFFVYGGRA KRNNFKSAED  
CMRTCGGA

$C_{284}H_{432}N_{84}O_{79}S_7$  6511.44

Trypsin inhibitor, pancreatic basic.

L-Arginyl-L-prolyl-L-aspartyl-L-phenylalanyl-L-cysteinyl-L-leucyl-L-glutamyl-L-prolyl-L-prolyl-L-tyrosyl-L-threonylglycyl-L-prolyl-L-cysteinyl-L-lysyl-L-alanyl-L-arginyl-L-isoleucyl-L-isoleucyl-L-arginyl-L-tyrosyl-L-phenylalanyl-L-tyrosyl-L-asparaginyl-L-alanyl-L-lysyl-L-alanylglycyl-L-leucyl-L-cysteinyl-L-glutamyl-L-threonyl-L-phenylalanyl-L-valyl-L-tyrosylglycylglycyl-L-cysteinyl-L-arginyl-L-alanyl-L-lysyl-L-arginyl-L-asparaginyl-L-asparaginyl-L-phenylalanyl-L-lysyl-L-seryl-L-alanyl-L-glutamyl-L-aspartyl-L-cysteinyl-L-methionyl-L-arginyl-L-threonyl-L-cysteinylglycylglycyl-L-alanine cyclic (5→55), (14→38), (30→51) tris(disulfide) [9087-70-1].

» Aprotinin is a polypeptide consisting of a chain of 58 amino acid residues, which inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin, and trypsin. Aprotinin is obtained from bovine tissues and purified by a suitable process, and is stored as a bulk solution or lyophilized powder. Its potency calculated on the dried basis is not less than 3 USP Aprotinin Units per mg. In addition, the method of manufacture is validated to result in not more than 0.2 µg of histamine per 3 USP Aprotinin Units using validated methods. The origin and sourcing of bovine material must be specified in compliance with FDA requirements. The manufacturing process is validated to demonstrate the clearance of potential infectious agents (i.e., viruses, TSE agents). One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.).

**Packaging and storage**—For lyophilized powder, preserve in tight containers, and store in a cold place. Protect from light. For bulk solution, preserve in tight containers at a temperature not exceeding 25°. Avoid freezing.

**Labeling**—The labeling states the source of material and the number of Kallikrein Inhibition Units per mg or the number of Kallikrein Inhibition Units per mL.

#### USP Reference standards (11)—

USP Aprotinin RS

USP Aprotinin System Suitability RS

USP Endotoxin RS

USP Trypsin Crystallized RS

#### Identification—

**A: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution**—Prepare a solution of Aprotinin in water having a concentration of about 15 USP Aprotinin Units per mL.

**Developing solvent system:** a mixture of glacial acetic acid and water (100 : 80) containing 100 g per L of sodium acetate.

**Cupric chloride solution**—Dissolve 1 g of cupric chloride in 100 mL of water.

**Spray reagent**—Dissolve 0.1 g of ninhydrin in a mixture containing 6 mL of *Cupric chloride solution*, 21 mL of glacial acetic acid, and 70 mL of alcohol.

**Procedure**—Proceed as directed in the chapter, except to spray the plate with the *Spray reagent*, and heat at 60° to visualize the spots.

**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Resolution solution*, as obtained in the test for *Limit of N-pyroglutamyl-aprotinin and related compounds*.

**Absorbance** (851)—Prepare a solution containing 3.0 USP Aprotinin Units per mL. The solution shows an absorption maximum at 277 nm. The absorbance at the maximum is not greater than 0.80.

**Safety**—Prepare a solution of Aprotinin that contains 4 USP Aprotinin Units per mL using a sufficient quantity of Water for Injection. It meets the requirements when tested as directed in the section *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88).

**Bacterial endotoxins** (85)—It contains not more than 0.14 USP Endotoxin Unit per USP Aprotinin Unit. Use a solution that contains 6 USP Aprotinin Units per mL.

**Loss on drying** (731)—[NOTE—This test should only be performed on the lyophilized powder.] Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

**Specific activity of the dry residue**—[NOTE—This test should only be performed when product is a concentrated solution.] Evaporate 25.0 mL of Aprotinin concentrated solution to dryness in a water bath, dry the residue at 110° for 15 hours, and weigh. From the weight of the residue and the activity determined in the *Assay*, calculate the number of USP Aprotinin Units per mg of dry residue. Not less than 3.0 USP Aprotinin Units per mg of dried residue is found.

**Limit of des-Ala-aprotinin and des-Ala-des-Gly-aprotinin—**

*Test solution*—Dilute a concentrated solution of Aprotinin with water, or weigh out Aprotinin and dissolve in water, to obtain a solution containing about 4 to 7 USP Aprotinin Units per mL.

*Standard solution*—Dilute USP Aprotinin RS with water to obtain a solution having a concentration similar to that of the *Test solution*.

*Capillary zone electrophoresis buffer*—Dissolve 8.21 g of monobasic potassium phosphate in 400 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 500 mL.

*Capillary zone electrophoresis system* (see *Capillary Electrophoresis* under *Biotechnology-Derived Articles—Test* (1047))—The capillary electropherograph is equipped with a 214-nm detector and a 45- to 60-cm uncoated fused silica capillary with an internal diameter of 75 µm with the temperature controlled at 25°. Apply a field strength of 0.2 kV/cm for 30 minutes, using *Capillary zone electrophoresis buffer* as the electrolyte in both buffer reservoirs. Electropherograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative migration times are about 0.98 for des-Ala-des-Gly-aprotinin, 0.99 for des-Ala-aprotinin, and 1 for aprotinin. The resolution,  $R_s$ , between the des-Ala-des-Gly-aprotinin and des-Ala-aprotinin peaks is not less than 0.8, and the resolution between the des-Ala-aprotinin and aprotinin peaks is not less than 0.5. The migration time for the aprotinin peak is between 19 and 25 minutes. The tailing factor,  $T$ , of the aprotinin peak is not more than 3 (see *Chromatography* (621) for calculation). The baseline is stable and shows little drift. Rinse the capillary for at least 1 minute with at least 10 total capillary volumes of 0.1 N sodium hydroxide, followed by at least 10 total capillary volumes of water, and by at least 20 capillary volumes of *Capillary zone electrophoresis buffer* between injections.

*Procedure*—Transfer a volume of the *Test solution*, approximately 15 nL, into the anodic end of the capillary (apply differential pressure of 3.5 kPa for 3 seconds either by vacuum or pressure), record an electropherogram, and measure

the peak areas. Calculate the percentage contents of des-Ala-des-Gly-aprotinin and des-Ala-aprotinin by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response corresponding to des-Ala-des-Gly-aprotinin or des-Ala-aprotinin; and  $r_s$  is the sum of the responses of des-Ala-des-Gly-aprotinin, des-Ala-aprotinin, and aprotinin peaks: not more than 8.0% des-Ala-des-Gly-aprotinin and not more than 7.5% des-Ala-aprotinin is found.

**Limit of N-pyroglutamyl-aprotinin and related compounds—**

*Solution A*—Prepare a filtered and degassed solution containing 3.52 g of monobasic potassium phosphate and 7.26 g of dibasic sodium phosphate dissolved in 1000 mL of water.

*Solution B*—Prepare a filtered and degassed solution containing 3.52 g of monobasic potassium phosphate, 7.26 g of dibasic sodium phosphate, and 66.07 g of ammonium sulfate dissolved in 1000 mL of water.

*Resolution solution*—Prepare a solution of USP Aprotinin System Suitability RS containing about 5 USP Aprotinin Units per mL in *Solution A*.

*Test solution*—Prepare a solution of Aprotinin having a concentration of about 5 USP Aprotinin Units per mL. Dilute with *Solution A*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 7.5-mm × 7.5-cm column that contains packing L52 and is maintained at a constant temperature of 40°. The flow rate is 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–21	92→64	8→36	linear gradient
21–30	64→0	36→100	linear gradient
30–31	0→92	100→8	linear gradient
31–40	92	8	re-equilibration

Chromatograph the *Resolution solution* as directed for *Procedure*: the retention time for aprotinin is between 17 and 20 minutes; the relative retention times are about 0.9 for *N*-pyroglutamyl-aprotinin, and 1.0 for aprotinin; the resolution,  $R$ , between *N*-pyroglutamyl-aprotinin and aprotinin is not less than 1.0; and the tailing factor for the aprotinin peak is not greater than 2.0.

*Procedure*—Inject 40 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of each impurity peak in the chromatogram by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each impurity peak; and  $r_s$  is the sum of the responses of all peaks in the chromatogram of the *Test solution*: not more than 1.0% *N*-pyroglutamyl-aprotinin is found; not more than 0.5% of any other impurity is found; and the sum of all unknown impurities is not more than 1.0%.

**Limit of high molecular weight proteins—**

*Mobile phase*—Prepare a filtered and degassed mixture of water, glacial acetic acid, and acetonitrile (6:2:2).

*Resolution solution*—Prepare an aprotinin solution that contains about 5 USP Aprotinin Units per mL with about 2% aprotinin oligomers. [NOTE—This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 hours and dissolving the solid at the specified concentration in water.]

*Test solution*—Prepare a solution of Aprotinin in water that contains about 5 USP Aprotinin Units per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a series of three 7.8-mm × 30-cm columns containing packing L33. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution* as directed for *Procedure*: the retention time for aprotinin is between 24.5 and 25.5 minutes; the relative retention times are about 0.9 for the dimer and 1.0 for aprotinin; the resolution,  $R$ , between the dimer peak and the aprotinin peak is not less than 1.3; and the tailing factor for the aprotinin peak is not greater than 2.5.

**Procedure**—Inject about 100  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of each oligomer peak in the chromatogram by the formula:

$$100r_i / r_s$$

in which  $r_i$  is the response of each peak having a retention time less than that of aprotinin monomer; and  $r_s$  is the sum of the responses of all peaks: the sum of all oligomers is not more than 1.0%.

#### Assay—

**0.0015 M Borate buffer**—Transfer about 0.93 g of boric acid into a 1000-mL volumetric flask, dissolve in 900 mL of water, adjust with 5 N sodium hydroxide to a pH of 8.0, dilute with water to volume, and mix. Transfer 100 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Prepare a solution of Aprotinin with 0.0015 M Borate buffer to obtain a solution having about 1.67 USP Aprotinin Units per mL (about 0.6 mg per mL).

**Trypsin solution**—Prepare a solution of USP Trypsin Crystallized RS containing about 4300 USP Trypsin Units per mL, using 0.001 N hydrochloric acid as the solvent. Use a freshly prepared solution, and keep in ice water.

**Trypsin and aprotinin solution**—To 4.0 mL of the *Trypsin solution*, add 1.0 mL of the *Assay preparation*. Dilute immediately with 0.0015 M Borate buffer to 40.0 mL. Allow to stand at room temperature for 10 minutes, and then keep in ice water. [NOTE—Use within 6 hours of preparation.]

**Dilute trypsin solution**—Dilute 0.5 mL of the *Trypsin solution* with 0.0015 M Borate buffer to 10.0 mL. Allow to stand at room temperature for 10 minutes, and then keep in ice water.

**Substrate solution**—Dissolve 69 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride in 10 mL of water. [NOTE—Use within 2 hours.]

**Procedure**—Mix 9.0 mL of 0.0015 M Borate buffer and 1.0 mL of *Substrate solution* in a jacketed glass vessel with a capacity of about 30 mL and that contains a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a buret, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjust to a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at  $25 \pm 0.1^\circ$ , add 1.0 mL of *Trypsin and aprotinin solution*, and start a timer. Maintain at a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS, and note the volume added every 30 seconds. Continue the reaction for 6 minutes. Determine the volume of 0.1 N sodium hydroxide added per second, in mL ( $n_1$ ). Carry out a similar titration using 1.0 mL of the *Dilute trypsin solution*. Determine the volume of 0.1 N sodium hydroxide added per second, in mL ( $n_2$ ). For the lyophilized powder, calculate the

aprotinin activity in USP Aprotinin Units per mg using the formula:

$$4000(2n_2 - n_1)/m$$

in which  $m$  is the quantity, in mg, of Aprotinin used to prepare 1 mL of the *Assay preparation*. For the concentrated solution, calculate the USP Aprotinin Units per mL using the following formula:

$$4000(2n_2 - n_1)D$$

in which  $D$  is the dilution factor of the concentrated solution used to prepare the *Assay preparation*.

## Aprotinin Injection

» Aprotinin Injection is a sterile solution of Aprotinin in Water for Injection that also contains sodium chloride. One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.). It contains not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label, expressed in Kallikrein Inhibition Units per mL.

**Packaging and storage**—Preserve in single-dose containers, store up to  $25^\circ$ , and avoid freezing.

#### USP Reference standards (11)—

USP Aprotinin RS  
USP Aprotinin System Suitability RS  
USP Endotoxin RS  
USP Trypsin Crystallized RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Limit of N-pyroglyutamyl-aprotinin and related compounds under Aprotinin*.

**B:** The determination of activity by the Assay is based on the specific inhibition of trypsin.

**Bacterial endotoxins** (85)—It contains not more than 0.14 USP Endotoxin Units per USP Aprotinin Unit.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.5 and 6.5.

**Particulate matter** (788): meets the requirements.

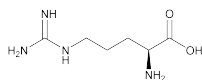
**Sodium chloride content**—Pipet 5.0 mL of Injection into 50 mL of water in a beaker. Add 10 mL of 25% nitric acid. Titrate with 0.1 N silver nitrate VS to a potentiometric endpoint, using a silver combination electrode. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride: between 42.5 mg and 47.5 mg of sodium chloride is found.

**Other requirements**—It meets the requirements under *Injections* (1) and for *Limit of N-pyroglyutamyl-aprotinin and related compounds under Aprotinin*.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins under Aprotinin*. The sum of all oligomers is not more than 1.5%.

**Assay**—Proceed as directed in the Assay under *Aprotinin*.

## Arginine



$C_6H_{14}N_4O_2$   
L-Arginine [74-79-3].

174.20

### DEFINITION

Arginine contains NLT 98.5% and NMT 101.5% of  $C_6H_{14}N_4O_2$ , as L-arginine, calculated on the dried basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Sample:** 80 mg of Arginine

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 3 mL of formic acid and 50 mL of glacial acetic acid

**Analysis:** Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with *Titrant*. Calculate the percentage of  $C_6H_{14}N_4O_2$  in the portion taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

$V$  = *Sample* titrant volume (mL)

$B$  = *Blank* titrant volume (mL)

$N$  = titrant normality (mEq/mL)

$F$  = equivalency factor: 87.10 mg/mEq

$W$  = weight of *Sample* (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.3%
- **CHLORIDE AND SULFATE, Chloride** (221): A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).
- **CHLORIDE AND SULFATE, Sulfate** (221): A 1.0-g portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (0.03%).
- **IRON** (241): NMT 30 ppm
- **HEAVY METALS, Method I** (231): NMT 15 ppm

#### Organic Impurities

#### • PROCEDURE

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Standard solution:** 0.05 mg/mL of USP L-Arginine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

**Sample solution:** 10 mg/mL of Arginine in 2 N hydrochloric acid

**System suitability solution:** 0.4 mg/mL each of USP L-Arginine RS and USP L-Lysine Hydrochloride RS in 0.1 N hydrochloric acid

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Isopropyl alcohol and ammonium hydroxide (7:3)

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *System suitability solution*

Proceed as directed under *Chromatography* (621), *Thin-Layer Chromatography*. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The chromatogram obtained from the *System suitability solution* exhibits two clearly separated spots.

#### Acceptance criteria

**Individual impurities:** Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*, NMT 0.5%

**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S): +26.3° to +27.7°

**Sample solution:** 80 mg/mL in 6 N hydrochloric acid

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

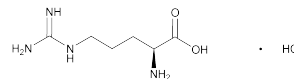
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)

USP L-Arginine RS

USP L-Lysine Hydrochloride RS

## Arginine Hydrochloride



$C_6H_{14}N_4O_2 \cdot HCl$

210.66

L-Arginine monohydrochloride;

L-(+)-Arginine monohydrochloride [1119-34-2].

### DEFINITION

Arginine Hydrochloride contains NLT 98.5% and NMT 101.5% of arginine hydrochloride ( $C_6H_{14}N_4O_2 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Sample:** 100 mg of Arginine Hydrochloride

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 50 mL of glacial acetic acid and 3 mL of 98% formic acid. Add 6 mL of mercuric acetate TS.

**Analysis:** Dissolve the *Sample* in 3 mL of 98% formic acid and 50 mL of glacial acetic acid. Add 6 mL of mercuric acetate TS and titrate with the *Titrant*. Calculate the percentage of arginine hydrochloride ( $C_6H_{14}N_4O_2 \cdot HCl$ ) in the *Sample* taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

$V$  = *Sample* titrant volume (mL)

$B$  = *Blank* titrant volume (mL)

$N$  = titrant normality (mEq/mL)

$F$  = equivalency factor, 105.3 mg/mEq

$W$  = weight of *Sample* (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
  - **CHLORIDE AND SULFATE**, Sulfate (221): A 1.6-g portion shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.03%).
  - **HEAVY METALS**, Method I (231)  
**Test preparation:** Proceed as directed in the chapter, except to dissolve 1.0 g in 20 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL.  
**Acceptance criteria:** NMT 20 ppm
  - **CHROMATOGRAPHIC PURITY**  
**System suitability solution:** 0.4 mg/mL each of USP Arginine Hydrochloride RS and USP L-Lysine Hydrochloride RS in water  
**Standard solution:** 0.05 mg/mL of USP Arginine Hydrochloride RS in water. [NOTE—This solution has a concentration equivalent to about 0.5% of that of the *Sample solution*.]  
**Sample solution:** 10 mg/mL of Arginine Hydrochloride in water  
**Chromatographic system**  
 (See *Chromatography* (621), *Thin-Layer Chromatography*).  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 5  $\mu$ L  
**Developing solvent system:** Isopropyl alcohol and ammonium hydroxide (70:30)  
**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
- Analysis**  
**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*  
 Proceed as directed in the chapter. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The *System suitability solution* exhibits two clearly separated spots.  
**Acceptance criteria:** Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*.  
**Individual impurities:** NMT 0.5%  
**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

- **OPTICAL ROTATION**, Specific Rotation (781S): +21.4° to +23.6° ( $t = 20^\circ$ )  
**Sample solution:** 80 mg/mL in 6 N hydrochloric acid
- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.2% of its weight.
- **CHLORIDE CONTENT**  
**Sample:** 350 mg of Arginine Hydrochloride  
**Titrimetric system**  
 (See *Titrimetry* (541).)  
**Mode:** Direct titration  
**Titrant:** 0.1 N silver nitrate VS  
**Endpoint detection:** Colorimetric  
**Blank:** 140 mL of water and 1 mL of dichlorofluorescein TS  
**Analysis:** Transfer the *Sample* to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Mix and titrate with the *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color.  
 Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

- $V$  = *Sample* titrant volume (mL)  
 $B$  = *Blank* titrant volume (mL)  
 $N$  = titrant normality (mEq/mL)

$F$  = equivalency factor, 35.45 mg/mEq  
 $W$  = weight of *Sample* (mg)  
**Acceptance criteria:** 16.5%–17.1%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Arginine Hydrochloride RS  
 USP L-Lysine Hydrochloride RS

## Arginine Hydrochloride Injection

» Arginine Hydrochloride Injection is a sterile solution of Arginine Hydrochloride in Water for Injection. It contains not less than 9.5 percent and not more than 10.5 percent of  $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2 \cdot \text{HCl}$ . It contains no antimicrobial agents.

NOTE—The chloride ion content of Arginine Hydrochloride Injection is approximately 475 mEq per L.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type II glass.

### USP Reference standards (11)—

USP Arginine Hydrochloride RS  
 USP Endotoxin RS

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

### Identification—

**A:** Transfer 1 mL of the Injection to a 200-mL volumetric flask, and dilute with water to volume. To 1 mL of this dilution add 2 mL of a solution of 0.02% 8-hydroxyquinoline in 3 N sodium hydroxide, and add 1 mL of 0.1% *N*-bromosuccinimide solution: an orange color is produced.

**B:** It meets the requirements of the tests for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.01 USP Endotoxin Unit per mg of arginine hydrochloride.

**pH** (791): between 5.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Color reagent**—Dissolve 28.0 g of potassium hydroxide and 2.0 g of potassium sodium tartrate in 100 mL of water. Cool, and add, in the order named, 100 mg of 2,4-dichloro-1-naphthol, 180 mL of alcohol, and 20.0 mL of 0.475% sodium hypochlorite solution. Mix by swirling, and allow to stand at room temperature for 1 hour before using. This *Color reagent* may be stored in a glass-stoppered bottle, in a refrigerator, for 2 months.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Arginine Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 40  $\mu$ g per mL.

**Assay preparation**—Pipet into a 100-mL volumetric flask a volume of Injection, equivalent to 200 mg of arginine hydrochloride, add water to volume, and mix. Pipet 5 mL of this solution into a 250-mL volumetric flask, add water to volume, and mix.

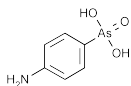
**Procedure**—Transfer 2.0-mL portions of the *Assay preparation* and the *Standard preparation*, respectively, to separate

flasks, and treat each as follows. Add 2.0 mL of potassium iodide solution (3 in 1000), mix, and allow to stand for 15 minutes. Add 6.0 mL of *Color reagent*, mix, and allow to stand for 15 minutes. Add 2.0 mL of sodium hypochlorite solution (19 in 10,000), mix, and allow to stand for 15 minutes. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_6H_{14}N_4O_2 \cdot HCl$  in each mL of the Injection taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which  $C$  is the concentration, in  $\mu g$  per mL, of USP Arginine Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Arsanilic Acid



$C_6H_8AsNO_3$  217.05  
p-Aminobenzenearsonic acid [98-50-0].

» Arsanilic Acid contains not less than 98.0 percent and not more than 102.0 percent of  $C_6H_8AsNO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—  
USP Arsanilic Acid RS

**Identification, Infrared Absorption** (197K).

**Loss on drying** (731)—Dry it in vacuum at 80° for 4 hours; it loses not more than 0.5% of its weight.

**Limit of o-arsanilic acid**—

*Mobile phase*—Dissolve 4.04 g of monobasic potassium phosphate in 985 mL of water, add 2 mL of phosphoric acid, and mix. Add 10 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Transfer about 67 mg of o-arsanilic acid, accurately weighed, to a 100-mL volumetric flask, add about 65 mL of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.0012 mg of o-arsanilic acid per mL.

*Test solution*—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 242-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu m$  base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the o-arsanilic acid peak is between 2.8 and

3.8; and the relative standard deviation for replicate injections is not more than 2.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu L$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the o-arsanilic acid peaks. Calculate the percentage of o-arsanilic acid in the portion of Arsanilic Acid taken by the formula:

$$5000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of o-arsanilic acid in the *Standard solution*;  $W$  is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the responses of the o-arsanilic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.12% is found.

**Limit of aniline**—

*Mobile phase*—Dissolve 7.76 g of monobasic potassium phosphate in 950 mL of water, add 50 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Transfer about 176 mg of aniline, accurately weighed, to a 25-mL volumetric flask, add about 1 mL of methanol, swirl, then add about 15 mL of water, and shake to dissolve. Dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.00045 mg of aniline per mL.

*Test solution*—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu m$  base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the aniline peak is between 2.3 and 3.3; and the relative standard deviation for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu L$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the aniline peaks. Calculate the percentage of aniline in the portion of Arsanilic Acid taken by the formula:

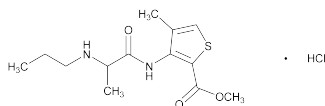
$$5000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of aniline in the *Standard solution*;  $W$  is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the responses of the aniline peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.045% is found.

**Assay**—Transfer about 125 mg of Arsanilic Acid, accurately weighed, to a 50-mL conical flask, and add 10.0 mL of a mixture of sulfuric acid, nitric acid, and perchloric acid (1000:50:50) and several glass beads. Digest on a hot plate for about 1 hour, increasing the temperature of the hot plate in steps until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, to the colorless solution add about 400 mg of hydrazine sulfate, and heat the flask vigorously on a hot plate until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and wash down the rim, neck, and insides of the flask with about 1 mL of water. Heat the flask again until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and transfer the colorless solution, with the aid of about 80 mL of water, to a 125-mL

conical flask. Add 10 mL of hydrochloric acid and several drops of 0.002 M potassium iodide, cool to between 0° and 5°, and titrate with 0.1 N potassium permanganate VS to a pale pink endpoint, maintaining the temperature between 0° and 5° during the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N potassium permanganate is equivalent to 10.852 mg of  $C_6H_8AsNO_3$ .

## Articaine Hydrochloride



$C_{13}H_{20}N_2O_3S \cdot HCl$  320.84  
2-Thiophenecarboxylic acid, 4-methyl-3-[[1-oxo-2-(propylamino)propyl]amino]-, methyl ester, monohydrochloride;  
Methyl 4-methyl-3-[2-(propylamino)propionamido]-2-thiophenecarboxylate, monohydrochloride [23964-57-0].

### DEFINITION

Articaine Hydrochloride contains NLT 98.5% and NMT 101.0% of  $C_{13}H_{20}N_2O_3S \cdot HCl$ , calculated on the dried basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION <197>

**Standard solution:** 12 mg/mL of USP Articaine RS in methylene chloride. Transfer 20  $\mu$ L of this solution onto a 300-mg disk.

**Sample solution:** Dissolve 100 mg of Articaine Hydrochloride in 5 mL of water. Add 3 mL of a saturated solution of sodium bicarbonate, and shake twice with 2 mL of methylene chloride. Combine the methylene chloride layers, dilute with methylene chloride to 5.0 mL, and dry over anhydrous sodium sulphate. Transfer 20  $\mu$ L of this solution onto a 300-mg disk.

#### B. IDENTIFICATION TESTS—GENERAL, Chloride <191>

### ASSAY

#### PROCEDURE

**Sample solution:** 250 mg of Articaine Hydrochloride to a 250-mL conical flask. Add 5.0 mL of 0.01 M hydrochloric acid and 50 mL of alcohol. Stir to dissolve.

**Analysis:** Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically, using a glass electrode. Calculate the volume of sodium hydroxide consumed by reading the volume added between the two points of inflection. Each mL of 0.1 M sodium hydroxide is equivalent to 32.08 mg of  $C_{13}H_{20}N_2O_3S \cdot HCl$ .

**Acceptance criteria:** 98.5%–101.0% on the dried basis.

### IMPURITIES

#### Inorganic Impurities

##### HEAVY METALS, Method I <231>

**Sample solution:** 200 mg/mL of Articaine Hydrochloride

**Acceptance criteria:** NMT 5 ppm

##### RESIDUE ON IGNITION <281>

**Sample:** 1 g

**Acceptance criteria:** NMT 0.1%

#### Organic Impurities

##### PROCEDURE

**Buffer solution:** 2.02 g of sodium 1-heptanesulfonate and 4.08 g of potassium dihydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.0.

**Mobile phase:** Acetonitrile and Buffer solution (1:3)

**Standard solution:** 2  $\mu$ g/mL of USP Articaine Related Compound A, and 1  $\mu$ g/mL each of USP Articaine Related Compound E RS and USP Articaine Hydrochloride RS in Mobile phase. [NOTE—This solution is also used to determine the reporting threshold limit.]

**Sample solution:** 1.0 mg/mL of Articaine Hydrochloride in Mobile phase

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Resolution:** NLT 1.2 between articaine related compound A and articaine related compound E

#### Analysis

**Samples:** Standard solution and Sample solution.

[NOTE—Run time is 5 times the retention time of articaine.]

Calculate the percentage of any articaine related compound A in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of articaine related compound A from the Sample solution

$r_S$  = response of articaine related compound A from the Standard solution

$C_S$  = concentration of USP Articaine Related Compound A RS in the Standard solution (mg/mL)

$C_U$  = concentration of Articaine Hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of each individual impurity in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of each individual impurity from the Sample solution

$r_S$  = response of articaine hydrochloride from the Standard solution

$C_S$  = concentration of USP Articaine Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = concentration of Articaine Hydrochloride in the Sample solution (mg/mL)

[NOTE—Disregard any peak below 0.05%.]

#### Acceptance criteria

**Individual impurities:** See Impurity Table 1.

**Total impurities:** NMT 0.5%. [NOTE—Excluding articaine related compound A.]



Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine acid <sup>a</sup>	0.6	0.1
Ethylarticaine <sup>b</sup>	0.7	0.1
Articaine related compound A <sup>c</sup>	0.8	0.2
Articaine related compound E <sup>d</sup>	0.86	0.1
Articaine acid-propionamide <sup>e</sup>	0.9	0.1
Articaine	1.0	—
Butylarticaine <sup>f</sup>	1.7	0.1
Dipropylarticaine <sup>g</sup>	2.1	0.1
3-Aminoarticaine <sup>h</sup>	2.6	0.1
Articaine isopropyl ester <sup>i</sup>	3.6	0.1
Bromo compound <sup>j</sup>	4.0	0.1
Any other individual impurity	—	0.10

<sup>a</sup> 4-Methyl-3-[[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid.

<sup>b</sup> Methyl 3-[[[(2*RS*)-2-(ethylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>c</sup> Methyl 4-methyl-3-[2-(propylamino)acetamido]thiophene-2-carboxylate.

<sup>d</sup> Methyl 3-[2-(isopropylamino)propanamido]-4-methylthiophene-2-carboxylate.

<sup>e</sup> 4-Methyl-*N*-propyl-3-[[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxamide.

<sup>f</sup> Methyl 3-[[[(2*RS*)-2-(butylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>g</sup> Methyl 3-[[[(2*RS*)-2-(dipropylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>h</sup> Methyl 3-amino-4-methylthiophene-2-carboxylate.

<sup>i</sup> 1-Methylethyl 4-methyl-3-[[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylate.

<sup>j</sup> Methyl 3-[[[(2*RS*)-2-bromopropanoyl]amino]-4-methylthiophene-2-carboxylate.

### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry at 105° for 5 h: it loses NMT 0.5% of its weight.
- **PH** (791)  
Sample solution: 10 mg/mL  
Acceptance criteria: 4.2–5.2

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Articaine RS  
USP Articaine Hydrochloride RS  
USP Articaine Related Compound A RS  
Methyl 4-methyl-3-[2-(propylamino)acetamido]thiophene-2-carboxylate.  
 $C_{12}H_{18}N_2O_3S$  270.35  
USP Articaine Related Compound E RS  
Methyl 3-[2-(isopropylamino)propanamido]-4-methylthiophene-2-carboxylate.  
 $C_{13}H_{20}N_2O_3S$  284.37

## Articaine Hydrochloride and Epinephrine Injection

### DEFINITION

Articaine Hydrochloride and Epinephrine Injection is a sterile solution of Articaine Hydrochloride and Epinephrine, in Water for Injection, and contains NLT 95.0% and NMT

105.0% of the labeled amount of articaine hydrochloride ( $C_{13}H_{20}N_2O_3S \cdot HCl$ ) and NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

### IDENTIFICATION

- **A.** The retention times of the articaine and epinephrine peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assays for *Articaine Hydrochloride* and *Epinephrine*, respectively.

### ASSAY

#### ARTICAINE HYDROCHLORIDE

**Buffer:** Glacial acetic acid and water (50:930). Adjust with 2 N sodium hydroxide to a pH of 3.4.

**Mobile phase:** Acetonitrile and *Buffer* (22:78)

**Standard stock solution:** 40 mg/mL of USP Articaine Hydrochloride RS in water

**Standard solution:** 0.8 mg/mL of USP Articaine Hydrochloride RS in *Mobile phase* from *Standard stock solution*

**Sample solution:** Equivalent to 0.8 mg/mL of articaine hydrochloride in *Mobile phase* from a portion of Injection

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

**Run time:** 2.5 times the retention time of articaine

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.2

**Relative standard deviation:** NMT 1.0%, from six injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of articaine hydrochloride ( $C_{13}H_{20}N_2O_3S \cdot HCl$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Articaine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of articaine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

#### • EPINEPHRINE

**Mobile phase:** Mix 50 mL of glacial acetic acid and 930 mL of water. Adjust with 2 N sodium hydroxide to a pH of 3.4. In this solution, dissolve 1.2 g of sodium 1-heptanesulfonate, and add 1.0 mL of 0.1 M edetate disodium and 0.298 g of potassium chloride. Add 150 mL of methanol.

**Diluent:** 0.5 mg/mL potassium metabisulfite in water

**System suitability solution:** 22 μg/mL of epinephrine from USP Epinephrine Bitartrate RS and 20 μg/mL of norepinephrine from USP Norepinephrine Bitartrate RS in *Diluent*

**Standard stock solution:** 0.55 mg/mL of epinephrine from USP Epinephrine Bitartrate RS in *Diluent*

**Standard solution:** Dilute a suitable volume of the *Standard stock solution* with *Diluent* to obtain a final concentration of *L* mg/mL of epinephrine, where *L* is the label claim of epinephrine in the Injection.

**Sample solution:** Use the Injection directly.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical

**Reference electrode:** Silver/silver chloride

**Working electrode:** Glassy carbon

**Potential:** +650 mV

**Detector temperature:**  $28 \pm 2^\circ$

**Column:** 4.0-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1 mL/min

**Injection size:** 2  $\mu$ L

**Run time:** 1.7 times the retention time of epinephrine

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for norepinephrine and epinephrine are 0.90 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between the norepinephrine and epinephrine peaks

**Tailing factor:** NMT 2.0 for the epinephrine peak

**Relative standard deviation:** NMT 1.0% for the epinephrine peak, from six injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of epinephrine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of epinephrine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

## PERFORMANCE TESTS

### • DELIVERABLE VOLUME (698)

For Articaine Hydrochloride and Epinephrine Injection packaged in single-dose containers: Meets the requirements

## IMPURITIES

### • ORGANIC IMPURITIES, LIMIT OF ARTICAINES RELATED COMPOUNDS

**Mobile phase, Standard stock solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay for Articaine Hydrochloride*.

**Standard solution:** 0.8 mg/mL of USP Articaine Hydrochloride RS from *Standard stock solution* and 40  $\mu$ g/mL of USP Articaine Related Compound B RS in *Mobile phase*

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 1 for relative retention times.]

**Suitability requirements**

**Tailing factor:** NMT 2.2 for the articaine peak

**Resolution:** NLT 1.25 between the articaine related compound B and articaine peaks

**Relative standard deviation:** NMT 1.0% for the articaine peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Articaine related compounds elute at relative retention times of NMT 2.0 with respect to the articaine peak.]

Calculate the percentage of articaine related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of each individual impurity from the *Sample solution*

$r_S$  = response of articaine from the *Standard solution*

$C_S$  = concentration of articaine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of articaine in the *Sample solution* (mg/mL)

[NOTE—Disregard any peak below 0.05%.]

**Acceptance criteria:** See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine related compound B	0.6	0.5
Articaine	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	0.5

### • ORGANIC IMPURITIES, LIMIT OF EPINEPHRINE RELATED COMPOUNDS

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Epinephrine*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Epinephrine related compounds elute between relative retention times of 0.35 and 1.0, with respect to the epinephrine peak.]

Calculate the percentage of epinephrine related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of each individual impurity from the *Sample solution*

$r_S$  = response of epinephrine from the *Standard solution*

$C_S$  = concentration of epinephrine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of epinephrine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epinephrine sulfonate <sup>a</sup>	0.46	7.5
Specified impurity	0.52	8
Epinephrine	1.0	—
Any other individual impurity	—	1
Total impurities	—	10

<sup>a</sup> 1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanesulfonic acid.

## SPECIFIC TESTS

### • PH (791): 2.7–5.2

### • BACTERIAL ENDOTOXINS TEST (85): NMT 0.7 USP Endotoxin Unit/mg of articaine hydrochloride

### • STERILITY (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

### • PARTICULATE MATTER IN INJECTIONS (788): Meets the requirements

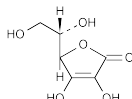
### • OTHER REQUIREMENTS: It meets the requirements under *Injections* (1).

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** <11>  
USP Articaïne Hydrochloride RS  
USP Articaïne Related Compound B RS  
4-Methyl-3-[[2-(propylamino)propanoyl]amino}thiophene-2-carboxylic acid.  
 $C_{12}H_{18}N_2O_3S$  270.35  
USP Endotoxin RS  
USP Epinephrine Bitartrate RS  
USP Norepinephrine Bitartrate RS

## Ascorbic Acid



$C_6H_8O_6$  176.12  
L-Ascorbic acid [50-81-7].

### DEFINITION

Ascorbic Acid contains NLT 99.0% and NMT 100.5% of  $C_6H_8O_6$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B.** A 20-mg/mL solution reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.

### ASSAY

#### • PROCEDURE

**Sample:** 400 mg of Ascorbic Acid

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N iodine VS

**Endpoint detection:** Visual

**Blank:** 100 mL of water and 25 mL of 2 N sulfuric acid.  
Add 3 mL of starch TS.

**Analysis:** Dissolve the *Sample* in a mixture of 100 mL of water and 25 mL of 2 N sulfuric acid. Add 3 mL of starch TS, and titrate immediately with *Titrant* until a persistent violet-blue color is obtained.

Calculate the percentage of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Ascorbic Acid taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

$V$  = sample titrant volume (mL)

$B$  = blank titrant volume (mL)

$N$  = titrant normality (mEq/mL)

$F$  = equivalency factor, 88.06 mg/mEq

$W$  = weight of *Sample* (mg)

**Acceptance criteria:** 99.0%–100.5%

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

- **HEAVY METALS** <231>

**Sample solution:** 1 g in 25 mL of water

**Acceptance criteria:** NMT 20 ppm

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** <781S>

**Sample solution:** 100 mg/mL in carbon dioxide-free water. Perform the test immediately after preparation of the *Sample solution*.

**Acceptance criteria:** +20.5° to +21.5°

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** <11>  
USP Ascorbic Acid RS

## Ascorbic Acid Injection

» Ascorbic Acid Injection is a sterile solution, in Water for Injection, of Ascorbic Acid prepared with the aid of Sodium Hydroxide, Sodium Carbonate, or Sodium Bicarbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

**Packaging and storage**—Preserve in light-resistant, single-dose containers, preferably of Type I or Type II glass.

**Labeling**—In addition to meeting the requirements for *Labeling* under *Injections* <1>, fused-seal containers of the Injection in concentrations of 250 mg per mL and greater are labeled to indicate that since pressure may develop on long storage, precautions should be taken to wrap the container in a protective covering while it is being opened.

### USP Reference standards <11>—

USP Ascorbic Acid RS

USP Endotoxin RS

### Identification—

**A:** To a volume of Injection, equivalent to 40 mg of ascorbic acid, add 4 mL of 0.1 N hydrochloric acid, then add 4 drops of methylene blue TS, and warm to 40°: the deep blue color becomes appreciably lighter or is completely discharged within 3 minutes.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay*.

**C:** It responds to the flame test for *Sodium* <191>.

**Bacterial endotoxins** <85>—It contains not more than 1.2 USP Endotoxin Units per mg of ascorbic acid.

**pH** <791>: between 5.5 and 7.0.

**Limit of oxalate**—Dilute a volume of Injection, equivalent to 50 mg of ascorbic acid, with water to 5 mL. Add 0.2 mL of acetic acid and 0.5 mL of calcium chloride TS: no turbidity is produced in 1 minute.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay—

**Mobile phase**—Dissolve 15.6 g of dibasic sodium phosphate and 12.2 g of monobasic potassium phosphate in 2000 mL of water, adjust with phosphoric acid to a pH of  $2.5 \pm 0.05$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ascorbic Acid RS in *Mobile phase*, and mix to obtain a solution having a known concentration of about 0.5 mg per mL. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 hours. Inject within 3 hours after removal from the refrigerator.]

**Assay preparation**—Dilute the Injection, quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.5 mg per mL. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 hours. Inject within 3 hours after removal from the refrigerator.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 245-nm detector and a 6-mm  $\times$  150-mm column that contains packing L39. The flow rate is about 0.6 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as

directed for *Procedure*: the column efficiency is not less than 3500 theoretical plates, the tailing factor is not more than 1.6, and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 4  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peak. Calculate the quantity, in mg, of ascorbic acid ( $C_6H_8O_6$ ) in each mL of the injection taken by the formula:

$$CD(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ascorbic Acid RS in the *Standard preparation*; *D* is the dilution factor; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ascorbic Acid Oral Solution

### DEFINITION

Ascorbic Acid Oral Solution is a solution of Ascorbic Acid in a hydroxylic organic solvent or an aqueous mixture thereof. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

### IDENTIFICATION

- **A.**  
**Sample solution:** A volume of Oral Solution equivalent to 40 mg of ascorbic acid  
**Analysis:** To the *Sample solution* add 4 mL of 0.1 N hydrochloric acid, then 4 drops of methylene blue TS, and warm to 40°.  
**Acceptance criteria:** The deep blue color becomes appreciably lighter or is completely discharged within 3 min.
- **B.**  
**Sample solution:** A volume of Oral Solution equivalent to 20 mg of ascorbic acid  
**Analysis:** To the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20). Add 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, then heat in a bath at 50°.  
**Acceptance criteria:** A blue color develops.

### ASSAY

#### • PROCEDURE

**Sample solution:** Transfer a volume of Oral Solution equivalent to 50 mg of ascorbic acid, previously diluted with water if necessary, to a 100-mL volumetric flask. Add 20 mL of metaphosphoric-acetic acid TS, dilute with water to volume, and mix.

**Blank:** A mixture of 5.5 mL of metaphosphoric-acetic acid TS and 15 mL of water

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** Standard dichlorophenol-indophenol VS

**Analysis:** Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, into a 50-mL conical flask. Add 5 mL of metaphosphoric-acetic acid TS, and titrate with *Titrant* until a rose-pink color persists for at least 5 s. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Oral Solution taken:

$$\text{Result} = [(V_S - V_B) \times F/W] \times 100$$

- $V_S$  = *Titrant* volume consumed by the *Sample solution* (mL)
- $V_B$  = *Titrant* volume consumed by the *Blank* (mL)
- $F$  = concentration of *Titrant* in terms of its equivalent of ascorbic acid (mg/mL)
- $W$  = nominal amount of ascorbic acid taken for *Analysis* (mg)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I <611>** (if present): 90.0%–110.0% of the labeled content of alcohol ( $C_2H_5OH$ )

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label Oral Solution that contains alcohol to state the alcohol content.

## Ascorbic Acid Tablets

### DEFINITION

Ascorbic Acid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

### IDENTIFICATION

- **A.**  
**Sample solution:** Triturate a quantity of finely powdered Tablets with diluted alcohol to make a solution of ascorbic acid with a concentration of 20 mg/mL, and filter.  
**Analysis:** Add alkaline cupric tartrate TS to a portion of the *Sample solution*.  
**Acceptance criteria:** The *Sample solution* reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.
- **B.**  
**Sample solution:** Use the *Sample solution* from Identification test A.  
**Analysis:** To 2 mL of the *Sample solution* add 4 drops of methylene blue TS, and warm to 40°.  
**Acceptance criteria:** The deep blue color of methylene blue becomes appreciably lighter or is completely discharged within 3 min.
- **C.**  
**Sample solution:** Use the *Sample solution* from Identification test A.  
**Analysis:** To 1 mL of the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20) and 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a bath at 50°.  
**Acceptance criteria:** A blue color develops.

### ASSAY

#### • PROCEDURE

**Sample stock solution:** Transfer NLT 20 Tablets to a 1000-mL volumetric flask containing 250 mL of metaphosphoric-acetic acids TS. Insert the stopper in the flask, and shake by mechanical means for 30 min or until the tablets have disintegrated completely. Dilute with water to volume.

**Sample solution:** Transfer a portion of the *Sample stock solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Quantitatively dilute the clear supernatant with water, if necessary, to obtain a solution containing 0.5 mg/mL of ascorbic acid.

**Blank:** A mixture of 5.5 mL of metaphosphoric-acetic acids TS and 15 mL of water

**Titrimetric system**(See *Titrimetry* <541>.)**Mode:** Direct titration**Titrant:** Standard dichlorophenol-indophenol VS**Endpoint detection:** Visual, a rose-pink color that persists for at least 5 s**Analysis:** Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, into 50-mL conical flask. Add 5 mL of metaphosphoric-acetic acids TS, and titrate with *Titrant*. Correct for the volume of the *Titrant* consumed by the *Blank*.Calculate the percentage of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) in the portion of the Tablets taken:

$$\text{Result} = [(V_S - V_B) \times F / W] \times 100$$

 $V_S$  = *Titrant* volume consumed by the *Sample solution* (mL) $V_B$  = *Titrant* volume consumed by the *Blank* (mL) $F$  = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL) $W$  = nominal weight of ascorbic acid taken for *Analysis* (mg)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION <711>****Medium:** Water; 900 mL**Apparatus 2:** 50 rpm**Time:** 45 min**Sample solution:** Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.**Analysis:** Proceed as directed in the *Assay*, conducting the procedure without delay and making any necessary modifications.

Calculate the percentage of ascorbic acid dissolved:

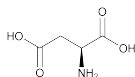
$$\text{Result} = (V_S - V_B) \times F \times [(V_M/a)/L] \times 100$$

 $V_S$  = *Titrant* volume consumed by the *Sample solution* (mL) $V_B$  = *Titrant* volume consumed by the *Blank* (mL) $F$  = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL) $V_M$  = volume of *Medium*, 900 mL $a$  = volume of the aliquot taken for *Analysis* $L$  = labeled amount of ascorbic acid (mg/Tablet)**Tolerances:** NLT 75% (Q) of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) is dissolved.**• UNIFORMITY OF DOSAGE UNITS <905>** Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

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**Aspartic Acid**

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 $C_4H_7NO_4$   
L-Aspartic acid [56-84-8].

133.10

**DEFINITION**Aspartic Acid contains NLT 98.5% and NMT 101.5% of aspartic acid ( $C_4H_7NO_4$ ), calculated on the dried basis.**IDENTIFICATION****• A. INFRARED ABSORPTION <197K>****ASSAY****• PROCEDURE****Sample:** 100 mg of Aspartic Acid**Titrimetric system**(See *Titrimetry* <541>.)**Mode:** Direct titration**Titrant:** 0.1 N sodium hydroxide VS**Endpoint detection:** Visual**Blank:** 50 mL of carbon dioxide-free water. Add 0.1 mL of bromothymol blue TS.**Analysis:** Transfer the *Sample* to a 125-mL flask, and dissolve in 50 mL of carbon dioxide-free water. Heat slightly if necessary. Cool, add 0.1 mL of bromothymol blue TS, and titrate with *Titrant* until the color changes from yellow to blue.Calculate the percentage of aspartic acid ( $C_4H_7NO_4$ ) in the portion of Aspartic Acid taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

 $V$  = sample titrant volume (mL) $B$  = blank titrant volume (mL) $N$  = titrant normality (mEq/mL) $F$  = equivalency factor, 133.1 mg/mEq $W$  = weight of *Sample* (mg)**Acceptance criteria:** 98.5%–101.5% on the dried basis**IMPURITIES****• RESIDUE ON IGNITION <281>** NMT 0.1%**• CHLORIDE AND SULFATE, Chloride <221>****Sample solution:** Dissolve 0.7 g of Aspartic Acid in 10 mL of diluted nitric acid, and dilute with water to 15 mL.**Acceptance criteria:** The *Sample solution* shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (NMT 0.02%).**• CHLORIDE AND SULFATE, Sulfate <221>****Sample solution:** Dissolve 0.8 g of Aspartic Acid in 4 mL of hydrochloric acid, and dilute with water to 15 mL.**Acceptance criteria:** The *Sample solution* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid (NMT 0.03%).**• IRON <241>** NMT 10 ppm**• HEAVY METALS, Method II <31>** NMT 10 ppm**• CHROMATOGRAPHIC PURITY****System suitability solution:** 10 mg each of USP Aspartic Acid RS and glutamic acid in 2 mL of ammonia TS. Dilute with water to 25.0 mL.**Standard solution:** Transfer 5 mg of USP Aspartic Acid RS to a 100-mL volumetric flask, dissolve in 2 mL of 17% ammonia solution (prepared by diluting ammonium hydroxide, 6 in 10), and dilute with water to volume.**Sample solution:** Transfer 0.1 g of Aspartic Acid to a 10-mL volumetric flask, dissolve in 2 mL of 17% ammonia solution (prepared by diluting ammonium hydroxide, 6 in 10), and dilute with water to volume.**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volume:** 5  $\mu$ L**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)**System suitability****Sample:** *System suitability solution***Suitability requirement:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

**Analysis**

**Samples:** *System suitability solution, Standard solution, and Sample solution*

Proceed as directed in the chapter, except dry the plate at 80° for 30 min, spray with *Spray reagent*, and heat at 80° for 30 min. Examine the plate under white light.

**Acceptance criteria:** No secondary spot from the *Sample solution* is larger or more intense than the principal spot from the *Standard solution*.

**Individual impurities:** NMT 0.5%

**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 80 mg/mL in 6 N hydrochloric acid

**Acceptance criteria:** +24.0° to +26.0°, at 20°

- **LOSS ON DRYING (731):** Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store protected from light.

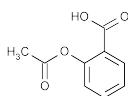
- **USP REFERENCE STANDARDS (11)**

USP Aspartic Acid RS

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**Aspirin**


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C<sub>9</sub>H<sub>8</sub>O<sub>4</sub> 180.16

Benzoic acid, 2-(acetyloxy)-.

Salicylic acid acetate [50-78-2].

» Aspirin contains not less than 99.5 percent and not more than 100.5 percent of C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Aspirin RS

**Identification**—

A: Heat it with water for several minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

B: *Infrared Absorption* (197K).

**Loss on drying (731)**—Dry it over silica gel for 5 hours: it loses not more than 0.5% of its weight.

**Readily carbonizable substances (271)**—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid Q*.

**Residue on ignition (281):** not more than 0.05%.

**Substances insoluble in sodium carbonate TS**—A solution of 500 mg in 10 mL of warm sodium carbonate TS is clear.

**Chloride (221)**—Boil 1.5 g with 75 mL of water for 5 minutes, cool, add sufficient water to restore the original volume, and filter. A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

**Sulfate**—Dissolve 6.0 g in 37 mL of acetone, and add 3 mL of water. Titrate potentiometrically with 0.02 M lead perchlorate, prepared by dissolving 9.20 g of lead perchlorate in water to make 1000 mL of solution, using a pH meter capable of a minimum reproducibility of ±0.1 mV (see pH (791)) and equipped with an electrode system con-

sisting of a lead-specific electrode and a silver-silver chloride reference glass-sleeved electrode containing a solution of tetraethylammonium perchlorate in glacial acetic acid (1 in 44) (see *Titrimetry* (541)): not more than 1.25 mL of 0.02 M lead perchlorate is consumed (0.04%). [NOTE—After use, rinse the lead-specific electrode with water, drain the reference electrode, flush with water, rinse with methanol, and allow to dry.]

**Heavy metals**—Dissolve 2 g in 25 mL of acetone, and add 1 mL of water. Add 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer (see *Heavy Metals* (231)), and allow to stand for 5 minutes: any color produced is not darker than that of a control made with 25 mL of acetone and 2 mL of *Standard Lead Solution* (see *Heavy Metals* (231)), treated in the same manner. The limit is 10 µg per g.

**Limit of free salicylic acid**—Dissolve 2.5 g in sufficient alcohol to make 25.0 mL. To each of two matched color-comparison tubes add 48 mL of water and 1 mL of a freshly prepared, diluted ferric ammonium sulfate solution (prepared by adding 1 mL of 1 N hydrochloric acid to 2 mL of ferric ammonium sulfate TS and diluting with water to 100 mL). Into one tube pipet 1 mL of a standard solution of salicylic acid in water, containing 0.10 mg of salicylic acid per mL. Into the second tube pipet 1 mL of the 1 in 10 solution of Aspirin. Mix the contents of each tube: after 30 seconds, the color in the second tube is not more intense than that in the tube containing the salicylic acid (0.1%).

**Assay**—Place about 1.5 g of Aspirin, accurately weighed, in a flask, add 50.0 mL of 0.5 N sodium hydroxide VS, and boil the mixture gently for 10 minutes. Add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N sodium hydroxide is equivalent to 45.04 mg of C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>.

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**Aspirin Boluses**


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» Aspirin Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Boluses to indicate that they are for veterinary use only.

**USP Reference standards (11)**—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

A: Crush 1 Bolus, boil a portion of the powder, equivalent to about 300 mg of aspirin, with 50 mL of water, cool, and add a drop of ferric chloride TS: a violet-red color is produced.

B: The retention time of the aspirin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution (711)**—

*Medium:* 0.5 M phosphate buffer, pH 7.4; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

*Diluting solution*—Prepare a mixture of acetonitrile and formic acid (99:1).

*Procedure*—Determine the amount of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) dissolved by employing UV absorption at the wavelength of the isosbestic point of aspirin and salicylic acid at 265 ± 2 nm on filtered portions of the solution under test, suitably

diluted with *Diluting solution*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of salicylic acid**—Using the chromatograms of the *Standard preparation* and the *Assay preparation*, obtained as directed in the *Assay*, calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Boluses taken by the formula:

$$100,000(C / W_A)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*;  $W_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Boluses taken, as determined in the *Assay*; and  $r_U$  and  $r_S$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.3% is found.

#### Assay—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Prepare a solution in *Diluting solution* having known concentrations of about 0.4 mg of USP Aspirin RS and 0.01 mg of USP Salicylic Acid RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 10 Boluses. Transfer an accurately weighed portion of the powder, equivalent to about 400 mg of aspirin, to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and stir by mechanical means for about 15 minutes. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for salicylic acid and 1.0 for aspirin, and the relative standard deviation of the aspirin peak response for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Boluses taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the aspirin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Capsules

» Aspirin Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

NOTE—Capsules that are enteric-coated or the contents of which are enteric-coated meet the requirements for *Aspirin Delayed-Release Capsules*.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

#### Identification—

**A:** Heat about 100 mg of the Capsule contents with 10 mL of water for several minutes, cool, and add 1 drop of ferric chloride TS: a violet-red color is produced.

**B:** Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour: the residue responds to *Identification test B* under *Aspirin*.

#### Dissolution (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Limit of free salicylic acid—

**Ferric chloride-urea reagent**—Dissolve by swirling, without the aid of heat, 60 g of urea in a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid. Adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2.

**Standard preparation**—Transfer 75.0 mg of salicylic acid, previously dried over silica gel for 3 hours and accurately weighed, to a 100-mL volumetric flask, add chloroform to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix. Transfer 10.0 mL of this last solution to a 50-mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether, dilute with chloroform to volume, and mix.

**Chromatographic column** (see *Chromatography* (621))—Proceed as directed under *Column Partition Chromatography*, packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 0.5 mL of 5 M phosphoric acid, and the upper segment is a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared *Ferric chloride-urea reagent*.

**Test preparation**—Weigh accurately a portion of the contents of the Capsules, as determined by the Assay, equivalent to 100 mg of aspirin, mix with 10 mL of chloroform by stirring for 3 minutes, and then transfer to the chromatographic column with the aid of a few mL of chloroform. Pass 50 mL of chloroform through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. Prepare as a receiver a 50-mL volumetric flask containing 10 mL of methanol and 2 drops of hydrochloric acid, and elute any salicylic acid from the column by passing 10 mL of a 1 in 10 solution of glacial acetic acid in ether that has been recently saturated with water, followed by 30 mL of chloroform. Dilute the eluate with chloroform to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, with a suitable spectrophotometer, using as the blank a solvent mixture of the same composition as that used for the *Standard preparation*: the absorbance of the *Test preparation* does not exceed that of the *Standard preparation* (0.75%, calculated on the labeled aspirin content).

**Assay**—[NOTE—In this assay use chloroform recently saturated with water.]

**Standard preparation**—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix. The concentration of USP Aspirin RS is about 50 µg per mL.

**Chromatographic column**—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* (621)), packing a chromatographic tube with a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared sodium bicarbonate solution (1 in 12).

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in chloroform, dilute with the latter solvent to volume, and mix.

**Procedure**—Without delay, concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Delayed-Release Capsules

» Aspirin Delayed-Release Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the Capsules or the contents thereof are enteric-coated.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Heat about 100 mg of the Capsule contents with 10 mL of water for several minutes, cool, and add 1 drop of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—Prepare the test specimen as follows. Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

**Dissolution** (711)—Proceed as directed for *Procedure for Method A* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

*Apparatus 1:* 100 rpm.

*Time:* 90 minutes, for *Buffer stage*.

**Diluent**—Prepare a mixture of 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1), and adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ .

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved by determining UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*), using a filtered portion of the solution under test, diluted, if necessary, with 0.1 N hydrochloric acid (analyzing the *Acid stage*) and with *Diluent* (analyzing the *Buffer stage*), in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—

**Mobile phase and Diluting solution**—Prepare as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation*, prepared as directed in the Assay, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution* prepared as directed for *Assay preparation*.

**Chromatographic system**—Use the *Chromatographic system* described in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure* in the Assay: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the Assay. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Capsules taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken, as determined in the Assay; and  $r_U$  and  $r_S$  are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.



**Assay—**

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) in the portion of Capsules taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Suppositories

» Aspirin Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>).

**Packaging and storage**—Preserve in well-closed containers, in a cool place.

**USP Reference standards** (11)—  
USP Aspirin RS

**Identification**—Transfer a portion of the melted Suppositories obtained in the *Assay*, equivalent to about 1 g of aspirin, to a 125-mL conical flask. Add 20 mL of alcohol, and warm until completely disintegrated. Cool in an ice bath for 5 minutes, filter, and evaporate the filtrate to dryness: the residue responds to *Identification tests A and B* under *Aspirin*.

**Limit of free salicylic acid—**

**Ferric chloride-urea reagent**—To a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid add 60 g of urea. Dissolve the urea by swirling and without the aid of heat, and adjust the resulting solution, if necessary, by the addition of 6 N hydrochloric acid to a pH of 3.2. Prepare on the day of use.

**Procedure**—Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube,

and uniformly pack with a mixture of about 1 g of chromatographic siliceous earth and 0.5 mL of 5 M phosphoric acid. Directly above this layer, pack a similar mixture of about 3 g of chromatographic siliceous earth and 2 mL of *Ferric chloride-urea reagent*. Transfer to a small beaker an accurately weighed portion of the cooled mass from the previously melted Suppositories obtained in the *Assay*, equivalent to 50 mg of aspirin, add 10 mL of chloroform, warm slightly, and stir until dissolved. With the aid of 5 mL of chloroform, transfer the mixture to the chromatographic adsorption column. Pass 50 mL of chloroform in several portions through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. If the purple zone reaches the bottom of the tube, discard the column, and repeat the test with a smaller quantity of melted Suppositories.

Elute the adsorbed salicylic acid into a 100-mL volumetric flask containing 20 mL of methanol and 0.2 mL of hydrochloric acid by passing two 10-mL portions of a 1 in 10 solution of glacial acetic acid in water-saturated ether, and then 30 mL of chloroform, through the column, and dilute the eluate with chloroform to volume. Dissolve a suitable, accurately weighed quantity of salicylic acid in chloroform to obtain a *Standard solution* containing 150 µg of salicylic acid per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 10 mL of methanol, 0.1 mL of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether. Add chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, using as the blank a solvent mixture of the same composition as that of the *Standard solution*: the absorbance of the solution from the Suppositories does not exceed that of the *Standard solution* (3.0%).

**Assay**—[NOTE—In this assay, use chloroform that recently was saturated with water.]

**Chromatographic column**—Uniformly pack a chromatographic tube, as described in the test for *Limit of free salicylic acid* for *Procedure*, with a mixture of about 3 g of chromatographic siliceous earth and 2 mL of sodium bicarbonate solution (1 in 12) prepared on the day of use.

**Standard preparation**—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, and add chloroform to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix.

**Assay preparation**—Tare a small dish and glass rod, place in the dish not fewer than 5 Suppositories, heat gently on a steam bath until melted, then stir, cool while stirring, and weigh. Transfer an accurately weighed portion of the mass, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add 40 mL of chloroform, mix, and add chloroform to volume.

**Procedure**—Pipet 5 mL of the *Assay preparation* into the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Without delay, elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform, and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in chloroform, dilute with the latter solvent to volume, and mix. Without delay, concomitantly determine the absorbances of the eluted *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) in the portion of Suppositories taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the

absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Tablets

» Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ). Tablets of larger than 81-mg size contain no sweeteners or other flavors.

NOTE—Tablets that are enteric-coated meet the requirements for *Aspirin Delayed-Release Tablets*.

**Packaging and storage**—Preserve in tight containers. Preserve flavored or sweetened Tablets of 81-mg size or smaller in containers holding not more than 36 Tablets each.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—Prepare the test specimen as follows. Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

**Dissolution** (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—

**Mobile phase and Diluting solution**—Prepare as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the *Assay*, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution* prepared as directed for *Assay preparation* in the *Assay*.

**Chromatographic system**—Use the *Chromatographic system* described in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution,  $R$ , between salicylic

acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the *Assay*, and  $r_U$  and  $r_S$  are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.3% is found. In the case of Tablets that are coated, not more than 3.0% is found.

**Assay**—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Buffered Aspirin Tablets

» Buffered Aspirin Tablets contain Aspirin and suitable buffering agents. Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

## USP Salicylic Acid RS

**Identification—**

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—

*Test specimen*—Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of chloroform for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness.

**Dissolution** (711)—

*Medium:* 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

*Apparatus 2:* 75 rpm. [NOTE—Where the Tablet is composed of multiple layers, a stainless steel wire helix may be used, if needed, to hold the Tablet in proper orientation in the apparatus.]

*Time:* 30 minutes.

*Procedure*—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved by employing UV absorption at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of methanol not to exceed 1% of the total volume of the Standard solution may be used to dissolve the Reference Standard prior to dilution with *Medium*.]

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

**Limit of free salicylic acid—**

*Mobile phase* and *Diluting solution*—Prepare as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the Assay, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

*Test solution*—Use the *Stock solution*, prepared as directed for Assay preparation in the Assay.

*Chromatographic system*—Prepare as directed in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation determined from salicylic acid is not more than 4.0%.

*Procedure*—Proceed as directed in the Assay. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the Assay; and  $r_U$  and  $r_S$  are the peak responses of salicylic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

**Assay—**

*Mobile phase*—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

*Diluting solution*—Prepare a mixture of acetonitrile and formic acid (99:1).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for Limit of free salicylic acid.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of aspirin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Aspirin Delayed-Release Tablets**

» Aspirin Delayed-Release Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the Tablets are enteric-coated.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification—**

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—Prepare the test specimen as follows. Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

**Dissolution** (711)—Proceed as directed for *Procedure* for Method B under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

*Apparatus 1*: 100 rpm.

*Time*: 90 minutes, for *Buffer stage*.

**Diluent**—Prepare a mixture of 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1), and adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ .

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved by determining UV absorbances at the wavelength of the isobestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*), using a filtered portion of the solution under test, diluted, if necessary, with 0.1 N hydrochloric acid (analyzing the *Acid stage*) and with *Diluent* (analyzing the *Buffer stage*), in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*.

**Uniformity of dosage units** (905): meet the requirements.

#### Limit of free salicylic acid—

**Mobile phase and Diluting solution**—Prepare as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the *Assay*, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution*, prepared as directed for *Assay preparation* in the *Assay*.

**Chromatographic system**—Use the *Chromatographic system* described in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution, *R*, between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C/Q_A)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*; *Q<sub>A</sub>* is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the *Assay*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

#### Assay—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector

and a 4.0-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Effervescent Tablets for Oral Solution

» Aspirin Effervescent Tablets for Oral Solution contain Aspirin and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate and/or carbonate. Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aspirin RS

USP Salicylic Acid RS

#### Identification—

**A**: Dissolve 1 Tablet in about 50 mL of 1 N hydrochloric acid, boil for about 5 minutes, and allow to cool. To 2 mL of the resulting solution add 2 or 3 drops of ferric chloride TS: a violet-red color is produced.

**B**: Add about one-half a Tablet to 50 mL of water in a flask, and immediately stopper with a stopper fitted with tubing so that the evolved gas passes through calcium hydroxide TS: a white precipitate forms.

**Solution time**—Two Tablets dissolve completely in 180 mL of water at  $17.5 \pm 2.5^\circ$  within 5 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301): not less than 5.0 mEq of acid is consumed by 1 Tablet.

**Limit of free salicylate**—Proceed as directed for *Limit of free salicylic acid* under *Buffered Aspirin Tablets*: not more than 8.0% is found.

**Assay**—Proceed as directed in the *Assay* under *Buffered Aspirin Tablets*.

## Aspirin Extended-Release Tablets

» Aspirin Extended-Release Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The labeling indicates the *Dissolution Test* with which the product complies.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—Prepare the test specimen as follows. Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

**Dissolution** (711)—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 60 rpm.

**Times:** 1 hour and 4 hours.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the isosbestic point at about 280 nm, using filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium.

**Tolerances**—The percentages of the labeled amount of  $C_9H_8O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 20% and 55%
4	not less than 80%

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** water; 1000 mL.

**Apparatus 2:** 30 rpm.

**Times:** 1, 2, 4, and 8 hours.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the isosbestic point at about 265 nm, using filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 5% of the total volume of the Standard solution may be used to bring the USP Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—The percentages of the labeled amount of  $C_9H_8O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 15% and 40%
2	between 25% and 60%
4	between 35% and 75%
8	not less than 70%

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—

**Mobile phase** and **Diluting solution**—Prepare as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the Assay, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution*, prepared as directed for Assay preparation in the Assay.

**Chromatographic system**—Use the *Chromatographic system* described in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure* in the Assay: the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the Assay. The relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the Assay; and  $r_U$  and  $r_S$  are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

**Assay**—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin, Alumina, and Magnesia Tablets

» Aspirin, Alumina, and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ), the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [ $Al(OH)_3$ ], and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magnesium hydroxide [ $Mg(OH)_2$ ].

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for aspirin and limit of free salicylic acid* exhibits a major peak for aspirin, the retention time of which corresponds with that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay for aspirin and limit of free salicylic acid*.

**B:** To a 0.7-g portion of finely powdered Tablets add 20 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate so obtained responds to the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification test B* with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution so obtained responds to the tests for *Aluminum* (191).

**Dissolution** (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium. [NOTE—Prepare the Standard solution at the time of use. An amount of methanol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium hydroxide, and for *Content Uniformity* with respect to aspirin.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

**Assay for aspirin and limit of free salicylic acid**—

**Mobile phase**—Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir. [NOTE—The composition of the *Mobile phase* may be adjusted if necessary (see *System Suitability* under *Chromatography* (621)).]

**Solvent mixture**—To 2 g of anhydrous citric acid add 990 mL of acetonitrile, 990 mL of chloroform, and 20 mL of formic acid, and stir for about 30 minutes. Allow to settle, and decant the clear solution into a suitable container. Use the clear solution as the *Solvent mixture*.

**Internal standard solution**—Dissolve phenacetin in *Solvent mixture* to obtain a solution having a concentration of about 2 mg per mL.

**Salicylic acid stock standard solution**—Dissolve a suitable quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL.

**Standard preparation**—Transfer about 325 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask. Add 10.0 mL of *Salicylic acid stock standard solution* and 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Immediately transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture*, cap the bottle, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for salicylic acid, 0.6 for aspirin, and 1.0 for phenacetin. [NOTE—Record each chromatogram until the chloroform peak appears at a relative retention time of about 1.8; the resolution,  $R$ , between the salicylic acid, aspirin, and internal standard peaks is not less than 2.0; the tailing factor for any of these peaks is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of aspirin and phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C / a)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*;  $a$  is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

**Assay for aluminum hydroxide**—

**Edetate disodium titrant**—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL

volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

**Procedure**—Pipet 50 mL of *Assay preparation* into a 250-mL beaker, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 50 mL of water for the *Assay preparation*, and make any necessary corrections. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium hydroxide—

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Pipet a volume of *Assay preparation*, equivalent to about 80 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and mix. Add 50 mL of ammonia-ammonium chloride buffer TS and 2 drops of eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS until the color changes to pure blue. Perform a blank determination, substituting for the *Assay preparation*, a volume of water equal to the volume of *Assay preparation* used, and make any necessary corrections. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

## Aspirin, Alumina, and Magnesium Oxide Tablets

» Aspirin, Alumina, and Magnesium Oxide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ), the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [ $\text{Al}(\text{OH})_3$ ], and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magnesium oxide ( $\text{MgO}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Tablets respond to the *Identification* tests under *Aspirin, Alumina, and Magnesia Tablets*.

**B:** Where the Tablets are composed of two layers, scrape a small amount of each layer into separate test tubes. Add 2 mL of water and 2 drops of methyl red TS to each tube, and shake for about 15 seconds: the solution from the aspirin-containing layer is red, and the solution from the buffer-containing layer is yellow.

**Dissolution** (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 1** (10-mesh screen): 100 rpm.

**Time:** 45 minutes.

Determine the amount of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) dissolved employing the following method.

**Alkaline detergent solution**—Prepare a suitable mixture of 1 N sodium hydroxide and a 30% solution of polyoxyethylene (23) lauryl ether (1000:0.5).

**pH 4.3 Buffer detergent**—Dissolve 12.9 g of citric acid monohydrate and 20.6 g of dibasic sodium phosphate heptahydrate in water to make 1000 mL of solution. Add 0.5 mL of a 30% solution of polyoxyethylene (23) lauryl ether, and mix.

**Standard preparation**—Dissolve a suitable quantity of USP Aspirin RS, accurately weighed, in *Medium* to obtain a solution having a known concentration of about 0.45 mg per mL.

**Procedure**—Use an automatic analyzer consisting of (1) a liquid sampler; (2) a proportioning pump; (3) a suitable fluorometer equipped with a 0.4-cm flow cell and suitable recording devices; and (4) a manifold consisting of the components illustrated in the diagram in the chapter *Automated Methods of Analysis* (16). With the sample line pumping pH 4.3 Buffer detergent, the other lines pumping their respective reagents, the fluorometer set at an excitation wavelength of 298 nm and an emission wavelength of 425 nm, adjust the system until a steady fluorescence baseline has been achieved. Start the sampler, and conduct determinations at a rate of 40 per hour, using a ratio of about 5:1 for sample and wash time. Record the fluorescence values of the *Standard preparation* and the solution under test. Calculate the quantity, in mg, of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) dissolved by the formula:

$$900C(F_U / F_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $F_U$  and  $F_S$  are the fluorescence values of the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amount of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium oxide, and for *Content Uniformity* with respect to aspirin.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

**Assay for aspirin and limit of free salicylic acid**—

**Mobile phase**—Prepare a suitable mixture of water, methanol, and phosphoric acid (700:300:30). Filter and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Solvent mixture**—Mix 20 mL of hydrochloric acid and 2000 mL of dehydrated alcohol.

**Standard aspirin preparation**—Dissolve a suitable quantity of USP Aspirin RS, accurately weighed, by blending in a 120-mL blender jar at high speed for about 1.5 minutes with an accurately measured volume of *Solvent mixture* to obtain a stock solution having a known concentration of about 5 mg per mL. Immediately transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. This solution contains about 0.25 mg per mL. [NOTE—Use these solutions within 1 hour.]

**Standard salicylic acid preparation**—Dissolve a suitable quantity of USP Salicylic Acid RS in dehydrated alcohol to obtain a stock solution having a known concentration of about 5 mg per mL. Transfer 3.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. Transfer 5.0 mL of this intermediate stock solution to a second 100-mL volumetric flask, dilute with

dehydrated alcohol to volume, and mix. This solution contains about 7.5 µg per mL.

**Resolution solution**—Transfer 5.0 mL of the stock solution used to prepare the *Standard aspirin preparation* to a 100-mL volumetric flask, add 5.0 mL of the intermediate stock solution used to prepare the *Standard salicylic acid preparation*, dilute with dehydrated alcohol to volume, and mix.

**Assay preparation**—Transfer an accurately counted number of Tablets, equivalent to about 2500 mg of aspirin, to a 120-mL blender jar containing 100.0 mL of *Solvent mixture*, and blend at high speed for about 1.5 minutes. Immediately filter a portion of the mixture thus obtained, and transfer 1.0 mL of the filtrate to a 100-mL volumetric flask. Immediately dilute with dehydrated alcohol to volume, and mix. [NOTE—Promptly inject this *Assay preparation* into the chromatograph as directed for *Procedure*.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 3-cm column that contains 5-µm packing L7. The flow rate is about 3.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the aspirin peak and the salicylic acid peak is not less than 2. Chromatograph the *Standard aspirin preparation*, and record the responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Standard salicylic acid preparation*, and record the responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard aspirin preparation*, the *Standard salicylic acid preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for aspirin and 1.0 for salicylic acid. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in each Tablet taken by the formula:

$$(10,000C/N)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard aspirin preparation*;  $N$  is the number of Tablets taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the aspirin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$1000(C/a)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Salicylic Acid RS in the *Standard salicylic acid preparation*;  $a$  is the quantity, in mg, of aspirin in the number of Tablets taken to prepare the *Assay preparation*, based on the labeled amount; and  $r_U$  and  $r_S$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard salicylic acid preparation*, respectively: not more than 3.0% is found.

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the *Assay under Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

**Procedure**—Pipet 20 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Eдетate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary corrections. Each mL of 0.05 to 3.900 mg of  $Al(OH)_3$ .

#### Assay for magnesium oxide—

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Pipet a volume of *Assay preparation*, equivalent to about 40 mg of magnesium oxide, into a 400-mL beaker, and add, with mixing, 20 mL of triethanolamine and 200 mL of water. Cool the solution for 10 minutes, while stirring, by immersion of the beaker in an ice bath. Remove the beaker from the ice bath, and add 15 mL of ammonia-ammonium chloride buffer TS and 2 drops of eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Titrate with 0.05 M edetate disodium VS to a blue endpoint, allowing about 60 seconds between drops of titrant as the endpoint is approached (after first color change is observed). [NOTE—The titration should be completed within 10 minutes after the addition of the buffer and indicator. If any precipitate is observed prior to titration, the solution should be discarded and a new solution prepared.] Perform a blank determination, substituting for the *Assay preparation*, a volume of water equivalent to the volume of *Assay preparation* used, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.015 mg of MgO.

## Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules

» Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Aspirin RS  
USP Caffeine RS  
USP Dihydrocodeine Bitartrate RS  
USP Salicylic Acid RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

#### Dissolution, Procedure for a Pooled Sample <711>—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 50 rpm.

**Time:** 45 minutes.

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay and limit of salicylic acid*.



**Standard preparation**—Prepare a solution in *Medium* containing known concentrations of about 0.002A mg of USP Aspirin RS, 0.002C mg of USP Caffeine RS, and 0.002D mg of USP Dihydrocodeine Bitartrate RS per mL, A, C, and D being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule.

**Test preparation**—Filter a portion of the solution under test.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ) dissolved by the same formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the relevant analyte obtained from the *Test preparation* and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_9H_8O_4$ ,  $C_8H_{10}N_4O_2$ , and  $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$  are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay and limit of salicylic acid—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate and 2.3 g of monobasic ammonium phosphate in 850 mL of water. Add 150 mL of acetonitrile, mix, degas, and adjust with phosphoric acid to a pH of 2.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of water and acetonitrile (53:46), and adjust with phosphoric acid to a pH of 2.5.

**Standard preparation**—Prepare a solution in *Diluent* containing known concentrations of about 0.001A mg of USP Aspirin RS, 0.001C mg of USP Caffeine RS, and 0.001D mg of USP Dihydrocodeine Bitartrate RS per mL, A, C, and D being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule. [NOTE—Use this solution within 3 hours.]

**Standard salicylic acid preparation**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a solution having a known concentration of about 0.005A  $\mu$ g per mL, A being the labeled amount, in mg, of aspirin per Capsule. [NOTE—Use this solution within 3 hours.]

**Resolution solution**—Prepare a solution in *Standard preparation* containing about 0.0001A mg of USP Salicylic Acid RS per mL, A being the labeled amount, in mg, of aspirin in each Capsule. [NOTE—Use this solution within 3 hours.]

**Assay preparation**—Transfer the contents of 10 Capsules to a 500-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this mixture to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation*. [NOTE—Use this solution within 3 hours.]

**Chromatographic system**—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.2 for caffeine, 0.3 for dihydrocodeine, 0.7 for aspirin, and 1.0 for salicylic acid; and the resolution, R, between the caffeine and dihydrocodeine peaks is not less than 2.5, between the dihydrocodeine and aspirin peaks is not less than 1.0, and between the aspirin and salicylic acid peaks is not less than 1.5. Chromatograph the *Standard preparation*, and record

the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0% for each analyte.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Assay preparation*, the *Standard preparation*, and the *Standard salicylic acid preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ) in each Capsule taken by the same formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the corresponding analyte peaks of the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of salicylic acid in the Capsules taken by the formula:

$$100(C / A)(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Salicylic Acid RS in the *Standard salicylic acid preparation*; A is the labeled amount, in mg, of aspirin in each Capsule taken; and  $r_U$  and  $r_S$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard salicylic acid preparation*, respectively: not more than 3.0% is found.

## Aspirin and Codeine Phosphate Tablets

» Aspirin and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Aspirin RS  
USP Codeine Phosphate RS  
USP Salicylic Acid RS

**Identification**—Dissolve a suitable quantity of USP Aspirin RS in the *Solvent mixture* prepared as directed under *Assay for aspirin and codeine phosphate and limit of free salicylic acid* to obtain a *Standard aspirin solution* containing about 3.3 mg per mL. Dissolve a suitable quantity of USP Codeine Phosphate RS in the *Solvent mixture* to obtain a *Standard codeine phosphate solution* containing about 1 mg per mL. Chromatograph these solutions as directed for *Procedure* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*. The retention times of the major peaks in the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*, correspond to those in the chromatograms of the *Standard aspirin solution* and the *Standard codeine phosphate solution*, respectively.

#### Dissolution (711)—

**Medium**: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2**: 75 rpm.

**Time**: 30 minutes.

Determine the amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved by employing the following method.

*Mobile phase, Solvent mixture, and Aspirin and codeine phosphate standard preparation*—Prepare as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*.

*Internal standard solution*—Dissolve phenacetin in methanol to obtain a solution having a concentration of about 0.07 mg per mL.

*Standard solution A*—Prepare a solution of USP Aspirin RS in *Solvent mixture* having an accurately known concentration of about 0.36 mg per mL.

*Standard solution B*—Transfer about 12 mg of USP Codeine Phosphate RS and 25 mg of USP Salicylic Acid RS, each accurately weighed, to a 50-mL volumetric flask, add 2.5 mL of methanol, and mix. Add *Medium* to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, add *Medium* to volume, and mix.

*Standard preparations A and B*—Pipet 10 mL of *Standard solution A* and 10 mL of *Standard solution B* into separate containers, add 3.0 mL of the *Internal standard solution* to each container, and mix.

*Test preparation*—Withdraw a portion of the solution under test and filter, discarding the few mL of the filtrate. Pipet 10 mL of the filtrate and 3.0 mL of the *Internal standard solution* into a suitable container, and mix.

*Chromatographic system*—Proceed as directed for *Chromatographic system* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*, except to use only the *Aspirin and codeine phosphate preparation* for evaluation of the suitability of the system.

*Procedure*—Proceed as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*, except to inject about 50  $\mu$ L of the *Standard preparations* and the *Test preparation*. The relative retention times are 0.3 for salicylic acid, 0.6 for aspirin, 0.8 for codeine phosphate, and 1.0 for phenacetin. Calculate the amount of codeine phosphate dissolved by comparison of the relative peak response ratios for the codeine phosphate peaks, obtained from *Standard preparation B* and the *Test preparation*. Calculate the percentage of aspirin dissolved by the formula:

$$[0.9C(R_U / R_S) + 0.9C'(R'_U / R'_S)(180.16 / 138.12)] / 3.25$$

in which C is the concentration, in  $\mu$ g per mL, of USP Aspirin RS in *Standard solution A*;  $R_U$  and  $R_S$  are the peak response ratios for the aspirin component obtained from the *Test preparation* and *Standard preparation A*, respectively;  $C'$  is the concentration, in  $\mu$ g per mL, of USP Salicylic Acid RS in *Standard solution B*;  $R'_U$  and  $R'_S$  are the peak response ratios for the salicylic acid component obtained from the *Test preparation* and *Standard preparation B*, respectively; and 180.16 and 138.12 are the molecular weights of aspirin and salicylic acid, respectively.

*Tolerances*—Not less than 75% (Q) of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to aspirin and codeine phosphate.

#### **Assay for aspirin and codeine phosphate and limit of free salicylic acid—**

*Mobile phase*—Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir. Pass through a membrane filter, and degas. [NOTE—The amounts of sodium 1-octanesulfonate, methanol, and acetonitrile may be varied to obtain acceptable chromatography.]

*Solvent mixture*—To 15 g of anhydrous citric acid add 200 mL of methanol and 20 mL of glacial acetic acid, dilute with chloroform to 1000 mL, and mix until the citric acid is dissolved.

*Internal standard solution*—Dissolve phenacetin in *Solvent mixture* to obtain a solution having a concentration of about 2 mg per mL.

*Salicylic acid stock standard solution*—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture*, and quantitatively dilute with *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL.

*Salicylic acid standard preparation*—Transfer 5.0 mL of *Salicylic acid stock standard solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

*Codeine phosphate stock standard solution*—Transfer about 325J mg of USP Codeine Phosphate RS, accurately weighed, to a 25-mL volumetric flask, J being the ratio of the labeled amount, in mg, of codeine phosphate to the labeled amount, in mg, of aspirin per Tablet. Dissolve in and dilute with *Solvent mixture* to volume, and mix.

*Aspirin and codeine phosphate standard preparation*—Transfer about 65 mg of USP Aspirin RS, accurately weighed, to a 10-mL volumetric flask. Add 5.0 mL of *Codeine phosphate stock standard solution*, 1.0 mL of *Salicylic acid stock standard solution*, and 1.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture*, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation*. Use on the day prepared.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the *Salicylic acid standard preparation* and the *Aspirin and codeine phosphate standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively; the resolution, R, between salicylic acid and aspirin, between aspirin and codeine, and between codeine and phenacetin is not less than 2.0; the tailing factor for each analyte peak is not more than 2.0; and the relative standard deviation of the ratios of the peak responses of salicylic acid, aspirin, and codeine to the peak response of phenacetin is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 5  $\mu$ L) of the *Salicylic acid standard preparation*, *Aspirin and codeine phosphate standard preparation*, and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Aspirin and codeine phosphate standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of aspirin and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U / R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; C is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Aspirin and codeine*

phosphate standard preparation; and  $R_U$  and  $R_S$  are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the Assay preparation and the Aspirin and codeine phosphate standard preparation, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the Salicylic acid standard preparation;  $a$  is the quantity, in mg, of aspirin in the portion of powdered Tablets taken, based on the labeled amount; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the Assay preparation and the Salicylic acid standard preparation, respectively: not more than 3.0% is found.

### Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets

» Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ), codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ), aluminum hydroxide [ $Al(OH)_3$ ], and magnesium hydroxide [ $Mg(OH)_2$ ].

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Aspirin RS  
USP Codeine Phosphate RS  
USP Salicylic Acid RS

#### Identification—

**A:** Tablets respond to the Identification test under Aspirin and Codeine Phosphate Tablets.

**B:** Tablets respond to the Identification tests under Alumina and Magnesia Tablets.

#### Dissolution (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 30 minutes.

**Mobile phase, Internal standard solution, Solvent mixture, Aspirin and codeine phosphate standard preparation, Standard solution A, Standard solution B, Standard preparations A and B, Test preparation, Chromatographic system, and Procedure**—Proceed as directed in the test for Dissolution under Aspirin and Codeine Phosphate Tablets.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) are dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements for Content Uniformity with respect to aspirin and codeine phosphate and for Weight Variation with respect to aluminum hydroxide and magnesium hydroxide.

**Acid-neutralizing capacity (301):** not less than 1.9 mEq per Tablet.

#### Assay for aspirin and codeine phosphate and limit of free salicylic acid—

**Mobile phase, Solvent mixture, Salicylic acid stock standard solution, Salicylic acid standard preparation, Aspirin and co-**

**deine phosphate standard preparation, and Chromatographic system**—Prepare as directed in the Assay for aspirin and codeine phosphate and limit of free salicylic acid under Aspirin and Codeine Phosphate Tablets.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of Internal standard solution and 45.0 mL of Solvent mixture, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the Assay preparation.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the Salicylic acid standard preparation, the Aspirin and codeine phosphate standard preparation, and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of powdered Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the Aspirin and codeine phosphate standard preparation; and  $R_U$  and  $R_S$  are the ratios of the peak responses of aspirin and phenacetin obtained from the Assay preparation and the Aspirin and codeine phosphate standard preparation, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ), in the portion of powdered Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U/R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the Aspirin and codeine phosphate standard preparation; and  $R_U$  and  $R_S$  are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the Assay preparation and the Aspirin and codeine phosphate Standard preparation, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the Salicylic acid standard preparation;  $a$  is the quantity, in mg, of aspirin in the portion of Tablets taken, determined as directed above; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the Assay preparation and the Salicylic acid standard preparation, respectively: not more than 3.0% is found.

#### Assay for aluminum hydroxide—

**Edetate disodium titrant**—Prepare and standardize as directed in the Assay under Ammonium Alum.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of Assay preparation into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of Edetate disodium titrant and 20 mL of acetic acid-ammonium acetate buffer TS. Add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the Assay prepa-

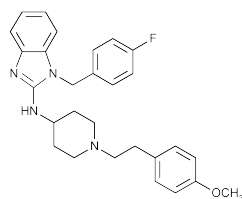
ration, and make any necessary correction. Each mL of 0.05 M Edetate disodium titrant is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

#### Assay for magnesium hydroxide—

**Assay preparation**—Prepare as directed in the Assay for aluminum oxide.

**Procedure**—Pipet a volume of Assay preparation, equivalent to about 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the Assay preparation, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

## Astemizole



$\text{C}_{28}\text{H}_{31}\text{FN}_4\text{O}$  458.57

1*H*-Benzimidazol-2-amine, 1-[(4-fluorophenyl)methyl]-*N*-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-1-(*p*-Fluorobenzyl)-2-[[1-(*p*-methoxyphenethyl)-4-piperidyl]amino]benzimidazole [68844-77-9].

» Astemizole contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{28}\text{H}_{31}\text{FN}_4\text{O}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Astemizole RS

**Identification, Infrared Absorption** (197K).

**Melting range** (741): between 175° and 178°.

**Loss on drying** (731)—Dry it at 105° in vacuum for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

#### Chromatographic purity—

**Solution A**—Prepare a filtered and degassed solution in water containing 17 g of tetrabutylammonium hydrogen sulfate per L. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Solution B**—Use filtered and degassed acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Mobile phase**—Use variable mixtures of Solution A and Solution B as directed for *Chromatographic system*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Astemizole RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 25 µg per mL.

**Resolution solution**—Dissolve an accurately weighed quantity of USP Astemizole RS and ketoconazole in methanol,

and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 25 µg and 250 µg per mL, respectively.

**Test solution**—Transfer about 100 mg of Astemizole, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 10-cm column that contains base-deactivated 3-µm packing L1. The flow rate is about 1 mL per minute. Equilibrate the system with acetonitrile and then with 95% Solution A and 5% Solution B, and hold at that composition for 5 minutes prior to injection. After injection, linearly change the composition to 80% Solution A and 20% Solution B over a period of 15 minutes. Maintain this composition for an additional 3 minutes. Purge the column with 100% Solution B for 5 minutes, and then equilibrate the system to the initial composition for 5 minutes prior to the following injection. Chromatograph the Resolution solution, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the astemizole and ketoconazole peaks is not less than 1.5.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Astemizole taken by the formula:

$$0.25(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the peak response of the Standard solution: not more than 0.25% of any individual impurity is found, and not more than 0.5% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, 0.13 M ammonium acetate, acetonitrile, and diethylamine (470:300:230:1.0), and adjust with glacial acetic acid to a pH of 7.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Astemizole RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 1.0 mg per mL.

**Assay preparation**—Transfer about 50 mg of Astemizole, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $\text{C}_{28}\text{H}_{31}\text{FN}_4\text{O}$  in the portion of Astemizole taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Astemizole RS in the Standard preparation; and  $r_U$  and  $r_S$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Astemizole Tablets

» Astemizole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of astemizole ( $C_{28}H_{31}FN_4O$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Astemizole RS

**Identification**—Transfer an amount of finely ground Tablets, equivalent to 100 mg of Astemizole, to a 100-mL volumetric flask, add methanol to volume, mix, and filter. Prepare a Standard solution of USP Astemizole RS in methanol having a concentration of 1 mg per mL. Separately apply 10  $\mu$ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, dioxane, methanol, and ammonium hydroxide (60:30:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, air-dry, and examine under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Dissolution** (711)—

*Medium*: simulated gastric fluid TS (without the enzyme); 800 mL.

*Apparatus 2*: 100 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of astemizole ( $C_{28}H_{31}FN_4O$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 285 nm of filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Astemizole RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of astemizole ( $C_{28}H_{31}FN_4O$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Chromatographic purity**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Astemizole.

*Test solution*—Use the Assay preparation.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 0.25% of any individual impurity is found; and the sum of all impurities is not more than 1.0%.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Astemizole.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of astemizole, to a 50-mL volumetric flask. Add 25 mL of *Mobile phase*, mix for 30 minutes, dilute with *Mobile phase* to volume, and centrifuge. Use the supernatant as the *Assay preparation*.

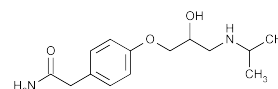
*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in mg, of astemizole ( $C_{28}H_{31}FN_4O$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_s)$$

in which C is the concentration, in mg per mL, of USP Astemizole RS in the *Standard preparation*; and  $r_U$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atenolol



$C_{14}H_{22}N_2O_3$  266.34  
Benzeneacetamide, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-; 2-[p-[2-Hydroxy-3-(isopropylamino)propoxy]-phenyl]-acetamide [29122-68-7].

### DEFINITION

Atenolol contains NLT 98.0% and NMT 102.0% of  $C_{14}H_{22}N_2O_3$ , calculated on the dried basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

*Sample solution*: 20  $\mu$ g/mL in methanol

### ASSAY

#### • PROCEDURE

**Mobile phase**: 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Degas this solution before use.

**Standard solution**: 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample solution**: 0.01 mg/mL of Atenolol in *Mobile phase*. Sonicate for 5 min for complete dissolution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 226 nm

**Column**: 3.9-mm  $\times$  30-cm; packing L1

**Flow rate**: 0.6 mL/min

**Injection size**: 10  $\mu$ L

#### System suitability

**Sample**: *Standard solution*

#### Suitability requirements

**Column efficiency**: NLT 5000 theoretical plates

**Tailing factor**: NMT 2.0

**Relative standard deviation**: NMT 2.0%

#### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{22}N_2O_3$  in the portion of Atenolol taken:

$$\text{Result} = (r_U / r_s) \times (C_s / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **CHLORIDE AND SULFATE**, *Chloride* (221)

Sample solution: Dissolve 1.0-g in 100 mL of 0.15 N nitric acid.

Acceptance criteria: Shows no more turbidity with 1 mL of silver nitrate TS than 1.4 mL of 0.020 N hydrochloric acid in 100 mL of 0.15 N nitric acid (0.1%)

#### Organic Impurities

##### • PROCEDURE

Mobile phase: Prepare as directed in the Assay.

Sample solution 1: 0.1 mg/mL of Atenolol in *Mobile phase*

Sample solution 2: 0.5 µg/mL of Atenolol, from *Sample solution 1* in *Mobile phase*

Chromatographic system: Proceed as directed in the Assay, except use the injection size listed below.

Injection size: 50 µL

#### Analysis

Samples: *Sample solution 1* and *Sample solution 2*

[NOTE—Chromatograph *Sample solution 1* for a period of time that is 6 times the retention time of the atenolol peak.]

Calculate the percentage of each impurity in *Sample solution 1*:

$$\text{Result} = 0.5(r_U/r_A)$$

$r_U$  = peak response of any individual impurity in *Sample solution 1*

$r_A$  = peak response of Atenolol in *Sample solution 2*

#### Acceptance criteria

Individual impurities: NMT 0.25%

Total impurities: NMT 0.5%

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* (741):

152°–156.5°

- **LOSS ON DRYING** (731): Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Atenolol RS

chromatogram of the *Standard preparation*, obtained as directed in the Assay.

**B**: *Ultraviolet Absorption* (197U)—

*Solution*: 10 µg of atenolol per mL.

*Medium*: methanol.

**Bacterial endotoxins** (85)—It contains not more than 33.3 USP Endotoxin Units per mg of atenolol.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.5 and 6.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

#### Assay—

*Citric acid buffer*—Transfer 2.5 g of citric acid to a 500-mL volumetric flask, add 400 mL of water, and swirl to dissolve. Adjust the solution with 2 N sodium hydroxide to a pH of 6.0, dilute with water to volume, and mix.

*Mobile phase*—Dissolve 930 mg of sodium octyl sulfate in 740 mL of water, add 8 mL of 3.6 N sulfuric acid, mix, and pass through a 1-µm or finer porosity filter. To the filtrate add 250 mL of acetonitrile, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 50 mg of USP Atenolol RS to a 100-mL volumetric flask, add 80 mL of *Citric acid buffer*, and sonicate for about 30 seconds to achieve dissolution. Dilute with *Citric acid buffer* to volume, and mix. Transfer 4.0 mL of this solution to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix. This solution contains about 0.2 mg of USP Atenolol RS per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to 2 mg of atenolol, to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{22}N_2O_3$  in each mL of the Injection taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Atenolol RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the atenolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atenolol Injection

» Atenolol Injection is a sterile solution of Atenolol in Water for Injection. It contains a suitable buffering agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a cool place or at controlled room temperature, protected from light. Avoid freezing.

**USP Reference standards** (11)—

USP Atenolol RS

USP Endotoxin RS

#### Identification—

**A**: The retention time of the main peak in the chromatogram of the *Assay preparation* corresponds to that in the

## Atenolol Oral Solution

### DEFINITION

Atenolol Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{14}H_{22}N_2O_3$ .

Prepare Atenolol Oral Solution at a 0.2% concentration, for example, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Atenolol	200 mg
Glycerin	5 mL
Vehicle for Oral Suspension	45 mL
Vehicle for Oral Solution, Sugar Free, a sufficient quantity to make	100 mL

Calculate the quantity of each ingredient required for the total volume and atenolol strength to be prepared. Mix the *Atenolol*, previously pulverized, and *Glycerin* to form a smooth paste. Incorporate the *Vehicle for Oral Suspension* or an equal volume of *Vehicle for Oral Solution, Sugar Free*. [NOTE—The *Vehicle for Oral Suspension* may be omitted.] Incorporate sufficient *Vehicle for Oral Solution, Sugar Free* in increments to bring to volume, and mix well. [NOTE—Do not use a sucrose-containing vehicle for oral solution.] Package, and label.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in amber, tight containers, and store at controlled room temperature.
- **LABELING:** Label it to state that it is to be shaken well before use, and discarded after 60 days. Label it to state that it is to be kept out of reach of children. Label it to indicate the nominal atenolol concentration.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded

## Atenolol Tablets

#### DEFINITION

Atenolol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>  
**Sample:** Mix a quantity of powdered Tablets, equivalent to 100 mg of atenolol, with 15 mL of methanol, heat the mixture to 50°, and shake for 5 min. Filter, and evaporate the filtrate on a water bath to dryness. Add 10 mL of 0.1 N hydrochloric acid to the residue, warm the solution, shake, and filter. To the filtrate add sufficient 1 N sodium hydroxide to make it alkaline, and extract the solution with 10 mL of chloroform, drying the chloroform extract over anhydrous sodium sulfate. Filter the dried chloroform solution, evaporate the filtrate on a water bath to dryness, and dry the residue at 105° for 1 h.
- **B.** The retention time of the atenolol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Mobile phase:** 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, and pass through a filter having a 0.5-μm or finer porosity. Degas this solution before use.  
**Standard solution:** 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample stock solution:** Transfer 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Mobile phase*, and sonicate for 15 min to disintegrate the Tablets. Dilute with *Mobile phase* to volume.

**Sample solution:** Centrifuge a portion of the *Sample stock solution*, and dilute a volume of the supernatant with *Mobile phase* to obtain a solution nominally containing 0.01 mg/mL of atenolol.

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 0.6 mL/min

**Injection size:** 10 μL

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 5000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{22}N_2O_3$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atenolol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION <711>

**Medium:** 0.1 N acetate buffer, pH 4.6 (prepared by mixing 44.9 parts (v/v) of 0.1 N sodium acetate with 55.1 parts (v/v) of 0.1 N acetic acid solution, and adjust with either diluted sodium hydroxide or diluted acetic acid to a pH of 4.6); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of  $C_{14}H_{22}N_2O_3$  dissolved by using the following method.

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay* under Atenolol.

**Standard solution:** 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample solution:** Pass a portion of the solution under test through a suitable 0.45-μm filter. Quantitatively dilute a measured volume of the filtrate with *Mobile phase* to obtain a solution estimated to contain about 0.01 mg/mL of atenolol.

**Analysis:** Proceed as directed in the *Assay*.

Calculate the percentage of  $C_{14}H_{22}N_2O_3$  dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (100/L)$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor for the *Sample solution*

$L$  = Tablet label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{14}H_{22}N_2O_3$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** <11>

USP Atenolol RS

## Atenolol and Chlorthalidone Tablets

» Atenolol and Chlorthalidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ).

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Atenolol RS

USP Chlorthalidone RS

### Identification—

**A:** Shake a quantity of powdered Tablets, equivalent to about 50 mg of chlorthalidone, with 5 mL of methanol for 15 minutes, and filter. Apply 10  $\mu$ L of this test solution, 10  $\mu$ L of a Standard solution of USP Chlorthalidone RS in methanol containing 10 mg per mL, and 10  $\mu$ L of a second Standard solution of USP Atenolol RS in methanol containing 10 mg per mL,  $J$  being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-butyl alcohol and 1 N ammonium hydroxide (5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light: the principal spots obtained from the test solution correspond in  $R_f$  value, size, and intensity to those obtained from the respective Standard solutions.

**B:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

Determine the amounts of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) dissolved by employing the following method.

*Mobile phase and Chromatographic system*—Prepare as directed in the *Assay*.

*Diluent*—Prepare a mixture of 1000 mL of acetonitrile and 32 mL of 3.6 N sulfuric acid.

*Standard solvent*—Prepare a mixture of water and *Diluent* (750: 225).

*Standard solution*—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in *Standard solvent* to obtain a solution having known concentrations of about 0.00085L mg of USP Atenolol RS and 0.00085L' mg of USP Chlorthalidone RS per mL,  $L$  and  $L'$  being the labeled amounts, in mg, of atenolol and chlorthalidone, respectively, per Tablet.

*Test solution*—Mix 10.0 mL of the filtered solution under test and 3.0 mL of *Diluent*.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of

atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) dissolved by the same formula:

$$1170C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard solution*; and  $r_U$  and  $r_S$  are the responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution*, respectively.

*Tolerances*—Not less than 80% ( $Q$ ) of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ) is dissolved in 45 minutes, and not less than 70% ( $Q$ ) of the labeled amount of chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Proceed as directed in the *Assay*, except to prepare the *Assay preparation* as follows. Transfer 1 Tablet to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.25 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablet. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*. Calculate the quantities, in mg, of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in the Tablet taken by the formula:

$$CV(r_U / r_S)$$

in which  $V$  is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation*; and the other terms are as defined in the *Assay*.

### Assay—

*Mobile phase*—Prepare a mixture of 740 mL of water, 250 mL of acetonitrile, 8 mL of 3.6 N sulfuric acid, and 930 mg of sodium octyl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in a mixture of water and acetonitrile (3:1) to obtain a solution having known concentrations of about 0.25 mg of USP Chlorthalidone RS and 0.25J mg of USP Atenolol RS per mL,  $J$  being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet.

*Assay preparation*—Transfer 10 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.5 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablets. Dilute with a mixture of water and acetonitrile (1:1) to volume, and mix. Pass a portion of this stock solution through a filter having a 0.5- $\mu$ m or finer porosity. Transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone; the resolution,  $R$ , between the atenolol and chlorthalidone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities,



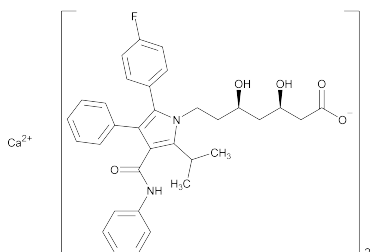
in mg, of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in each Tablet taken by the formula:

$$2C(V/10)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*;  $V$  is the volume, in mL, of the volumetric flask used to prepare the stock solution for the *Assay preparation*; and  $r_U$  and  $r_S$  are the responses for the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

NOTE—If a trailing peak or shoulder is observed on the chlorthalidone peak with a relative retention time of not more than 1.1 in the chromatograms of both the *Standard preparation* and the *Assay preparation*, sum the areas for the chlorthalidone peak with the trailing peak or shoulder to report the peak responses for chlorthalidone.

## Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$  1209.42

$C_{66}H_{68}CaF_2N_4O_{10}$  1155.34

1*H*-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)- $\beta,\delta$ -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-, calcium salt (2:1), trihydrate [ $R$ -( $R^*$ , $R^*$ )]-; Calcium ( $\beta R, \delta R$ )-2-( $p$ -fluorophenyl)- $\beta,\delta$ -dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoate (1:2), trihydrate [344423-98-9].  
Anhydrous [134523-03-8].

### DEFINITION

Atorvastatin Calcium contains NLT 98.0% and NMT 102.0% of  $C_{66}H_{68}CaF_2N_4O_{10}$ , calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

#### • B. CALCIUM

**Diluent:** Methanol, water, and hydrochloric acid (75:25:2)

**Blank:** *Diluent*

**Sample solution:** 0.05 mg/mL of Atorvastatin Calcium in *Diluent*

#### Analysis

**Samples:** *Sample solution* and *Blank*

#### Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** Calcium emission line at 422.7 nm

**Flame:** Air-acetylene

**Acceptance criteria:** The *Sample solution* exhibits a significant absorption at the calcium emission line at 422.7 nm.

### ASSAY

#### • PROCEDURE

**Buffer:** 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of  $5.0 \pm 0.1$ .

**Solution A:** Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (21:12:67)

**Solution B:** Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (61:12:27)

**Diluent:** *N,N*-dimethylformamide

**Mobile phase:** See the gradient table below.

[NOTE—If necessary, adjust the *Mobile phase* by increasing or decreasing the percentage of acetonitrile or the pH of the ammonium acetate solution to achieve a retention time of 26–34 min for the atorvastatin peak.]

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	100	0
70	20	80
85	0	100
100	0	100
105	100	0
115	100	0

**System suitability solution:** 0.05 mg/mL of USP Atorvastatin Calcium RS and 0.06 mg/mL of USP Atorvastatin Related Compound B RS in *Diluent*

**Standard solution:** 0.4 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. [NOTE—Use sonication if necessary.]

**Sample solution:** 0.4 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[NOTE—If significant fronting of the peaks for atorvastatin related compound B and atorvastatin is observed, use the following *Diluent* to prepare the *Sample solution*, *Standard solution*, and *System suitability solution*: acetonitrile, stabilizer-free tetrahydrofuran, and water (1:1:2).]

**Mode:** LC

**Detector:** UV 244 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin, *System suitability solution*

**Tailing factor:** NMT 1.6, *Standard solution*

**Relative standard deviation:** NMT 0.6%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{66}H_{68}CaF_2N_4O_{10}$  in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities****• HEAVY METALS****Diluent:** Methanol and water (9:1)**Sample solution:** Dissolve 250 mg of the sample in 30 mL of *Diluent*.**Standard lead solution:** Prepared as directed under *Heavy Metals* (231).**Reference solution:** Dilute 0.5 mL of the *Standard lead solution* with *Diluent* to 30 mL.**Blank solution:** 20 mL of *Diluent***Monitor solution:** Dissolve 250 mg of Atorvastatin Calcium in 0.5 mL of the *Standard lead solution*, and dilute with *Diluent* to 30 mL.**Analysis****Samples:** *Sample solution*, *Reference solution*, *Blank solution*, and *Monitor solution*

To each solution, add 2 mL of pH 3.5 Acetate Buffer, prepared as directed under *Heavy Metals* (231). Mix, add to 1.2 mL of thioacetamide–glycerin base TS, and mix immediately. Pass the solutions through a membrane filter of 0.45-μm pore size. Compare the spots on the filters obtained with the different solutions: the brown color of the spot from the *Sample solution* is not more intense than that of the spot from the *Reference solution*. The test is invalid if the *Reference solution* does not show a slight brown color compared to the *Blank solution*, or if the color of the *Monitor solution* is not at least as intense as the color of the *Reference solution*.

**Acceptance criteria:** NMT 20 ppm**Organic Impurities****• PROCEDURE****Buffer, Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed for the *Assay*.**Standard solution:** 1.5 μg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound D RS in *Diluent***Sample solution:** 1 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]**Analysis****Samples:** *Standard solution* and *Sample solution*  
Chromatograph the *Standard solution*, and identify the components on the basis of their relative retention times, given in *Impurity Table 1*.

Calculate the percentage of each of the atorvastatin related compounds A, B, C, and D in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of the relevant atorvastatin related compound from the *Sample solution* $r_S$  = peak response of the relevant atorvastatin related compound from the *Standard solution* $C_S$  = concentration of the relevant atorvastatin related compound in the *Standard solution* (mg/mL) $C_U$  = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of any other individual impurity from the *Sample solution* $r_T$  = sum of the responses of all the peaks from the *Sample solution*

[NOTE—Disregard any peak observed in the blank; the reporting level for impurities is 0.05%.]

**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 1.0%. [NOTE—This total does not include atorvastatin related compound E, as determined in the test for *Enantiomeric Purity*.]**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atorvastatin related compound A <sup>a</sup>	0.8	0.3
Atorvastatin related compound B <sup>b</sup>	0.9	0.3
Atorvastatin	1.0	n/a
Atorvastatin related compound C <sup>c</sup>	1.2	0.3
Atorvastatin related compound D <sup>d, e</sup>	2.1	0.1
Any other individual impurity	—	0.1

<sup>a</sup> Desfluoro impurity.<sup>b</sup> 3S,5R isomer.<sup>c</sup> Difluoro impurity.<sup>d</sup> Epoxide impurity.<sup>e</sup> Atorvastatin related compound D may undergo a transformation equilibrium with its cyclic hemiketal form. The cyclic hemiketal of atorvastatin related compound D elutes about 1–2 min before atorvastatin related compound D. Use the sum of the areas of the two peaks as a peak response for atorvastatin related compound D in the *Standard solution* and the *Sample solution*.**SPECIFIC TESTS****• ENANTIOMERIC PURITY****Mobile phase:** Hexane, dehydrated alcohol, and trifluoroacetic acid (940:60:1)**System suitability stock solution:** 5 mg/mL of USP Atorvastatin Calcium RS and 37.5 μg/mL of USP Atorvastatin Related Compound E RS in methanol. [NOTE—Atorvastatin related compound E is the 3S,5S enantiomer of atorvastatin.]**System suitability solution:** Transfer 2.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.**Sample solution:** Transfer 10 mg of Atorvastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 244 nm**Column:** 4.6-mm × 25-cm; packing L51**Flow rate:** 1.0 mL/min**Injection size:** 20 μL**System suitability****Samples:** *System suitability solution*

[NOTE—The elution order of the peaks is atorvastatin related compound E followed by atorvastatin.]

**Resolution:** NLT 2.0 between the peaks for atorvastatin related compound E and atorvastatin**Analysis****Samples:** *Sample solution*

Calculate the percentage of atorvastatin related compound E in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

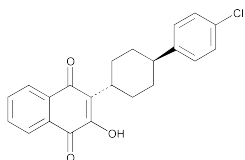
 $r_U$  = peak response for atorvastatin related compound E $r_T$  = sum of the responses of the peaks for atorvastatin related compound E and atorvastatin**Acceptance criteria:** NMT 0.3% of atorvastatin related compound E

- **WATER DETERMINATION, Method Ia** (921): 3.5%–5.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Atorvastatin Calcium RS
  - USP Atorvastatin Related Compound A RS  
Desfluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.  
 $C_{66}H_{70}CaN_4O_{10}$  1119.38
  - USP Atorvastatin Related Compound B RS  
3*S*,5*R* Isomer, or (3*S*,5*R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.  
 $C_{66}H_{68}CaF_2N_4O_{10}$  1155.34
  - USP Atorvastatin Related Compound C RS  
Difluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.  
 $C_{66}H_{66}F_4N_4O_{10}$  1191.34
  - USP Atorvastatin Related Compound D RS  
Epoxide impurity, or 3-(4-fluorobenzoyl)-2-isobutryl-3-phenyl-oxirane-2-carboxylic acid phenylamide.  
 $C_{26}H_{22}FNO_4$  431.46
  - USP Atorvastatin Related Compound E RS  
3*S*,5*S* Enantiomer, or (3*S*,5*S*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.  
 $C_{66}H_{68}CaF_2N_4O_{10}$  1155.34

## Atovaquone



$C_{22}H_{19}ClO_3$  366.84  
1,4-Naphthalenedione, 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-, *trans*-.  
2-[*trans*-4-(*p*-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone [95233-18-4].

» Atovaquone contains not less than 97.5 percent and not more than 101.5 percent of  $C_{22}H_{19}ClO_3$ , calculated on the anhydrous and organic solvent-free basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Atovaquone RS  
USP Atovaquone Related Compound A RS  
*cis*-2[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.

#### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

#### Heavy metals—

**Test preparation**—Thoroughly mix 1.0 g of Atovaquone with 0.5 g of magnesium oxide in a silica crucible. Ignite to dull redness until a homogeneous white or grayish-white mass is obtained. If the mixture remains colored after 30 minutes, allow to cool, mix using a fine glass rod, and repeat the ignition. If necessary, repeat the operation. Heat the residue at 800° for about 1 hour. Cool, take up the residue in two 5-mL portions of 6 N hydrochloric acid, add 0.1 mL of phenolphthalein TS, and then add 13.5 N ammonium hydroxide until a pink color is obtained. Cool, add glacial acetic acid until the solution is decolorized, and add 0.5 mL in excess. Filter, if necessary, and wash the filter with water. Dilute with water to 20 mL.

**Standard preparation**—Add 1.0 mL of *Standard Lead Solution* (see *Special Reagents* under *Heavy Metals* (231)) to 0.5 g of magnesium oxide, and dry between 100° and 105°. Proceed as directed for *Test preparation*, starting with “Ignite to dull redness”.

**Blank preparation**—Proceed as directed for *Test preparation*, omitting the Atovaquone.

**Procedure**—Transfer 12.0 mL of the *Test preparation* to a 50-mL color-comparison tube, 10.0 mL of the *Standard preparation* to another, and 10.0 mL of the *Blank preparation* to a third. Then add 2.0 mL of the *Test preparation* to the *Standard preparation* as well as to the *Blank preparation*. Add 2 mL of pH 3.5 Acetate Buffer (see *Heavy Metals* (231)) to each of the three tubes, mix, add 1.2 mL of thioacetamide–glycerin base TS, and mix. Allow to stand for 2 minutes, and view downward over a white surface: the solution from the *Standard preparation* is slightly brown when compared with the solution from the *Blank preparation*, and the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (10 µg per g).

#### Limit of residual organic solvents—

**Standard solution**—Transfer 1.0 mL of methanol and 1.0 mL of glacial acetic acid to a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

**Test solution**—Transfer about 100 mg of Atovaquone, accurately weighed, to a 2-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 2.8-m column that contains 10% liquid phase G16 on support S2. The carrier gas is nitrogen, flowing at a rate of about 42.5 mL per minute. The column temperature is maintained at about 180° and the detector block temperature is maintained at about 250°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for methanol and 1.0 for acetic acid; the resolution, *R*, between methanol and acetic acid is not less than 14; the column efficiency calculated from the acetic acid peak is not less than 700; and the tailing factor for acetic acid is not less than 0.8.

**Procedure**—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for methanol and acetic acid. Calculate the percentage, by weight, of methanol and acetic acid in the portion of Atovaquone taken by the formula:

$$0.1(G/W)(r_U / r_S)$$

in which *G* is either 0.79, the specific gravity of methanol, or 1.05, the specific gravity of glacial acetic acid, as appropriate; *W* is the weight, in mg, of Atovaquone taken to pre-

pare the *Test solution*; and  $r_U$  and  $r_S$  are the peak area responses of methanol or acetic acid, as appropriate, obtained from the *Test solution* and the *Standard solution*, respectively; not more than 0.2% of methanol or of acetic acid is found.

**Related compounds**—Using the chromatograms of the *Assay preparation* and the *Resolution solution* obtained in the *Assay*, calculate the percentage of atovaquone related compounds in the portion of Atovaquone taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the individual peak response of a related compound, if any, in the chromatogram of the *Assay preparation*; and  $r_S$  is the sum of the responses of all the peaks in the chromatogram of the *Assay preparation*, including the atovaquone peak. Not more than 1.0% of any related compound with a retention time corresponding to that of atovaquone related compound A, as determined from the chromatogram of the *Resolution solution*, is found; not more than 0.5% of any related compound with a retention time of 0.63 or 1.8 relative to that of atovaquone is found; and not more than 0.3% of any related compound with a retention time of 0.89 relative to that of atovaquone is found. Not more than 0.2% of any other individual related compound is found; and the sum of all other such related compounds is not more than 1.0%. The sum of all related compounds is not more than 1.5%.

#### Assay—

**Mobile phase**—Prepare a mixture of acetonitrile, water, methanol, and phosphoric acid (525:300:175:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of acetonitrile and water (80:20).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Atovaquone RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.25 mg per mL.

**Resolution solution**—Prepare a solution in *Diluent* containing about 0.25 mg of USP Atovaquone RS and 0.02 mg of USP Atovaquone Related Compound A RS per mL. Store in a low-actinic glass container.

**Assay preparation**—Transfer about 25 mg of Atovaquone, accurately weighed, to a low-actinic, 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.85 for atovaquone related compound A and 1.0 for atovaquone; and the resolution,  $R$ , between atovaquone related compound A and atovaquone is not less than 4. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 9000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{19}ClO_3$  in the portion of Atovaquone taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Atovaquone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the atovaquone peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atovaquone Oral Suspension

» Atovaquone Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of atovaquone ( $C_{22}H_{19}ClO_3$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Atovaquone RS

USP Atovaquone Related Compound A RS

*cis*-2[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.

#### Identification—

**A: Ultraviolet Absorption** (197U)—

**Medium:** a mixture of methanol and water (1:1).

**Solution**—Transfer 5.0 mL of the *Assay preparation* and 5.0 mL of the *Standard preparation*, prepared in the *Assay*, to separate 50-mL volumetric flasks, dilute with *Medium* to volume, and mix.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Uniformity of dosage units (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.5 and 7.0.

#### Sedimentation—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS—Transfer 50 mL of well-mixed Oral Suspension to a glass-stoppered graduated cylinder, and allow to stand for 16 hours. Measure the volume, if any, of clear liquid observed in the cylinder: not more than 1 mL of clear liquid is found.

**Related compounds**—Using the chromatograms of the *Resolution solution*, the *Standard preparation*, and the *Assay preparation* obtained in the *Assay*, calculate the percentage of atovaquone-related compounds, based on the labeled strength of atovaquone, by the formula:

$$\frac{(25,000/3)C(r_i / r_S)100D}{SF_i L}$$

in which  $C$  is the concentration, in mg per mL, of USP Atovaquone RS in the *Standard preparation*;  $D$  is the density of Oral Suspension, in g per mL (1.04 g per mL at 20° to 25°);  $S$  is the weight, in g, of Oral Suspension taken to prepare the *Assay preparation*;  $L$  is the labeled amount, in mg per mL, of atovaquone in the Oral Suspension;  $F_i$  is the response factor of an individual atovaquone related compound relative to the response of atovaquone, specifically, 1.08 for any peak observed at a relative retention time of about 0.65, 0.85 for any peak observed at a retention time corresponding to that of atovaquone related compound A, as determined from the chromatogram of the *Resolution solution*, and 1.0 for any other related compound peak;  $r_i$  is the individual peak response of an atovaquone related compound, if any, in the chromatogram of the *Assay preparation*; and  $r_S$  is the peak response of atovaquone in the chromatogram of the *Standard preparation*. Disregard any peak having a relative retention time of about 0.3, which is due to photodegradation during preparation of the *Assay preparation*. Not more than 0.5% of an atovaquone related com-

pound with a relative retention time of about 0.65 is found; not more than 1.0% of atovaquone related compound A is found; not more than 0.3% of an atovaquone related compound with a relative retention time of about 0.88 is found; not more than 0.2% of any other atovaquone related compound is found; and the sum of all related compounds is not more than 2.0%.

#### Assay—

**Mobile phase**—Prepare a mixture of acetonitrile, water, methanol, and phosphoric acid (480:360:160:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Prepare a solution in 0.1 M methanolic sodium hydroxide containing about 0.09 mg of USP Atovaquone RS and 0.01 mg of USP Atovaquone Related Compound A RS per mL. Store in a low-actinic glass container.

**Standard preparation**—Transfer about 30 mg of USP Atovaquone RS, accurately weighed, to a low-actinic 10-mL volumetric flask, and add 2 mL of water and 6 mL of 0.1 M methanolic sodium hydroxide. Sonicate for about 5 minutes or until the material has dissolved. Allow to cool, dilute with 0.1 M methanolic sodium hydroxide to volume, and mix. Transfer 3.0 mL of this solution to a low-actinic 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. [NOTE—Minimize exposure of this solution to light.]

**Assay preparation**—Transfer approximately 5.2 g of the well-mixed Oral Suspension, accurately weighed, to a low-actinic 250-mL volumetric flask. Add 50 mL of water, swirl for about 5 minutes, add 150 mL of 0.1 M methanolic sodium hydroxide, and sonicate for about 15 minutes. Allow to cool, dilute with 0.1 M methanolic sodium hydroxide to volume, and mix. Immediately filter a 20-mL portion, discarding the first 5 mL of the filtrate. Transfer 3.0 mL of the clear filtrate to a low-actinic 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. [NOTE—Minimize exposure of this solution to light.]

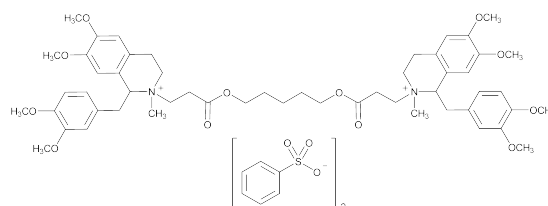
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 12.5-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.86 for atovaquone related compound A and 1.0 for atovaquone. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation*, the *Resolution solution*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of atovaquone (C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub>) in each mL of the Oral Suspension taken by the formula:

$$(25,000/3)(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Atovaquone RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the atovaquone peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atracurium Besylate



C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub> 1243.48  
 Isoquinolinium, 2,2'-[1,5-pentanediyldis[oxo(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-, dibenzenesulfonate;  
 2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate, pentamethylene ester [64228-81-5].

#### DEFINITION

Atracurium Besylate contains NLT 96.0% and NMT 102.0% of C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub>, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention times of the three main isomeric peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.

**Solution A:** Acetonitrile, methanol, and *Buffer* (20:5:75)

**Solution B:** Acetonitrile, methanol, and *Buffer* (20:30:50)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

**Standard solution:** 1 mg/mL of USP Atracurium Besylate RS in *Solution A*

**Sample solution:** 1 mg/mL of Atracurium Besylate in *Solution A*

#### Chromatographic system

(See *Chromatography*, (621) *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: Standard solution

[NOTE—Refer to Table 2 for relative retention times.]

Suitability requirements

**Resolution:** NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

**Relative standard deviation:** NMT 2.0%, for the *cis-cis* isomer peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of atracurium besylate (C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub>) in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Sample solution

$r_S$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Standard solution

$C_S$  = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Atracurium Besylate in the Sample solution (mg/mL)

**Acceptance criteria:** 96.0%–102.0%, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

## IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%
- **HEAVY METALS**, Method II (231): 20 ppm
- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Chromatographic system, and Sample solution: Proceed as directed in the Assay.

**Standard solution:** 0.01 mg/mL of USP Atracurium Besylate RS in Solution A

**System suitability solution:** 1 mg/mL of USP Atracurium Besylate RS in Solution A

System suitability

Sample: System suitability solution

Suitability requirements

**Resolution:** NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

Analysis

Samples: Standard solution and Sample solution

Record the chromatograms, and measure all of the peak responses, except the three main isomeric peaks.

Calculate the percentage of each impurity in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the Sample solution

$r_T$  = sum of peak responses due to the atracurium *cis-cis*, *trans-trans*, and *cis-trans* isomers from the Standard solution

$C_S$  = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Atracurium Besylate in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. [NOTE—Disregard any peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity E <sup>a</sup>	0.2	1.0	1.5
Impurity F <sup>b</sup>	0.25	1.0	1.0
Impurity G (laudanidine) <sup>c</sup>	0.3	2.0	1.0
Impurity D	0.45 <sup>d</sup> and 0.5 <sup>e</sup>	1.0	1.5 <sup>p</sup>
Atracurium <i>trans-trans</i> isomer	0.8	—	—
Atracurium <i>cis-trans</i> isomer	0.9	—	—
Atracurium <i>cis-cis</i> isomer	1.0	—	—
Impurity A	1.04 <sup>f</sup> and 1.08 <sup>g</sup>	1.0	1.5 <sup>p</sup>
Impurity I	1.07 <sup>g</sup> and 1.12 <sup>k</sup>	1.0	1.0 <sup>p</sup>
Impurity H	1.07 <sup>h</sup> and 1.12 <sup>i</sup>	1.0	1.0 <sup>p</sup>
Impurity K <sup>j</sup>	1.09 and 1.12	1.0	1.0 <sup>p</sup>
Impurity B <sup>m</sup>	1.15	1.0	0.1
Impurity C	1.2 <sup>n</sup> and 1.3 <sup>o</sup>	1.0	1.0 <sup>p</sup>
Any individual impurity	—	1.0	0.1
Total impurities	—	—	3.5

<sup>a</sup> 3-[1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinio]propanoate.

<sup>b</sup> 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>c</sup> 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline.

<sup>d</sup> *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>e</sup> *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>f</sup> *trans-trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>g</sup> *cis-trans* Isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>h</sup> *cis-trans* Isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>i</sup> *cis-cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>j</sup> 2,2'-[(Hexane-1,5)-diylbis(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>k</sup> *cis-cis* Isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>l</sup> *cis-cis* Isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>m</sup> Pentane-1,5-diyl bis[3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]propanoate].

<sup>n</sup> *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.

<sup>o</sup> *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.

<sup>p</sup> Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

## • LIMIT OF IMPURITY J (Methyl Benzenesulfonate)

Buffer, Solution A, and Solution B: Prepare as directed in the Assay.

**Standard stock solution:** 0.2 mg/mL of Impurity J (methyl benzenesulfonate) in acetonitrile

**Standard solution:** 1 µg/mL of Impurity J (methyl benzenesulfonate) in Solution A from Standard stock solution

**Sample solution:** 10 mg/mL of Atracurium Besylate in Solution A

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	75	25
25	75	25
30	55	45
38	0	100
45	0	100

#### Chromatographic system

Mode: LC

Detector: UV 217 nm

Column: 4.6-mm × 25-cm; 5-μm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 100 μL

#### Analysis

Samples: Standard solution and Sample solution

Measure the responses for the Impurity J (methyl benzenesulfonate) peaks.

Acceptance criteria: NMT 0.01%, the peak response of the Sample solution being NMT that of the Standard solution

#### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 5.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place. [NOTE—Atracurium Besylate is unstable at room temperature.]
- **USP REFERENCE STANDARDS** (11)  
USP Atracurium Besylate RS

## Atracurium Besylate Injection

#### DEFINITION

Atracurium Besylate Injection is a sterile solution containing NLT 90.0% and NMT 115.0% of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ). It contains an amount of the *trans-trans* isomer equivalent to NLT 5.0% and NMT 6.5% of the labeled amount of atracurium besylate, an amount of the *cis-trans* isomer equivalent to NLT 34.5% and NMT 38.5% of the labeled amount of atracurium besylate, and an amount of the *cis-cis* isomer equivalent to NLT 55.0% and NMT 60.0% of the labeled amount of atracurium besylate.

[NOTE—The Injection is unstable at room temperature. Store all samples in the refrigerator. Analyze all preparations as soon as possible, or use a refrigerated injector.]

#### IDENTIFICATION

- **A.** The retention times of the peaks of the three atracurium besylate isomers from the Sample solution correspond to those from the Standard solution, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

Buffer: 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.

Solution A: Acetonitrile, methanol, and Buffer (20:5:75)

Solution B: Acetonitrile, methanol, and Buffer (20:30:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Standard solution: 1 mg/mL of USP Atracurium Besylate RS in Solution A

Sample solution: Nominally equivalent to 1 mg/mL of atracurium besylate from Injection in Solution A

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

#### System suitability

Sample: Standard solution

[NOTE—Refer to Table 2 under Organic Impurities for relative retention times.]

#### Suitability requirements

Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

Relative standard deviation: NMT 2.0%, for the *cis-cis* isomer peak

#### Analysis

Samples: Standard solution and Sample solution

Measure the responses for the three atracurium besylate isomer peaks.

Calculate the percentage of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ) in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Sample solution

$r_S$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Standard solution

$C_S$  = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of atracurium besylate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–115.0% of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ). It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

#### IMPURITIES

##### • ORGANIC IMPURITIES

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution: 1 mg/mL of USP Atracurium Besylate RS in Solution A

**Standard solution:** 0.02 mg/mL of USP Atracurium Besylate RS in *Solution A*, from *Standard stock solution*

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Standard solution*

$C_S$  = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atracurium besylate in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzenesulfonic acid <sup>a</sup>	0.08	—	—
Acidic compound	0.22	1.0	6.0
Impurity G (laudanosine)	0.29	2.0	3.0
<i>cis-</i> and <i>trans-</i> isomers of the hydroxy compound	0.44 <sup>b</sup> and 0.50 <sup>c</sup>	1.0	6.0 <sup>f</sup>
Atracurium <i>trans-trans</i> isomer	0.8	—	—
Atracurium <i>cis-trans</i> isomer	0.9	—	—
Atracurium <i>cis-cis</i> isomer	1.0	—	—
<i>cis-</i> and <i>trans-</i> isomers of the monoacrylate	1.28 <sup>d</sup> and 1.33 <sup>e</sup>	1.0	3.0 <sup>f</sup>
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	15.0

<sup>a</sup> For identification purposes only.

<sup>b</sup> *cis* isomer of the hydroxy compound.

<sup>c</sup> *trans* isomer of the hydroxy compound.

<sup>d</sup> *cis* isomer of the monoacrylate.

<sup>e</sup> *trans* isomer of the monoacrylate.

<sup>f</sup> Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

**SPECIFIC TESTS**

• **PH** (791): 3.00–3.65

• **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 5.56 USP Endotoxin Units/mg of atracurium besylate.

• **INJECTIONS** (1): Meets the requirements

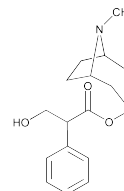
**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, in a refrigerator, and protect from freezing. Protect from light.

• **USP REFERENCE STANDARDS** (11)

USP Atracurium Besylate RS

## Atropine



$C_{17}H_{23}NO_3$  289.37

Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-;

1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol (±)-tropate (ester) [51-55-8].

**DEFINITION**

Atropine contains NLT 99.0% and NMT 100.5% of atropine ( $C_{17}H_{23}NO_3$ ), calculated on the anhydrous basis.

[**CAUTION**—Handle Atropine with exceptional care, because it is highly potent.]

**IDENTIFICATION**

• **A.**

**Standard:** 36 mg of USP Atropine Sulfate RS

**Sample:** 30 mg

**Analysis:** Dissolve the *Standard* and *Sample* in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through separate filters of 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide.

**Acceptance criteria:** The IR absorption spectrum, determined in a 1-mm cell, of the solution of the *Sample* exhibits maxima only at the same wavelengths as that of the solution of the *Standard*.

• **B.**

**Sample solution:** A solution (1 in 50) in 3 N hydrochloric acid

**Analysis:** Add gold chloride TS to the *Sample solution*.

**Acceptance criteria:** A lusterless precipitate is formed (distinction from hyoscyamine, which, similarly treated, yields a lustrous precipitate).

**ASSAY**

• **PROCEDURE**

**Sample:** 400 mg of Atropine

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS to a green endpoint, using 1 drop of crystal violet TS. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of atropine ( $C_{17}H_{23}NO_3$ ).

**Acceptance criteria:** 99.0%–100.5% on the anhydrous basis



**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **LIMIT OF FOREIGN ALKALOIDS AND OTHER IMPURITIES**  
 Standard solution: 24 mg/mL of USP Atropine Sulfate RS in methanol  
 Sample solution A: 20 mg/mL of Atropine in methanol  
 Sample solution B: 1 mg/mL of Atropine in methanol  
**Chromatographic system**  
 (See *Chromatography* (621), *Thin-Layer Chromatography*.)  
**Mode:** TLC  
**Adsorbent:** 0.5-mm layer of chromatographic silica gel  
**Application volume:** See *Analysis*.  
**Developing solvent system:** Chloroform, acetone, and diethylamine (5:4:1)  
**Spray reagent:** Potassium iodoplatinate TS  
**Analysis**  
*Samples:* Standard solution, 5  $\mu$ L; Sample solution A, 25  $\mu$ L; Sample solution B, 1  $\mu$ L  
 Apply the *Samples* to the TLC plate. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Allow the solvent to evaporate. Locate the spots on the plate by spraying with *Spray reagent*.  
**Acceptance criteria:** NMT 0.2%; the  $R_f$  value of the principal spot of each *Sample solution* corresponds to that of the *Standard solution*; no secondary spot of *Sample solution A* exhibits intensity equal to or greater than the principal spot of *Sample solution B*.
- **READILY CARBONIZABLE SUBSTANCES TEST** (271)  
 Sample solution: 200 mg in 5 mL of 2 N sulfuric acid  
**Acceptance criteria:** The solution has no more color than *Matching Fluid A*, and the solution is colored no more than light yellow upon the addition of 0.2 mL of nitric acid.

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Angular Rotation** (781)  
 Sample solution: 1 g, previously dried at 105° for 1 h, in sufficient 50% alcohol (w/w) to obtain a volume of 20 mL at 25° (using a 200-mm tube)  
**Acceptance criteria:**  $-0.70^\circ$  to  $+0.05^\circ$  (limit of hyoscyamine)
- **MELTING RANGE OR TEMPERATURE** (741): 114°–118°
- **WATER DETERMINATION, Method I** (921): NMT 0.2%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Atropine Sulfate RS

**Atropine Sulfate**

( $C_{17}H_{23}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · H<sub>2</sub>O 694.83  
 Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-, sulfate (2:1) (salt), monohydrate.  
 1 $\alpha$ H,5 $\alpha$ H-Tropan-3- $\alpha$ -ol (±)-tropate (ester), sulfate (2:1) (salt) monohydrate [5908-99-6].  
 Anhydrous 676.83 [55-48-1].

» Atropine Sulfate contains not less than 98.5 percent and not more than 101.0 percent of ( $C_{17}H_{23}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>, calculated on the anhydrous basis.

*Caution—Handle Atropine Sulfate with exceptional care, since it is highly potent.*

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Atropine Sulfate RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** A solution (1 in 20) meets the requirements of the tests for *Sulfate* (191).

**Melting temperature, Class Ia** (741): not lower than 187°, determined after drying at 120° for 4 hours. [NOTE—Since anhydrous Atropine Sulfate is hygroscopic, determine its melting temperature promptly on a specimen placed in the capillary tube immediately after drying.]

**Angular rotation** (781A)—The observed rotation, in degrees, multiplied by 200, and divided by the length, in mm, of the polarimeter tube used, is between  $-0.60^\circ$  and  $+0.05^\circ$  (limit of hyoscyamine).

*Test solution*—Dissolve 1 g, accurately weighed, in water to make a volume of 20 mL at 25°.

**Acidity**—Dissolve 1.0 g in 20 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.30 mL is required to produce a yellow color.

**Water, Method I** (921): not more than 4.0%.

**Residue on ignition** (281): not more than 0.2%.

**Other alkaloids**—Dissolve 150 mg in 10 mL of water. To 5 mL of the solution add a few drops of platinic chloride TS: no precipitate is formed. To the remaining 5 mL of the solution add 2 mL of 6 N ammonium hydroxide, and shake vigorously: a slight opalescence may develop but no turbidity is produced.

**Assay**—Dissolve about 1 g of Atropine Sulfate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 67.68 mg of ( $C_{17}H_{23}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>.

**Atropine Sulfate Injection**

» Atropine Sulfate Injection is a sterile solution of Atropine Sulfate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of ( $C_{17}H_{23}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · H<sub>2</sub>O.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Atropine Sulfate RS

USP Endotoxin RS

**Identification** (see *Thin-Layer Chromatographic Identification Test* (201))—

**Adsorbent:** chromatographic silica gel.

**Developing solvent:** mixture of chloroform and diethylamine (9:1).

*Test preparation*—Use undiluted. Apply 15  $\mu$ L.

*Detection reagent:* potassium iodoplatinate TS.

*Procedure*—Proceed as directed for *Procedure* under *Thin-Layer Chromatographic Identification Test* (201), the spots on the plate located by spraying with *Detection reagent*.

**Bacterial endotoxins** (85)—It contains not more than 55.6 USP Endotoxin Units per mg of atropine sulfate.

**pH** (791): between 3.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Acetate buffer*—Prepare a solution in water containing in each L 0.05 mole of sodium acetate and 2.9 mL of glacial acetic acid.

*Mobile phase*—Transfer 5.1 g of tetrabutylammonium hydrogen sulfate to a 1-L volumetric flask, add 50 mL of acetonitrile, and dilute with *Acetate buffer* to volume. Adjust with 5 N sodium hydroxide to a pH of  $5.5 \pm 0.1$ .

*Standard preparation*—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 80 µg per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of atropine sulfate, to a 25-mL volumetric flask, dilute with water to volume, and mix.

*Resolution solution*—Prepare a solution in water containing about 2.5 µg of *p*-hydroxybenzoic acid per mL. Dilute one volume of this solution with four volumes of the *Standard preparation*.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and 30-cm  $\times$  3.9-mm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%. In a similar manner, chromatograph the *Resolution solution*: the retention time of *p*-hydroxybenzoic acid is about 1.6 relative to that of atropine, and the resolution, *R*, between the *p*-hydroxybenzoic acid and atropine peaks is not less than 2.2.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$  in each mL of the Injection taken by the formula:

$$(694.85/676.83)(25C/V)(r_U/r_S)$$

in which 694.85 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; *C* is the concentration, in mg per mL, of USP Atropine Sulfate RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atropine Sulfate Ophthalmic Ointment

**DEFINITION**

Atropine Sulfate Ophthalmic Ointment is Atropine Sulfate in a suitable ophthalmic ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of atropine sulfate monohydrate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ . It is sterile.

**IDENTIFICATION**

- A. IDENTIFICATION—ORGANIC NITROGENOUS BASES <181>**

**Sample solution:** Transfer a portion of Ophthalmic Ointment, equivalent to 50 mg of atropine sulfate, to a suitable separator, and dissolve in 25 mL of ether. Add 25 mL of 0.01 N hydrochloric acid, shake vigorously, allow the layers to separate, and discard the organic phase. Heat the aqueous phase gently on a steam bath while passing nitrogen through the solution to expel any residual ether.

**Analysis:** Proceed as directed in the chapter, beginning with "In a second separator dissolve 50 mg..."

**Acceptance criteria:** Meets the requirements

- B. IDENTIFICATION TESTS—GENERAL, Sulfate <191>**

**Sample solution:** Transfer 5 g of Ophthalmic Ointment to a separator, dissolve in 50 mL of ether, and extract with 20 mL of water.

**Acceptance criteria:** Meets the requirements

**ASSAY**

- PROCEDURE**

**Buffer:** 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide, as necessary.

**Internal standard solution:** 0.5 mg/mL of homatropine hydrobromide in water. [NOTE—Prepare fresh daily.]

**Standard stock solution:** 0.1 mg/mL of USP Atropine Sulfate RS in water

**Standard solution:** 0.5 mg/mL of atropine sulfate prepared as follows. Pipet 10 mL of *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride. [NOTE—Prepare fresh daily.]

**Sample solution:** Nominally 0.5 mg/mL of atropine sulfate prepared as follows. Transfer Ophthalmic Ointment, equivalent to 10 mg of atropine sulfate, to a separator containing 50 mL of ether. Shake to dissolve, extract with three 25-mL portions of 0.1 M sulfuric acid, collect the acid extracts in a 100-mL volumetric flask, and dilute with 0.1 M sulfuric acid to volume. Pipet 10 mL of this solution and treat as follows. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  1.8-m glass; packed with a 3% phase G3 on support S1AB

**Temperatures**

**Column:** 225°

**Injection port:** 250°

**Detector:** 250°

**Flow rate:** 25 mL/min

**Carrier gas:** Nitrogen

**Injection volume:** 1 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 4.0

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of atropine sulfate monohydrate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$  in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

*R<sub>U</sub>* = peak area ratio of atropine to homatropine from the *Sample solution*

- $R_S$  = peak area ratio of atropine to homatropine from the *Standard solution*  
 $C_S$  = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85  
 $M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83  
**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** <71>: Meets the requirements
- **METAL PARTICLES IN OPHTHALMIC OINTMENTS** <751>: Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** <11>  
USP Atropine Sulfate RS

## Atropine Sulfate Ophthalmic Solution

**DEFINITION**

Atropine Sulfate Ophthalmic Solution is a sterile, aqueous solution of Atropine Sulfate. It contains NLT 93.0% and NMT 107.0% of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ . It may contain suitable stabilizers and antimicrobial agents.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197M>  
**Standard:** 36 mg USP Atropine Sulfate RS  
**Sample:** Ophthalmic Solution, equivalent to 30 mg, evaporated to dryness  
**Analysis:** Dissolve the *Sample* and *Standard* in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through separate filters of 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide.  
**Acceptance criteria:** The IR absorption spectrum, determined in a 1-mm cell, of the solution of the *Sample* exhibits maxima only at the same wavelengths as that of the solution of the *Standard*.
- **B. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>  
**Sample solution:** Evaporate to dryness a quantity of Ophthalmic Solution. Prepare a solution from the residue that contains the equivalent of 50 mg of atropine sulfate/mL.  
**Acceptance criteria:** Meets the requirements

**ASSAY**• **PROCEDURE**

**Buffer:** 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide.  
**Internal standard solution:** 0.5 mg/mL of homatropine hydrobromide in water. [NOTE—Prepare fresh daily.]  
**Standard stock solution:** 0.1 mg/mL of USP Atropine Sulfate RS in water

**Standard solution:** 0.5 mg/mL of atropine sulfate prepared as follows. Pipet 10 mL of *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride. [NOTE—Prepare fresh daily.]

**Sample solution:** Nominally 0.5 mg/mL of atropine sulfate prepared as follows. Ophthalmic Solution, equivalent to 10 mg of atropine sulfate, in a 100-mL volumetric flask. Dilute with water to volume. Pipet 10 mL of this solution and treat as follows. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  1.8-m glass; packed with a 3% phase G3 on support S1AB

**Temperatures**

**Column:** 225°

**Injection port:** 250°

**Detector:** 250°

**Flow rate:** 25 mL/min

**Carrier gas:** Nitrogen

**Injection volume:** 1  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 4.0

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of atropine sulfate

$[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$  in each mL of Ophthalmic Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak area ratio of atropine sulfate to homatropine hydrobromide from the *Sample solution*  
 $R_S$  = peak area ratio of atropine sulfate to homatropine hydrobromide from the *Standard solution*

$C_S$  = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85  
 $M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83

Acceptance criteria: 93.0%–107.0%

#### SPECIFIC TESTS

- **PH** (791): 3.5–6.0
- **STERILITY TESTS** (71): Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

## Atropine Sulfate Tablets

#### DEFINITION

Atropine Sulfate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ .

#### IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181)  
**Sample:** A quantity of Tablets, equivalent to 5 mg of atropine sulfate  
**Analysis:** Triturate with 10 mL of water for a few min, and filter into a small separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract with 50 mL of chloroform. Filter the chloroform layer, and evaporate to dryness.  
**Acceptance criteria:** The residue meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Sulfate** (191): A filtered solution of Tablets meets the requirements of the tests.

#### ASSAY

##### PROCEDURE

**Buffer:** 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide, as necessary.

**Internal standard solution:** 0.5 mg/mL of homatropine hydrobromide in water. [NOTE—Prepare fresh daily.]

**Standard solution:** 0.1 mg/mL of USP Atropine Sulfate RS in water. Pipet 10 mL of this solution into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride. [NOTE—Prepare fresh daily.]

**Sample solution:** Transfer an equivalent to 1 mg of atropine sulfate, from NLT 20 finely powdered Tablets, to a separator. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  1.8-m glass; packed with a 3% phase G3 on support S1AB

#### Temperatures

**Column:** 225°

**Injection port:** 250°

**Detector:** 250°

**Flow rate:** 25 mL/min

**Carrier gas:** Nitrogen

**Injection volume:** 1  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 4.0

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$  in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak area ratio of atropine sulfate to homatropine hydrobromide from the *Sample solution*

$R_S$  = peak area ratio of atropine sulfate to homatropine hydrobromide from the *Standard solution*

$C_S$  = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atropine sulfate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85

$M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701)  
**Time:** 15 min  
**Acceptance criteria:** Meet the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

## Activated Attapulgate

» Activated Attapulgate is a highly heat-treated, processed, native magnesium aluminum silicate.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Activated Attapulgate responds to the *Identification* test for *Colloidal Activated Attapulgate*, the characteristic peak, however, being much less intense.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 4.0% of its weight.

**Loss on ignition** (733)—When ignited at 1000° for 1 hour, it loses between 4.0% and 12.0% of its weight.

**Volatile matter**—When ignited at 600° for 1 hour, it loses between 3.0% and 7.5% of its weight on the dried basis.

**Powder fineness**—Proceed as directed in the test for *Powder fineness* under *Colloidal Activated Attapulgate*. The dry weight of the residue is not more than 0.10% of the weight of the specimen taken.

**Acid-soluble matter**—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.25 g is found (25%).

**Other requirements**—It meets the requirements of the tests for *Microbial limits*, *pH*, *Carbonate*, *Arsenic and Lead*, and *Adsorptive capacity* under *Colloidal Activated Attapulgit*.

## Colloidal Activated Attapulgit

» Colloidal Activated Attapulgit is a purified native magnesium aluminum silicate.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Add 2 g in small portions to 100 mL of water, with vigorous agitation. Allow to stand for at least 12 hours to ensure complete hydration. Place 2 mL of the resulting mixture on a suitable glass slide, and allow to air-dry at room temperature to produce a uniform film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that the ethylene glycol saturates the desiccator chamber. Allow to stand for 12 hours. Record the X-ray diffraction pattern (see *X-Ray Diffraction* <941>), and calculate the *d* values: several peaks are observed; the characteristic peak corresponds to a *d* value between 10.3 and 10.7 Angstrom units.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the test for absence of *Escherichia coli*.

**pH** <791>—Disperse 1.0 g in 10 mL of carbon dioxide-free water, and mix: the pH of the mixed dispersion so obtained is between 7.0 and 9.5.

**Loss on drying** <731>—Dry it at 105° to constant weight: it loses between 5.0% and 17.0% of its weight.

**Loss on ignition** <733>—When ignited at 1000° for 1 hour, it loses between 17.0% and 27.0% of its weight.

**Volatile matter**—When ignited at 600° for 1 hour, it loses between 7.5% and 12.5% of its weight on the dried basis.

**Powder fineness**—Add 50 g to 450 mL of water containing 5 g of sodium pyrophosphate, and stir for 10 minutes. Pour the resulting dispersion slowly through a No. 325 standard sieve (see *Particle Size Distribution Estimation by Analytical Sieving* <786>), and carefully wash the residue until clean. Dry the residue at 105° to constant weight: the dry weight of the residue so obtained is not more than 0.30% of the weight of the specimen taken.

**Acid-soluble matter**—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.15 g is found (15%).

**Carbonate**—Mix 1.0 g with 15 mL of 0.5 N sulfuric acid: no effervescence occurs.

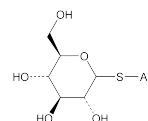
**Arsenic and Lead**—To 5.0 g add 50 mL of 1 N nitric acid, and boil for 30 minutes, adding 1 N nitric acid at times to maintain the volume. Filter into a 100-mL volumetric flask, wash the filter with water, and dilute the combined filtrate and washings with water to volume.

**Arsenic**—Determine the arsenic in the solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* <851>), using a graphite furnace to volatilize the arsenic, as directed by the manufacturer of the instrument used, and measuring the absorbance at 189.0 nm against a standard: not more than 2 ppm is found.

**Lead**—Determine the lead in the solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* <851>), using a graphite furnace to volatilize the lead, as directed by the manufacturer of the instrument used, and measuring the absorbance at 283.3 nm against a standard: not more than 0.001% is found.

**Adsorptive capacity**—To 10 mL of a 1 in 10 suspension of the specimen in water add 80 mL of methylene blue solution (1 in 1000), and shake. Add 10 mL of barium chloride solution (1 in 50), and shake. Allow to stand for 15 minutes. Transfer 40 mL of the supernatant to a 50-mL centrifuge tube, and centrifuge. To 5 mL of the clear supernatant add 495 mL of water, and mix: the color of the solution so obtained is not deeper than that of a solution containing 1.5 µg of methylene blue per mL.

## Aurothioglucose



$C_6H_{11}AuO_5S$  392.18  
Gold, (1-thio-D-glucopyranosato)-.  
(1-Thio-D-glucopyranosato)gold [12192-57-3].

» Aurothioglucose contains not less than 95.0 percent and not more than 105.0 percent of  $C_6H_{11}AuO_5S$ , calculated on the dried basis. It is stabilized by the addition of a small amount of Sodium Acetate.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**USP Reference standards** <11>—

USP Aurothioglucose RS

**Identification**—

**A:** Dissolve a suitable quantity in water to obtain a solution containing 4 mg per mL. Apply 10 µL of this solution and 10 µL of an aqueous Standard solution of USP Aurothioglucose RS containing 4 mg per mL to a suitable thin-layer chromatographic glass microfilament sheet (see *Chromatography* <621>) impregnated with silicic acid and a suitable fluorescing substance. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, and ethyl acetate (3:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the sheet from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the *R<sub>F</sub>* value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**B:** To a portion of the filtrate obtained in the Assay add barium chloride TS: a heavy, white precipitate is formed.

**Specific rotation** <781S>: between +65° and +75°.

**Test solution:** 10 mg per mL, in water.

**Loss on drying** <731>—Dry it over phosphorus pentoxide for 24 hours: it loses not more than 1.0% of its weight.

**Assay**—Accurately weigh about 1 g of Aurothioglucose, and dissolve in 100 mL of water in a 300-mL Kjeldahl flask. Slowly add 10 mL of nitric acid, and when the reaction has subsided, boil the mixture for 5 minutes. Filter, wash well the separated gold with hot water, dry, and ignite to constant weight. The weight of the gold so obtained, multiplied by 1.991, represents the weight of  $C_6H_{11}AuO_5S$  in the portion of Aurothioglucose taken.

## Aurothioglucose Injectable Suspension

» Aurothioglucose Injectable Suspension is a sterile suspension of Aurothioglucose in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_{11}AuO_5S$ . It may contain suitable thickening agents.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Protect from light.

**USP Reference standards** (11)—  
USP Aurothioglucose RS

**Identification**—Transfer a volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a centrifuge separator containing 20 mL of ethyl acetate and 50 mL of water. Shake the mixture thoroughly, and centrifuge until the liquid phases have been clearly separated. Withdraw the lower, aqueous phase, and filter, discarding the first 10 mL of the filtrate. Collect the filtrate in a glass-stoppered vessel, and proceed as directed in *Identification* test A under *Aurothioglucose*, beginning with “apply 10  $\mu$ L of this solution.”

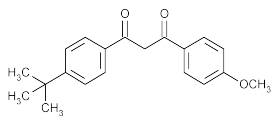
**Bacterial endotoxins** (85)—It contains not more than 7.14 USP Endotoxin Units per mg of aurothioglucose.

**Sterility** (71)—It meets the requirements.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer with a pipet, calibrated to contain rather than to deliver, an accurately measured volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a beaker containing 400 mL of acetone. Wash the pipet into the beaker with a small quantity of acetone, mix, allow the solids to settle, and decant the supernatant through a filter. Wash the solids with another 400-mL portion of acetone, and repeat the decantation. Transfer the solids to the filter with the aid of acetone, then transfer the filter and its contents to a short-necked, 300-mL Kjeldahl flask, add 5 mL of water, and proceed as directed in the *Assay* under *Gold Sodium Thiomalate*, beginning with “add 20 mL of nitric acid.” The weight of gold so obtained, multiplied by 1.991, represents the weight of  $C_6H_{11}AuO_5S$  in the portion of Injectable Suspension taken.

## Avobenzone



$C_{20}H_{22}O_3$  310.39

1,3-Propanedione, 1-[4-(1,1-dimethylethyl)phenyl]-3-(4-methoxyphenyl)-.

1-(*p*-*tert*-Butylphenyl)-3-(*p*-methoxyphenyl)-1,3-propanedione [70356-09-1].

» Avobenzone contains not less than 95.0 percent and not more than 105.0 percent of  $C_{20}H_{22}O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Avobenzone RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 5  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 360 nm do not differ by more than 3.0%.

**Melting range**, *Class I* (741): between 81° and 86°.

**Loss on drying** (731)—Dry it in vacuum at 70° for 4 hours; it loses not more than 0.5% of its weight.

**Chromatographic purity**—

*Test solution*—Proceed as directed for *Assay preparation* in the *Assay*.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed in the *Assay*.

*Procedure*—Inject a volume (about 1  $\mu$ L) of *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Avobenzone taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each individual peak, other than the avobenzone peak, in the chromatogram of the *Test solution*; and  $r_s$  is the sum of the responses of all of the peaks in the chromatogram of the *Test solution*: not more than 3.0% of any individual impurity is found, and the sum of all of the impurities is not more than 4.5%.

**Assay**—

*Standard preparation*—Dilute an accurately measured quantity of USP Avobenzone RS in acetone, and dilute quantitatively, and stepwise if necessary, with acetone to obtain a solution having a known concentration of about 50 mg per mL.

*Assay preparation*—Transfer about 500 mg of Avobenzone, accurately weighed, to a 10-mL volumetric flask, dilute with acetone to volume, and mix.

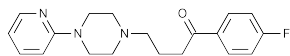
*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm  $\times$  25-m fused silica capillary column coated with phase G1. The column temperature is maintained at about 200° until the time of injection, then increased at a rate of 4° per minute to 280°. The injection port temperature is maintained at 200°, and the detector temperature is maintained at about 280°. Helium is used as the carrier gas. The split ratio is 50:1. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between avobenzone and any adjacent peak is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 1  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{22}O_3$  in the portion of Avobenzone taken by the formula:

$$10C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Avobenzone RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Azaperone



$C_{19}H_{22}FN_3O$  327.40

1-Butanone, 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-

4'-Fluoro-4-[4-(2-pyridyl)-1-piperazinyl]butyrophenone  
[1649-18-9].

» Azaperone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{22}FN_3O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Azaperone RS

**Identification**, *Infrared Absorption* (197K): previously dried.

**Melting range** (741): between 92° and 95°.

**Loss on drying** (731)—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—Dissolve an accurately weighed quantity of USP Azaperone RS in a mixture of acetone and methylene chloride (5:1) to obtain a solution having a concentration of 0.50 mg per mL (*Standard solution A*). Quantitatively dilute a volume of *Standard solution A* with the same solvent mixture to obtain a solution having a concentration of 0.25 mg per mL (*Standard solution B*). Prepare a test solution by dissolving an accurately weighed quantity of Azaperone in a mixture of acetone and methylene chloride (5:1) to obtain a solution containing 50 mg per mL. Separately apply 1  $\mu$ L each of *Standard solution A*, *Standard solution B*, and the test solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.2-mm layer of chromatographic silica gel mixture with chemically bonded amino groups, and allow the spots to dry. Develop the chromatograms in a solvent system consisting of a mixture of cyclohexane, acetone, and methanol (65:30:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow the plate to air-dry. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots, other than any spot at the origin, observed in the chromatogram of the test solution with those of the principal spots in the chromatograms of *Standard solution A* and *Standard solution B*: the sum of the intensities of the secondary spots obtained from the test solution corresponds to not more than the intensity of the principal spot in the chromatogram of *Standard solution A* (1.0%).

**Assay**—Dissolve about 120 mg of Azaperone, accurately weighed, in 50 mL of a mixture of methyl ethyl ketone and glacial acetic acid (7:1). Add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 16.37 mg of  $C_{19}H_{22}FN_3O$ .

## Azaperone Injection

» Azaperone Injection is a sterile solution of Azaperone in Water for Injection, prepared with the aid of Tartaric Acid. It may contain a suitable preservative and a stabilizing agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{19}H_{22}FN_3O$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Azaperone RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for azaperone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**pH** (791): between 4.0 and 5.6.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture containing 6 volumes of acetonitrile and 4 volumes of 0.01 M dibasic potassium phosphate, and adjust by the addition of dilute phosphoric acid (1 in 10) to a pH of  $7.8 \pm 0.1$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of benzophenone in methanol containing about 0.5 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Azaperone RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution*, dilute quantitatively with methanol to 10.0 mL, and mix.

*Assay preparation*—Dilute an accurately measured volume of Injection quantitatively with methanol to obtain a solution containing about 0.5 mg of azaperone per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution*, dilute quantitatively with methanol to 10.0 mL, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with 243-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the azaperone and internal standard peaks is not less than 2.7; and the relative standard deviation for replicate injections is not more than 2.0%.

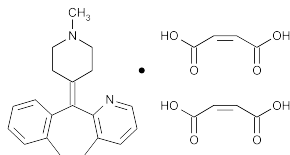
*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of azaperone ( $C_{19}H_{22}FN_3O$ ) in each mL of the Injection taken by the formula:

$$(C/L/D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Azaperone RS in the *Standard preparation*; L is the labeled quantity, in mg, of azaperone in each mL of the Injection; D is the concentration, in mg per mL, of azaperone in the *Assay preparation*, based on the volume of Injection taken

and the extent of dilution; and  $R_U$  and  $R_S$  are the ratios of the azaperone peak to the benzophenone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Azatadine Maleate



$C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  522.55

5*H*-Benzo[5,6]cyclohepta[1,2-*b*]pyridine, 6,11-dihydro-11-(1-methyl-4-piperidylidene)-, (*Z*)-2-butenedioate (1:2).

6,11-Dihydro-11-(1-methyl-4-piperidylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine maleate (1:2) [3978-86-7].

» Azatadine Maleate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Azatadine Maleate RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 40 µg per mL.

*Medium:* 0.25 N hydrochloric acid in methanol.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours; it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—Dissolve an accurately weighed quantity in a solvent mixture consisting of toluene and methanol (1:1), to obtain a solution having a known concentration of about 7 mg of the test specimen per mL. Similarly prepare a Standard solution of USP Azatadine Maleate RS in the same medium having a known concentration of about 7 mg per mL. Apply 100-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, isopropyl alcohol, and diethylamine (10:10:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by visualization under short-wavelength UV light. Separately transfer the silica gel mixture containing the principal spot from each track to suitable stoppered centrifuge tubes. [NOTE—Take care to separate the principal spots from any adjacent spots.] Similarly transfer an equal amount of silica gel from a blank section of the plate to a separate, suitable stoppered centrifuge tube. To each of the three tubes add 15.0 mL of a solvent mixture consisting of methanol and 0.5 N hydrochloric acid (4:1), shake vigorously for about 15 minutes, and centrifuge. Concomitantly determine the absorbances of the supernatant test solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 284 nm, with a suitable spectrophotometer, using the solution obtained

from the blank section of the plate as the blank. Calculate the chromatographic purity taken by the formula:

$$100(A_U / A_S)(C_S / C_U)$$

in which  $A_U$  and  $A_S$  are the absorbances of the test solution and the Standard solution, respectively; and  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of the Standard solution and the test solution, respectively. The chromatographic purity is not less than 98.0%.

**Assay**—Dissolve about 650 mg of Azatadine Maleate, accurately weighed, in 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 26.13 mg of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ .

## Azatadine Maleate Tablets

» Azatadine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Azatadine Maleate RS

**Identification**—Transfer 15.0 mL of the *Standard preparation* and 15.0 mL of the *Assay preparation*, respectively, prepared as directed in the *Assay*, to separate 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge. Transfer the solvent hexane extracts (upper phase) from each centrifuge tube to separate 50-mL conical flasks fitted with glass stoppers. Evaporate the solvent hexane extracts on a steam bath under a stream of nitrogen to dryness, pipet 1 mL of solvent hexane into each flask, insert the stoppers, and mix by use of a vortex mixer (or equivalent) until the residues have dissolved. Use these solutions as the Standard solution and the test solution, respectively. Apply separately 100 µL each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of toluene, isopropyl alcohol, and diethylamine (10:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value and intensity of the principal spot in the chromatogram of the test solution correspond to those obtained from the chromatogram of the Standard solution.

**Dissolution** (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure**—Determine the amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 283 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Azatadine Maleate RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  is dissolved in 30 minutes.



**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

**Standard preparation**—Dissolve an accurately weighed quantity of USP Azatadine Maleate RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

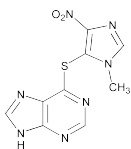
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 mg of azatadine maleate, to a 50-mL flask fitted with a glass stopper. Add 25.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake the mixture by mechanical means for about 30 minutes. Filter the mixture into a suitable glass-stoppered vessel, discarding the first 5 mL of the filtrate.

**Procedure**—Separately transfer 15.0 mL of the *Standard preparation*, 15.0 mL of the *Assay preparation*, and 15.0 mL of 0.1 N hydrochloric acid to provide the reagent blank to three 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge until the supernatants (solvent hexane phase) are clear. With the aid of separate syringes, transfer the supernatants to separate 50-mL centrifuge tubes fitted with glass stoppers. Rinse each syringe with 10 mL of solvent hexane, and add the rinse to the aqueous phase from which the respective supernatant was removed. Insert the stoppers, rotate each tube for about 10 minutes, and centrifuge. Transfer each supernatant to the respective supernatant previously collected. Pipet 15 mL of 0.1 N hydrochloric acid into each centrifuge tube containing the combined supernatants, insert the stoppers, rotate each tube for about 15 minutes, and centrifuge. Remove and discard the supernatants. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer zeroed with 0.1 N hydrochloric acid, using the prepared reagent blank. Calculate the quantity, in mg, of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$25C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Azatadine Maleate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Azathioprine



$C_9H_7N_7O_2S$  277.26

1*H*-Purine, 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-. 6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine [446-86-6].

» Azathioprine contains not less than 98.0 percent and not more than 101.5 percent of  $C_9H_7N_7O_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Azathioprine RS

USP Mercaptopurine RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** The principal spot in the test preparation chromatogram in the test for *Limit of mercaptopurine* shows the same  $R_f$  value as that obtained with the solution of USP Azathioprine RS.

**Acidity or alkalinity**—Shake 2.0 g with 100 mL of water for 15 minutes, and filter: 20.0 mL of the filtrate requires for neutralization not more than 0.10 mL of 0.020 N hydrochloric acid or not more than 0.10 mL of 0.020 N sodium hydroxide, methyl red TS being used as the indicator.

**Loss on drying** (731)—Dry it in vacuum at 105° for 5 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Limit of mercaptopurine**—Prepare three solutions in 6 N ammonium hydroxide containing, respectively, 20 mg of Azathioprine per mL, 20 mg of USP Azathioprine RS per mL, and 200 µg of USP Mercaptopurine RS, on the anhydrous basis, per mL. Apply 5-µL volumes of the solutions at points about 2 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of microcrystalline cellulose. Allow the spots to dry, and develop the chromatogram in a suitable chamber, using butyl alcohol, previously saturated with 6 N ammonium hydroxide, as the solvent, until the solvent front has moved about 15 cm from the point of application. Remove the plate, air-dry, and locate the spots by viewing under short- and long-wavelength UV light: any spot in the chromatogram from Azathioprine, other than the principal spot, is not more intense than the spot in the chromatogram obtained with USP Mercaptopurine RS (1.0%).

**Assay**—Dissolve about 300 mg of Azathioprine, accurately weighed, in 80 mL of dimethylformamide. Add 5 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with 0.1 N tetrabutylammonium hydroxide VS, using a magnetic stirrer, and taking precautions to prevent absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 27.73 mg of  $C_9H_7N_7O_2S$ .

## Azathioprine Oral Suspension

**DEFINITION**

Azathioprine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ).

Prepare Azathioprine Oral Suspension of 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Azathioprine	5 g
Vehicle: a mixture of Vehicle for Oral Solution, (regular or sugar-free), <i>NF</i> and Vehicle for Oral Suspension, <i>NF</i> (1:1), a sufficient quantity to make	100 mL

If using tablets, comminute them to a fine powder in a suitable mortar, or add *Azathioprine* powder to the mortar. Add about 10 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

**[CAUTION]**—Avoid skin contact or inhalation of azathioprine by using protective gloves and a fume hood or surgical mask.]

**ASSAY****• PROCEDURE**

**Mobile phase:** Dissolve 1.1 g of sodium-1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust with 1 N hydrochloric acid to a pH of 3.5.

**Standard solution:** 25 mg of USP Azathioprine RS, in a 50-mL volumetric flask. Add 1.5 mL of methanol and 0.5 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min. Dilute with methanol to volume. Transfer 10 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^{\circ}$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the Sample into a 100-mL volumetric flask, and dilute with Mobile phase to volume.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

[NOTE—The retention time for the azathioprine peak is about 4 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.3% for replicate injections

**Analysis**

**Samples:** Standard solution and Sample solution  
Calculate the percentage of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of azathioprine in the Standard solution ( $\mu$ g/mL)

$C_U$  = nominal concentration of azathioprine in the Sample solution ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH** <791>: 3.8–4.8

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature, or in a cold place.

**• LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded

**• USP REFERENCE STANDARDS** <11>  
USP Azathioprine RS

**Azathioprine Tablets**

» Azathioprine Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ).

**Packaging and storage**—Protect from light.

**USP Reference standards** <11>—

USP Azathioprine RS

**Identification**—Tablets meet the requirement of the *Thin-layer Chromatographic Identification Test* <201>, the test solution being the filtrate obtained by shaking a quantity of powdered Tablets, equivalent to about 200 mg of azathioprine, with 10 mL of 6 N ammonium hydroxide, the Standard solution being a solution of USP Azathioprine RS in 6 N ammonium hydroxide containing 20 mg per mL, and 5- $\mu$ L portions of each solution being spotted on a thin-layer chromatographic plate coated with a 0.25-mm layer of microcrystalline cellulose, and butyl alcohol, previously saturated with 6 N ammonium hydroxide, being used for developing.

**Dissolution** <711>—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_9H_7N_7O_2S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 280 nm of filtered portions of the solution under test, suitably diluted with Medium, if necessary, in comparison with a Standard solution having a known concentration of USP Azathioprine RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_9H_7N_7O_2S$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

**Mobile phase**—Dissolve 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, add 300 mL of methanol, and mix. Adjust the solution with 1 N hydrochloric acid to a pH of 3.5. Filter the solution through a 0.8- $\mu$ m solvent-resistant membrane, and degas, making adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 25 mg of USP Azathioprine RS, accurately weighed, to a 50-mL volumetric flask. Add about 1.5 mL of methanol and 0.5 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 minutes. Dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 50 mg of azathioprine, and transfer to a 100-mL volumetric flask. Add 25 mL of methanol and 1.0 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 minutes. Dilute with methanol to volume, and mix. Allow the excipients to settle, transfer 10.0 mL of the supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 800 theoretical plates, the tailing factor for the azathioprine peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of azathioprine ( $C_9H_7N_7O_2S$ ) in the portion of Tablets taken by the formula:

$$500(C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Azathioprine RS in the Standard preparation, and  $r_U$  and  $r_S$  are the peak responses for azathioprine obtained from the Assay preparation and the Standard preparation, respectively.

## Azathioprine Sodium for Injection

» Azathioprine Sodium for Injection is a sterile solid prepared by the freeze-drying of an aqueous solution of Azathioprine and Sodium Hydroxide. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), at controlled room temperature.

### USP Reference standards (11)—

USP Azathioprine RS  
USP Mercaptopurine RS  
USP Endotoxin RS

**Completeness of solution** (641)—The contents of 1 container are soluble in 10 mL of water, to give a clear, bright yellow solution, essentially free from foreign matter.

**Identification**—The principal spot in the chromatogram of the specimen under examination obtained in the test for *Limit of mercaptopurine* shows the same  $R_f$  value as that obtained with the solution of USP Azathioprine RS.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mg of azathioprine.

**pH** (791): between 9.8 and 11.0, the contents of 1 container being dissolved in 10 mL of water.

**Water, Method I** (921): not more than 7.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

**Limit of mercaptopurine**—Prepare solutions in dimethylformamide containing, respectively, 10 mg of Azathioprine Sodium for Injection per mL, 10 mg of USP Azathioprine RS per mL, and 100  $\mu$ g of USP Mercaptopurine RS per mL. Apply 15  $\mu$ L of the USP Mercaptopurine RS solution and 5- $\mu$ L portions of the other two solutions at points about 2 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 250- $\mu$ m layer of microcrystalline cellulose. Allow the spots to dry, and develop the chromatogram in a suitable chamber, using butyl alcohol, previously saturated with 5 N ammonium hydroxide, as the solvent, until the solvent front has moved about 15 cm from the point of application. Remove the plate, air-dry, and locate the spots by viewing under short- and long-wavelength UV light: any spot in the chromatogram from azathioprine, other than the principal spot, is not more intense than the spot in the chromatogram obtained with USP Mercaptopurine RS (3.0%).

**Other requirements**—It meets the requirements under *Injections* (1) and *Uniformity of Dosage Units* (905).

### Assay—

**Standard preparation**—Transfer 25 mg of USP Azathioprine RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 2.5 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix.

**Assay preparation**—Transfer the contents of 1 vial of Azathioprine Sodium for Injection with the aid of water to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into another 100-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix.

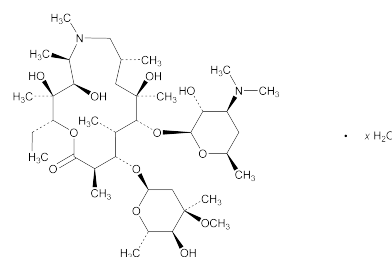
**Procedure**—Transfer 20 mL each of the *Standard preparation* and the *Assay preparation*, separately, to polarographic cells, and deaerate for 10 minutes with nitrogen that previously has been saturated with 0.1 N sulfuric acid. Blanket the solution with saturated nitrogen, insert the dropping mercury electrode of a suitable polarograph, and record the

polarogram from  $-0.60$  volt to  $-1.00$  volt, using a saturated calomel electrode as the reference electrode. Determine the height of the diffusion current as the difference between the residual current and diffusion current plateau. Calculate the quantity, in mg, of azathioprine ( $C_9H_7N_7O_2S$ ) in the volume of solution from the vial used for the *Assay preparation* taken by the formula:

$$0.1 C(i_d)_U / (i_d)_S$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Azathioprine RS in the *Standard preparation*; and  $(i_d)_U$  and  $(i_d)_S$  are the diffusion currents of the *Assay preparation* and the *Standard preparation*, respectively.

## Azithromycin



$C_{38}H_{72}N_2O_{12}$  748.98

$C_{38}H_{72}N_2O_{12} \cdot H_2O$  767.00

$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$  785.02

1-Oxa-6-azacyclopentadecan-15-one, 13-[(2,6-dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-, [2R(2R\*,3S\*,4R\*,5R\*,8R\*,10R\*,11R\*,12S\*,13S\*,14R\*)];

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one;

9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A

Anhydrous [83905-01-5].

Monohydrate [121470-24-4].

Dihydrate [117772-70-0].

### DEFINITION

Azithromycin is anhydrous or contains one or two molecules of water of hydration. It contains the equivalent of NLT 945  $\mu$ g and NMT 1030  $\mu$ g of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a water bath, and dry at  $80^\circ$  for 30 min under vacuum. Perform the test on the residues.
- **B.** The retention time of the azithromycin peak in the *Sample solution* corresponds to that in the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of

acetonitrile. Adjust with 6 mL of 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ , and filter.

**Standard stock solution:** 0.165 mg/mL of USP Azithromycin RS in acetonitrile. [NOTE—Sonicate as necessary.]

**Standard solution:** 3.3 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**Sample stock solution:** 0.165 mg/mL of Azithromycin in acetonitrile. [NOTE—Sonicate as necessary.]

**Sample solution:** 3.3 µg/mL of azithromycin from the *Sample stock solution* in *Mobile phase*

**System suitability stock solution:** 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). [NOTE—Dissolve in acetonitrile, using 10% of the final volume. Swirl and sonicate to dissolve. Dilute with *Mobile phase* to volume.]

**System suitability solution:** 3.3 µg/mL of azithromycin from the *Sample stock solution* and 3.2 µg/mL of azaerythromycin A from the *System suitability stock solution* in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Detector type:** Dual glassy carbon electrodes

**Detector mode:** Oxidative screen mode

**Detector settings:** Electrode 1 set at  $+0.70 \pm 0.05$  V, electrode 2 set at  $+0.82 \pm 0.05$  V, background current optimized to  $85 \pm 15$  nanoamperes

#### Column

**Guard column:** 4.6-mm × 5-cm; 5-µm packing L29

**Analytical column:** 4.6-mm × 15-cm; 5-µm packing L29 or 3-µm packing L49 without the guard column

**Flow rate:** 1.5 mL/min

**Injection size:** 50 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively. The relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor range:** 0.9–1.5 for azithromycin, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of  $C_{38}H_{72}N_2O_{12}$  in each mg of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution*

$C_U$  = concentration of Azithromycin in the *Sample solution*

$P$  = potency of USP Azithromycin RS (µg/mg of azithromycin)

**Acceptance criteria:** 945–1030 µg/mg on the anhydrous basis

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.3%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

- **HEAVY METALS, Method II** (231): NMT 25 ppm

#### Organic Impurities

- **PROCEDURE 1** [NOTE— Use *Organic Impurities, Procedure 2* when the impurity profile includes azithromycin related compound F.]

[NOTE— Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Proceed as directed in the *Assay*.

**Solution A:** 2.7 mg/mL of monobasic potassium phosphate in water. Adjust with 10 N potassium hydroxide to a pH of  $7.5 \pm 0.1$ .

**Diluent:** Acetonitrile and *Solution A* (1:3)

**Standard stock solution:** 45 µg/mL of USP Desosaminylazithromycin RS, 105 µg/mL of USP *N*-Demethylazithromycin RS, and 160 µg/mL of USP Azithromycin RS in acetonitrile

**Standard solution:** 0.9 µg/mL of USP Desosaminylazithromycin RS, 2.1 µg/mL of USP *N*-Demethylazithromycin RS, and 3.2 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Diluent*

**Sample solution:** 33 mg of Azithromycin in a 100-mL volumetric flask. Add 5 mL of acetonitrile, and sonicate for about 20 s to dissolve. Dilute with *Diluent* to volume.

[NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Detector type:** Dual glassy carbon electrodes

**Detector mode:** Oxidative screen mode

**Detector settings:** Electrode 1 set at  $+0.70 \pm 0.05$  V, electrode 2 set at  $+0.85 \pm 0.05$  V, background current optimized to  $95 \pm 25$  nanoamperes

[NOTE— In general, maintain electrode 1 at 0.12 V less than electrode 2, and maintain the electrodes at a constant temperature of about 26°.]

#### Column

**Guard column:** 4.6-mm × 5-cm; 5-µm packing L29

**Analytical column:** 4.6-mm × 15-cm; 5-µm packing L29 or 3-µm packing L49 without the guard column.

**Flow rate:** 0.4 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates for the azithromycin peak

**Tailing factor:** NMT 1.5 for each peak

**Relative standard deviation:** NMT 5% for each of these compounds

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE— Record the *Sample solution* chromatograms for NLT 3.3 times the retention time of the azithromycin peak.]

Calculate the percentages of desosaminylazithromycin and *N*-demethylazithromycin in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak area for the relevant analyte from the *Sample solution*

$r_S$  = peak area for the relevant analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$F$  = unit conversion (0.001 mg/µg)

Calculate the percentages of other related substances in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

- $r_U$  = peak area of each additional impurity from the *Sample solution*  
 $r_S$  = peak area of the azithromycin peak from the *Standard solution*  
 $C_S$  = concentration of USP Azithromycin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\text{mg/mL}$ )  
 $F$  = unit conversion ( $0.001 \text{ mg}/\mu\text{g}$ )

**Acceptance criteria:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desosaminylazithromycin	0.38	0.3
N-demethylazithromycin	0.54	0.7
Total impurities	—	3.0

- PROCEDURE 2** [NOTE— Use *Organic Impurities, Procedure 2* when the impurity profile includes azithromycin related compound F.]

**Solution A:** 1.8 mg/mL of anhydrous dibasic sodium phosphate in water. Adjust with 1 N sodium hydroxide or 10% phosphoric acid to a pH of 8.9.

**Solution B:** Acetonitrile and methanol (3:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

**Solution C:** 1.73 mg/mL of monobasic ammonium phosphate. Adjust with ammonia TS to a pH of  $10.0 \pm 0.05$ .

**Solution D:** Methanol, acetonitrile, and *Solution C* (7:6:7)

**System suitability solution:** 0.0165 mg/mL of USP Azithromycin Related Compound F RS and 0.027 mg/mL of USP Desosaminylazithromycin RS in *Solution D*

**Standard solution:** 86  $\mu\text{g/mL}$  of USP Azithromycin RS in *Solution D*

**Sample solution:** 8.6 mg/mL of Azithromycin in *Solution D*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 60°

**Flow rate:** 1 mL/min

**Injection size:** 50  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Peak-to-valley ratio:** NLT 1.4, *System suitability solution*

[NOTE— The calculation formula for peak-to-valley ratio is:

$$\text{Result} = H_P/H_V$$

$H_P$  = height above the baseline of the desosaminylazithromycin peak

$H_V$  = height above the baseline of the lowest point of the curve separating the desosaminylazithromycin and azithromycin related compound F peaks.]

**Tailing factor:** 0.8–1.5, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE— Disregard peaks eluting before azithromycin 3'-N-oxide and after 3-deoxyazithromycin (azithromycin B). Disregard peaks with a response less than 0.1 times the response of the azithromycin peak in the *Standard solution* (0.1%).]

Calculate the percentage of each related compound in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times 100/F_2$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of azithromycin in the *Sample solution* ( $\text{mg/mL}$ )

$F_1$  = unit conversion factor ( $0.001 \text{ mg}/\mu\text{g}$ )

$F_2$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria:** See *Impurity Table 2*.

#### SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation <781S>:**  $-45^\circ$  to  $-49^\circ$ , at 20°

**Sample solution:** 20 mg/mL, in dehydrated alcohol

- CRYSTALLINITY <695>:** Meets the requirements except, where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions

- pH <791>:** 9.0–11.0

**Sample stock solution:** 4 mg/mL in methanol

**Sample solution:** 2 mg/mL from the *Sample stock solution* in a mixture of methanol and water (1:1)

- WATER DETERMINATION, Method I <921>**

Where it is labeled as **anhydrous**: NMT 2.0%

Where it is labeled as the **dihydrate**: 4.0%–5.0%

Where it is labeled as the **monohydrate**: 1.8%–4.0%, except that it may be 4.0%–6.5% when the requirements of the *Loss on Drying* test are met

- LOSS ON DRYING:** Where it is labeled as Azithromycin monohydrate and has a water content of 4.0%–6.5% (see *Thermal Analysis* <891>).

[NOTE—The quantity taken for this procedure may be adjusted, if necessary, for instrument sensitivity.]

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis in an appropriately calibrated instrument, using about 10 mg of Azithromycin. Heat the specimen at the rate of 10°/min between ambient temperature and 150° in an atmosphere of nitrogen at a constant flow rate of about 35 mL/min. From the thermogram plot the derivatives of the loss on drying (percent loss/min), identify the inflection points of the two weight loss steps at about 70° and 130°.

**Acceptance criteria:** It loses NMT 4.5% of its weight between ambient temperature and the inflection point at about 70°, and 1.8%–2.6% between the inflection point at about 70° and the inflection point at about 130°.

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor (F <sub>2</sub> )	Acceptance Criteria, NMT (%)
Azithromycin <i>N</i> -oxide <sup>a</sup>	0.29	0.43	0.5
3'-( <i>N,N</i> -Didemethyl)-3'- <i>N</i> -formylazithromycin <sup>b</sup>	0.37	1.7	0.5
3'-( <i>N,N</i> -Didemethyl) azithromycin (aminoazithromycin) <sup>c</sup>	0.43	1.0	0.5
Azithromycin related compound F <sup>d</sup>	0.51	3.8	0.5
Desosaminylazithromycin <sup>e</sup>	0.54	1.0	0.3
<i>N</i> -Demethylazithromycin <sup>f</sup>	0.61	1.0	0.7
Azithromycin C (3'- <i>O</i> -demethylazithromycin) <sup>g</sup>	0.73	1.0	0.5
3'-De(dimethylamino)-3'-oxoazithromycin <sup>h</sup>	0.76	1.5	0.5
Azaerythromycin A <sup>i</sup>	0.83	1.0	0.5
Azithromycin impurity P <sup>j</sup>	0.92	1.0	0.2
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin <sup>k</sup>	1.23	1.0	0.5
3'- <i>N</i> -Demethyl-3'- <i>N</i> -[(4-methylphenyl)sulfonyl]-azithromycin <sup>l</sup>	1.26	1.0	0.5
3-Deoxyazithromycin (azithromycin B) <sup>m</sup>	1.31	1.0	1.0

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> 3'-*N*-Demethyl-3'-*N*-formylazithromycin.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>i</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>j</sup> Specified unidentified impurity.

<sup>k</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one dihydrate.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>m</sup> (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

Impurity Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor (F <sub>2</sub> )	Acceptance Criteria, NMT (%)
Any individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> 3'-*N*-Demethyl-3'-*N*-formylazithromycin.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>i</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>j</sup> Specified unidentified impurity.

<sup>k</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one dihydrate.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>m</sup> (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
  - **LABELING:** Label it to indicate whether it is anhydrous, or the monohydrate or the dihydrate. The amorphous form is so labeled. Where the quantity of azithromycin is indicated in the labeling of any preparation containing Azithromycin, this shall be understood to be in terms of anhydrous azithromycin (C<sub>38</sub>H<sub>72</sub>N<sub>2</sub>O<sub>12</sub>). The labeling states with which *Organic Impurities* procedure the article complies, if other than *Procedure 1*.
  - **USP REFERENCE STANDARDS** (11)
    - USP Azithromycin RS
    - USP Azaerythromycin A RS
    - USP Azithromycin Identity RS

A mixture of azithromycin, 3'-(*N,N*-dimethyl-3'-*N*-formylazithromycin, 3'-*N*-demethyl-3'-*N*-formylazithromycin (Rotamer 1), 3'-*N*-demethyl-3'-*N*-formylazithromycin (Rotamer 2), 3'-de(dimethylamino)-3'-oxoazithromycin, 2-desethyl-2-propylazithromycin, 3-deoxyazithromycin and 3'-*N*-demethyl-3'-*N*-[(4-methylphenyl)sulfonyl]azithromycin.

  - USP Desosaminylazithromycin RS
  - USP *N*-Demethylazithromycin RS
- (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,

14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{37}H_{70}N_2O_{12}$  734.96

USP Azithromycin Related Compound F RS

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{38}H_{70}N_2O_{13}$  762.97

## Azithromycin Capsules

### DEFINITION

Azithromycin Capsules contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

### IDENTIFICATION

- A.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ , and pass through a suitable filter.

**Standard stock solution:** 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.

**Standard solution:** 3.3  $\mu$ g/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**System suitability stock solution:** 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile, using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

**System suitability solution:** 3.2  $\mu$ g/mL of azaerythromycin A from the *System suitability stock solution* and 3.3  $\mu$ g/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Remove, as completely as possible, the contents of NLT 20 Capsules. Prepare a 1-mg/mL solution of anhydrous azithromycin in acetonitrile. Dissolve a portion of the mixed Capsule contents first in 70% of the final volume of acetonitrile, and shake by mechanical means for 30 min. Dilute with acetonitrile to volume. Place 40 mL of the resulting suspension in a centrifuge tube, and centrifuge. Use the supernatant to prepare the *Sample solution*.

**Sample solution:** 3.2  $\mu$ g/mL of azithromycin from the *Sample stock solution* in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:**  $+0.70 \pm 0.05$  V

**Electrode 2:**  $+0.82 \pm 0.05$  V

**Background current:**  $85 \pm 15$  nanoamperes

#### Columns

**Guard:** 4.6-mm  $\times$  5-cm; 5- $\mu$ m packing L29

**Analytical:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L29 or 3- $\mu$ m packing L49 without the guard column

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* ( $\mu$ g/mL)

$P$  = potency of azithromycin in USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Medium:** pH 6.0 sodium phosphate buffer (Prepare 6 L of 0.1 M dibasic sodium phosphate. Adjust with about 40 mL of hydrochloric acid to a pH of  $6.0 \pm 0.05$ , and add 600 mg of trypsin); 900 mL

**Apparatus 2:** 100 rpm

**Time:** 45 min

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.3 mg/mL of USP Azithromycin RS in *Medium*. Sonicate briefly to dissolve.

**Standard solution:** 3.84  $\mu$ g/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.5- $\mu$ m or finer pore size. Transfer 2.0 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer 4.0 mL of this solution to a second 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) dissolved using the procedure in the *Assay*, making any necessary modifications.

Calculate the percentage of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 5.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Where packaged in unit-of-use containers, each container contains six 250-mg Capsules, and the label indicates the intended sequential day of use for each Capsule.
- **USP REFERENCE STANDARDS** (11)
  - USP Azaerythromycin A RS
  - USP Azithromycin RS

# Azithromycin for Injection

## DEFINITION

Azithromycin for Injection is a sterile, dry mixture of azithromycin and a suitable stabilizing agent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

## IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

**Buffer:** 6.7 mg/mL of dibasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (52:48). Adjust with 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ .

**Diluent:** Acetonitrile and water (52:48)

**System suitability solution:** 1 mg/mL each of USP Azaerythromycin A RS and USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and then dilute with water to volume.

**Standard solution:** 1 mg/mL of USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and dilute with water to volume.

**Sample solution:** Equivalent to 1 mg/mL of azithromycin from Azithromycin for Injection in *Diluent* prepared as follows. Reconstitute 3 vials individually as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Columns**

**Guard:** 4.6-mm  $\times$  1-cm; 5- $\mu$ m packing L67

**Analytical:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L67

**Temperature**

**Column:** 40°

**Autosampler:** 15°

**Flow rate:** 1 mL/min

**Injection size:** 15  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.68 and 1.0, respectively.]

## Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Tailing factor:** NLT 0.9 and NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

## Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

## IMPURITIES

[NOTE—The test for *Limit of Azithromycin N-Oxide*, *Desosaminylazithromycin*, and *N-Demethylazithromycin* does not quantify aminoazithromycin, formamido analog, methylformamido analog, and 3'-de(dimethylamino)-3'-oxoazithromycin. If these impurities are part of the impurity profile, the *Limit of Aminoazithromycin*, *Formamido Analog*, *Methylformamido Analog*, and *3'-De(dimethylamino)-3'-oxoazithromycin* test is recommended in addition to the test for *Limit of Azithromycin N-Oxide*, *Desosaminylazithromycin*, and *N-Demethylazithromycin*.]

- **LIMIT OF AZITHROMYCIN N-OXIDE, DESOSAMINYLAZITHROMYCIN, AND N-DEMETHYLAZITHROMYCIN**

**Buffer:** 3.5 g/L of dibasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (23:77). Adjust with 5 N potassium hydroxide to a pH of  $10.55 \pm 0.05$ .

**Standard stock solution:** 50  $\mu$ g/mL of USP Azithromycin N-oxide RS, 45  $\mu$ g/mL of USP Desosaminylazithromycin RS, and 160  $\mu$ g/mL each of USP N-Demethylazithromycin RS and USP Azithromycin RS in acetonitrile. Sonicate if necessary to dissolve.

**Standard solution:** 1  $\mu$ g/mL of azithromycin N-oxide, 0.9  $\mu$ g/mL of desosaminylazithromycin, 3.2  $\mu$ g/mL each of N-demethylazithromycin and azithromycin from *Standard stock solution* in *Mobile phase*

**Sample solution:** Equivalent to 0.3 mg/mL of azithromycin in *Mobile phase* from Azithromycin for Injection

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual series glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:**  $+0.70 \pm 0.05V$

**Electrode 2:**  $+0.82 \pm 0.05V$

**Background current:**  $95 \pm 25$  nanoamperes

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L49

**Flow rate:** 1 mL/min

**Injection size:** 50  $\mu$ L

**Autosampler temperature:** 5°

**System suitability**

**Sample:** *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

## Suitability requirements

**Tailing factor:** NMT 2.0 for azithromycin and NMT 2.6 for N-demethylazithromycin



**Relative standard deviation:** NMT 10.0% for azithromycin *N*-oxide, desosaminylazithromycin, *N*-demethylazithromycin, and azithromycin

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each specified impurity from the *Sample solution*

$r_S$  = peak response of each specified impurity from the *Standard solution*

$C_S$  = concentration of the relevant impurity USP RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of relevant USP RS (mg/mg)

**Acceptance criteria:** See Table 1. The reporting level for impurities is 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azithromycin <i>N</i> -oxide <sup>a</sup>	0.17	1.0
Desosaminylazithromycin <sup>b</sup>	0.27	0.3
Erythromycin A oxime <sup>c,d</sup>	0.35	—
<i>N</i> -Demethylazithromycin <sup>e</sup>	0.50	1.0
Azaerythromycin A <sup>c,f</sup>	0.85	—
Azithromycin	1.00	—

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-(dimethylazinoyl)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>c</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>d</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-6-[[[3,4,6-Trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethylloxacyclotetradecan-2-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>f</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

#### • LIMIT OF AMINOAZITHROMYCIN, FORMAMIDO ANALOG, METHYLFORMAMIDO ANALOG, AND 3'-DE(DIMETHYLAMINO)-3'-OXOAZITHROMYCIN (if present)

**Buffer:** 3.5 g/mL of dibasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (46:54). Adjust with 10 N potassium hydroxide to a pH of 11.0  $\pm$  0.1.

**Diluent:** Acetonitrile and water (46:54)

**Blank:** Use the *Diluent*.

**Standard stock solution:** 0.09 mg/mL of USP Desosaminylazithromycin RS, 0.21 mg/mL of USP *N*-

Demethylazithromycin RS, and 0.30 mg/mL of USP Azithromycin RS in acetonitrile

**Standard solution:** 0.0018 mg/mL of desosaminylazithromycin, 0.0042 mg/mL of *N*-demethylazithromycin, and 0.006 mg/mL of azithromycin in *Diluent*

**Sample solution:** Equivalent to 0.6 mg/mL of azithromycin from Azithromycin for Injection in *Diluent*. Reconstitute 3 vials individually, as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*. The *Sample solution* must be injected immediately after preparation.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual series glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:** +0.70  $\pm$  0.05V

**Electrode 2:** +0.82  $\pm$  0.05V

**Background current:** 95  $\pm$  25 nanoamperes

#### Columns

**Guard:** 4.6-mm  $\times$  1-cm; 5- $\mu$ m packing L67

**Analytical:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L67

#### Temperature

**Column:** 40°

**Autosampler:** 15°

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.5 between desosaminylazithromycin and *N*-demethylazithromycin

**Tailing factor:** NMT 1.5 for azithromycin

**Relative standard deviation:** NMT 5% for azithromycin

#### Analysis

**Samples:** *Blank*, *Standard solution*, and *Sample solution*

Disregard any peaks corresponding to those obtained from the *Blank*.

Calculate the percentage of each impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether <sup>a,b</sup>	0.20	—
3'-( <i>N,N</i> -Didemethyl)azithromycin (aminoazithromycin) <sup>c</sup> + 3'-( <i>N,N</i> -didemethyl)-3'- <i>N</i> -formylazithromycin (formamido analog) <sup>d</sup>	0.25	1.0
Azithromycin F <sup>a,e</sup>	0.30	—
Desosaminylazithromycin <sup>f,g</sup>	0.31	—
3'- <i>N</i> -Demethyl-3'- <i>N</i> -formylazithromycin (methylformamido analog) <sup>h</sup>	0.32	1.0
<i>N</i> -Demethylazithromycin <sup>f,i</sup>	0.35	—
Erythromycin A oxime <sup>a,j</sup>	0.42	—
Azaerythromycin A <sup>a,k</sup>	0.63	—
3'-De(dimethylamino)-3'-oxoazithromycin <sup>l</sup>	0.72	1.0
3'-Demethyl-3'- <i>N</i> -[(4-methylphenyl)sulfonyl]azithromycin <sup>a,m</sup>	0.85	—
Azithromycin	1.00	—
Azithromycin B (3-Deoxyazithromycin) <sup>a,n</sup>	1.64	—

<sup>a</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>b</sup> (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,Z)-12-[[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxo-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-amino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>d</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12-hexamethyl-14-hydroxymethyl-11-[[[3-dimethylamino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>f</sup> These impurities are controlled using the Limit of Azithromycin *N*-Oxide Desosaminylazithromycin, and *N*-Demethylazithromycin test. They are listed here for information only.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>i</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>j</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,E)-6-[[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

<sup>k</sup> 9-Deoxo-9a-aza-9a-homoerythromycin A.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-oxo-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>m</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-[*N*-(4-acetamidophenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>n</sup> (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>o</sup> Total impurities includes desosaminylazithromycin and *N*-demethylazithromycin.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other unspecified impurity	—	0.2
Total impurities <sup>o</sup>	—	3.0

<sup>a</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>b</sup> (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,Z)-12-[[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxo-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-amino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>d</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12-hexamethyl-14-hydroxymethyl-11-[[[3-dimethylamino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>f</sup> These impurities are controlled using the Limit of Azithromycin *N*-Oxide Desosaminylazithromycin, and *N*-Demethylazithromycin test. They are listed here for information only.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>i</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>j</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,E)-6-[[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

<sup>k</sup> 9-Deoxo-9a-aza-9a-homoerythromycin A.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-oxo-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>m</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3-[*N*-(4-acetamidophenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>n</sup> (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>o</sup> Total impurities includes desosaminylazithromycin and *N*-demethylazithromycin.

## SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.7 USP Endotoxin Units/mg of azithromycin.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements
- **PH (791):** 6.4–6.8, determined in a solution constituted as directed in the labeling
- **WATER DETERMINATION, Method I (921):** NMT 2.0%
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections (1)*.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described under *Injections (1)*, *Containers for Sterile Solids*. Store at controlled room temperature.
- **LABELING:** It meets the requirements for *Injections (1)*, *Labeling*.

• **USP REFERENCE STANDARDS** <11>

- USP Azaerythromycin A RS  
9-Deoxy-9a-aza-9a-homoerythromycin A.  
 $C_{37}H_{70}N_2O_{12}$  734.96  
USP Azithromycin RS  
USP Azithromycin N-Oxide RS  
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylaziridinyl)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.  
 $C_{38}H_{72}N_2O_{13}$  764.98  
USP N-Demethylazithromycin RS  
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.  
 $C_{37}H_{70}N_2O_{12}$  734.96  
USP Desosaminylazithromycin RS  
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.  
 $C_{30}H_{58}N_2O_9$  590.79  
USP Endotoxin RS

## Azithromycin for Oral Suspension

### DEFINITION

Azithromycin for Oral Suspension is a dry mixture of Azithromycin and one or more buffers, sweeteners, diluents, anticaking agents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

### IDENTIFICATION

- **A.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ , and pass through a suitable filter.

**Diluent:** Dissolve 2.2 g of monobasic potassium phosphate in 1590 mL of water, and add 600 mL of isopropyl alcohol, 480 mL of alcohol, and 330 mL of acetonitrile. Adjust with 10 N potassium hydroxide to a pH of  $8.4 \pm 0.1$ , and shake by mechanical means for 30 min.

**Standard stock solution:** 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.

**Standard solution:** 3.3  $\mu$ g/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**System suitability stock solution:** 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

**System suitability solution:** 3.2  $\mu$ g/mL of azaerythromycin A from the *System suitability stock solution* and 3.3  $\mu$ g/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample stock solution 1** (where packaged in a single-unit container): 2 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Transfer the contents of a container of Azithromycin for Oral Suspension to a suitable volumetric flask. Add *Diluent* equal to 70% of the volume of the flask, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume. Transfer 40 mL of this suspension to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 1*.

**Sample stock solution 2** (where packaged in a multiple-unit container): 0.4 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Constitute Azithromycin for Oral Suspension as directed in the labeling. Transfer a suitable aliquot of the suspension so obtained, freshly mixed and free from air bubbles to a suitable volumetric flask to obtain to obtain a final concentration of 0.4 mg/mL. Add *Diluent* equal to 70% of the final volume, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Transfer 40 mL of the suspension so obtained to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 2*.

**Sample solution 1:** 3.2  $\mu$ g/mL of azithromycin from *Sample stock solution 1* in *Mobile phase*

**Sample solution 2:** 4  $\mu$ g/mL of azithromycin from *Sample stock solution 2* in *Mobile phase*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:**  $+0.70 \pm 0.05$  V

**Electrode 2:**  $+0.82 \pm 0.05$  V

**Background current:**  $85 \pm 15$  nanoamperes

### Column

**Guard:** 4.6-mm  $\times$  5-cm; 5- $\mu$ m packing L29

**Analytical:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L29 or 3- $\mu$ m packing L49 without the guard column

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Where packaged in a single-unit container**

Calculate the percentage of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from *Sample solution 1*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in *Sample solution 1* (mg/mL)

$P$  = potency of azithromycin in USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Where packaged in a multiple-unit container**

Calculate the percentage of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from *Sample solution 2*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in *Sample solution 2* (mg/mL)

$P$  = potency of azithromycin in USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DELIVERABLE VOLUME** (698): Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for a solid packaged in single-unit containers

**SPECIFIC TESTS**

• **pH** (791)

For a solid packaged in single-unit containers: 9.0–11.0, in the suspension constituted as directed in the labeling

For a solid packaged in multiple-unit containers: 8.5–11.0, in the suspension constituted as directed in the labeling

- **WATER DETERMINATION, Method I** (921): NMT 1.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Azithromycin RS  
USP Azaerythromycin RS

## Azithromycin Tablets

**DEFINITION**

Azithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Solution A:** A solution containing 4.4 mg/mL of dibasic potassium phosphate and 0.5 mg/mL of sodium 1-oc-tanesulfonate. Adjust with phosphoric acid to a pH of  $8.20 \pm 0.05$ .

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (9:3:8)

**Solution B:** 1.7 mg/mL of monobasic ammonium phosphate. Adjust with ammonium hydroxide to a pH of  $10.00 \pm 0.05$ .

**Diluent A:** Methanol, acetonitrile, and *Solution B* (7:6:7)

**Standard solution:** Dissolve USP Azithromycin RS in *Diluent A* using about 75% of the final volume, sonicate to dissolve, dilute with *Diluent A* to volume, and mix to obtain a solution having a known concentration of 0.4 mg/mL of azithromycin.

**System suitability stock solution:** 0.2 mg/mL of USP Azaerythromycin A RS in acetonitrile. [NOTE—Sonicate if necessary to dissolve.]

**System suitability solution:** 0.02 mg/mL of azaerythromycin A from the *System suitability stock solution* and

0.02 mg/mL of azithromycin from the *Standard solution* in *Diluent A*

**Sample stock solution:** Weigh and finely powder NLT 20 Tablets. Transfer an equivalent to 667 mg of azithromycin to a 200-mL volumetric flask. Add 75 mL of *Diluent A*, and sonicate for NLT 15 min. Shake by mechanical means for NLT 15 min. Allow the solution to equilibrate to room temperature, dilute with *Diluent A* to volume, and mix.

**Sample solution:** 0.4 mg/mL of azithromycin from the *Sample stock solution* in *Diluent A*. Pass through a filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 50°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Identify the peaks by their relative retention times, which are 0.64 and 1.0 for azaerythromycin A and azithromycin, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{38}H_{72}N_2O_{12}$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of azithromycin from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg)

$F$  = unit conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISSOLUTION** (711)

**Medium:** pH 6.0 phosphate buffer; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Solution A and Mobile phase:** Proceed as directed in the *Assay*.

**Diluent:** 17.5 mg/mL of dibasic potassium phosphate. Adjust with phosphoric acid to a pH of  $8.00 \pm 0.05$ . Prepare a mixture of this solution and acetonitrile (80:20).

**Standard stock solution:** Dissolve USP Azithromycin RS in *Medium* to obtain a solution having a known concentration of about (L/1000) mg/mL, where L is the Tablet label claim, in mg.

**Standard solution:** Dilute the *Standard stock solution* with *Diluent* to obtain a solution having a known concentration of about (L/2000) mg/mL, where L is the Tablet label claim, in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Dilute a portion of the filtrate with *Diluent* to obtain a

solution having a theoretical concentration of about (L/2000) mg/mL, where L is the Tablet label claim, in mg, assuming complete dissolution.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 50°

**Flow rate:** 1.5 mL/min

**Injection size:** 50 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements:**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amount of C<sub>38</sub>H<sub>72</sub>N<sub>2</sub>O<sub>12</sub> dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of azithromycin from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of C<sub>38</sub>H<sub>72</sub>N<sub>2</sub>O<sub>12</sub> is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### PROCEDURE

[NOTE— Use low-actinic glassware. Refrigerate the *Standard solution* and the *Sample solution* after preparation and during analysis, using a refrigerated autosampler set at 4°. The solutions must be analyzed within 24 h of preparation.]

**Solution B, Diluent A, and System suitability solution:** Proceed as directed in the *Assay*.

**Solution C:** 1.8 mg/mL of dibasic sodium phosphate in water

**Solution D:** Acetonitrile and methanol (3:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution C (%)	Solution D (%)
0	50	50
25	50	50
30	45	55
40	40	60
55	35	65
60	35	65
61	50	50
70	50	50

**Diluent B:** Methanol and *Solution B* (1:1)

**Blank:** Use *Diluent A*.

**Standard stock solution:** Use the *Standard solution* as directed in the *Assay*.

**Standard solution:** 0.02 mg/mL of azithromycin from the *Standard stock solution* in *Diluent A*

**Sensitivity solution:** 0.004 mg/mL of azithromycin from the *Standard solution* in *Diluent A*

**Sample stock solution:** Weigh and finely powder NLT 20 Tablets. Transfer an equivalent to 1335 mg of azithromycin to a 100-mL volumetric flask. Add 75 mL of acetonitrile, and sonicate for NLT 15 min. Shake by

mechanical means for NLT 15 min. Allow the solution to equilibrate to room temperature, dilute with acetonitrile to volume, and mix.

**Sample solution:** Centrifuge an aliquot of the *Sample stock solution* for 15 min. Transfer 3.0 mL of the supernatant to a 10-mL volumetric flask, dilute with *Diluent B* to volume, and mix to obtain a solution having a nominal concentration of about 4 mg/mL of azithromycin. Pass through a filter of 0.45-μm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 60°

**Flow rate:** 0.8 mL/min

**Autosampler temperature:** 4°

**Injection size:** 100 μL

#### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

**Suitability requirements**

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

#### Analysis

**Samples:** *Blank* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS (μg/mg)

$F_1$  = unit conversion factor, 0.001 mg/μg

$F_2$  = relative response factor (see *Impurity Table 1*)

#### Acceptance criteria

[NOTE—The reporting level for impurities is 0.1%. Disregard any peaks in the *Sample solution* that correspond to peaks in the *Blank*.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin 3'-N-oxide	0.28	0.45	1.0
3'-(N,N-Didemethyl)-3'-N-formylazithromycin	0.38	1.9	1.0
3'-(N,N-Didemethyl) azithromycin (aminoazithromycin)	0.40	0.52	0.5
Desosaminylazithromycin	0.47	1.1	0.5

<sup>a</sup> 3'-(N-Demethyl)-3'-N-formylazithromycin.

<sup>b</sup> These compounds are synthetic process impurities of azithromycin. They are controlled in the drug substance and are listed here for information only. The total impurities specification does not include these impurities.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin related compound F <sup>a</sup>	0.53	4.8	1.0
3'-N-Demethyl-azithromycin	0.57	0.53	0.7
3'-De(dimethyl-amino)-3'-oxo-azithromycin	0.78	1.6	1.0
6-Demethylazithromycin (azaerythromycin A) <sup>b</sup>	0.82	—	—
Azithromycin	1.0	—	—
3-Deoxyazithromycin (azithromycin B) <sup>b</sup>	1.3	—	—
3'-N-Demethyl-3'-N-[(4-methylphenyl)sulfonyl]azithromycin <sup>b</sup>	1.4	—	—
Any other unspecified impurity	—	1.0	0.2
Total impurities <sup>b</sup>	—	—	5.0

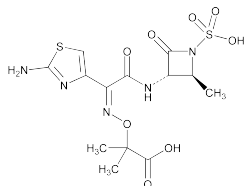
<sup>a</sup> 3'-(N-Demethyl)-3'-N-formylazithromycin.

<sup>b</sup> These compounds are synthetic process impurities of azithromycin. They are controlled in the drug substance and are listed here for information only. The total impurities specification does not include these impurities.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Azaerythromycin A RS  
USP Azithromycin RS

### Aztreonam



$C_{13}H_{17}N_5O_8S_2$  435.43  
Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methyl-, [2S-[2 $\alpha$ ,3 $\beta$ (Z)]]-;  
(Z)-2-[[[1-(2-Amino-4-thiazolyl)[[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropanoic acid [78110-38-0].

#### DEFINITION

Aztreonam, which may be anhydrous or hydrated, contains NLT 92.0% and NMT 105.0% of  $C_{13}H_{17}N_5O_8S_2$ , calculated on the anhydrous and solvent-free basis.

#### IDENTIFICATION

- **INFRARED ABSORPTION** <197K>: If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the reference

standard in equal volumes of methanol. [NOTE—To achieve a complete dissolution, it is suggested to use about 25 mL of methanol for each 50 mg of material, and stir the mixture for 40 min at room temperature.] Evaporate the solutions to dryness under vacuum, and dry at 40° for 4 h under vacuum. Perform the test on the residues.

#### ASSAY

##### • PROCEDURE

[NOTE—Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to prevent isomerization of aztreonam Z-isomer to aztreonam E-isomer.]

**Buffer:** 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (1:4)

**System suitability solution:** 1 mg/mL of USP Aztreonam RS and 1 mg/mL of USP Aztreonam E-Isomer RS in *Mobile phase*

**Standard solution:** 1 mg/mL of USP Aztreonam RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Aztreonam in *Mobile phase*

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

##### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for aztreonam and aztreonam E-isomer are 1.0 and 1.8, respectively.]

##### Suitability requirements

**Resolution:** NLT 2.0 between aztreonam and aztreonam E-isomer, *System suitability solution*

**Tailing factor:** NMT 2 for aztreonam, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)

$P$  = potency of USP Aztreonam RS ( $\mu$ g/mg)

$F$  = unit conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 92.0%–105.0% on the anhydrous and solvent-free basis

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION** <281>: NMT 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

- **HEAVY METALS, Method II** <231>: NMT 30 ppm

##### Organic Impurities

##### • PROCEDURE

[NOTE— Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to

prevent isomerization of aztreonam Z-isomer to aztreonam E-isomer.]

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of aztreonam from the *Standard solution*  
 $C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)  
 $P$  = potency of USP Aztreonam RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = unit conversion factor, 0.001  $\text{mg}/\mu\text{g}$

#### Acceptance criteria

Individual impurities: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Open-ring aztreonam <sup>a</sup> and open-ring desulfated aztreonam <sup>b,c</sup>	0.55	1.0
Aztreonam (Z-isomer)	1.0	—
Desulfated aztreonam <sup>d</sup>	1.6	1.5
Aztreonam E-isomer <sup>e</sup>	1.8	0.5
Aztreonam ethyl ester <sup>f</sup>	3.9	1.5
Any individual unspecified impurity	—	0.1
Total impurities	—	3.0

<sup>a</sup> (2S,3S)-2-[(Z)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido]-3-(sulfoamino)butanoic acid.

<sup>b</sup> (2S,3S)-3-Amino-2-[(Z)-2-[2-aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido]butanoic acid.

<sup>c</sup> Open-ring aztreonam and open-ring desulfated aztreonam coelute. The limit is for the sum of these two impurities.

<sup>d</sup> (Z)-2-[[[(2-Amino-4-thiazolyl){[(2S,3S)-2-methyl-4-oxo-3-azetidinyl]carbamoyl}methylene]amino]oxy]-2-methylpropionic acid.

<sup>e</sup> (E)-2-[[[(2-Amino-4-thiazolyl){[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino]oxy]-2-methylpropionic acid.

<sup>f</sup> Ethyl (Z)-2-[[[(2-amino-4-thiazolyl){[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino]oxy]-2-methylpropionate.

#### SPECIFIC TESTS

- STERILITY TESTS (71):** Where the label states that Aztreonam is sterile, it meets the requirements for *Test for Sterility of the Product to Be Examined—Membrane Filtration*, using Fluid A, to which 23.4 g of sterile arginine has been added to each 1000 mL.
- WATER DETERMINATION, Method I (921):** NMT 2.0%; if labeled as the hydrated form: 12.0%–18.0%. [NOTE—The term hydrated form refers to the  $\alpha$ -form of Aztreonam, which is not a stoichiometric hydrate.]
- BACTERIAL ENDOTOXINS TEST (85):** Where the label states that aztreonam is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.
- LIMIT OF ALCOHOL**

[NOTE—This test is to be performed if alcohol is used while manufacturing Aztreonam.]

**Standard solution:** 0.004 mL/mL of alcohol from USP Alcohol Determination—Alcohol RS and 0.004 mL/mL of acetonitrile from USP Alcohol Determination—Aceto-

nitrile RS in dimethylformamide. [NOTE—The *Standard solution* contains 0.4% alcohol and 0.4% acetonitrile.]

**Sample solution:** 80 mg/mL of Aztreonam and 0.004 mL/mL of acetonitrile in dimethylformamide.

[NOTE—Dissolve Aztreonam in dimethylformamide using 20% of the final volume. Add a suitable aliquot of USP Alcohol Determination—Acetonitrile RS, and dilute with dimethylformamide to volume. The concentration of acetonitrile in the *Sample solution* is 0.4%.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm  $\times$  30-m; phase G43

**Film thickness:** 3.0  $\mu\text{m}$

**Temperature**

**Injector:** 210°

**Detector:** 280°

**Column:** See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	200	4

**Carrier gas:** He

**Linear velocity:** 35 cm/s

**Injection mode:** Split

**Split ratio:** 5:1

**Injection size:** 0.5  $\mu\text{L}$

#### System suitability

[NOTE—The relative retention times for alcohol and acetonitrile are 1.0 and 1.3, respectively.]

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between alcohol and acetonitrile

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alcohol in the portion of Aztreonam taken:

$$\text{Result} = (R_U/R_S) \times (C_S \times D/C_U) \times F \times 100$$

$R_U$  = peak response ratio of alcohol to acetonitrile from the *Sample solution*

$R_S$  = peak response ratio of alcohol to acetonitrile from the *Standard solution*

$C_S$  = concentration of alcohol in the *Standard solution* (mL/mL)

$D$  = density of alcohol (g/mL)

$C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)

$F$  = unit conversion factor, 1000 mg/g

**Acceptance criteria:** NMT 4%

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is the hydrated form, the label so indicates.
- USP REFERENCE STANDARDS (11)**
  - USP Alcohol Determination—Acetonitrile RS  
C<sub>2</sub>H<sub>3</sub>N 41.05
  - USP Alcohol Determination—Alcohol RS  
C<sub>2</sub>H<sub>5</sub>OH 46.07

USP Aztreonam RS  
 Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-  
 [(2-methyl-4-oxo-1-sulfo-3-azetidiny]amino]-2-oxo-  
 ethylidene]amino]oxy]-2-methyl-, [2S-[2 $\alpha$ ,3 $\beta$ (Z)]]-  
 $C_{13}H_{17}N_5O_8S_2$  435.43  
 USP Aztreonam E-Isomer RS  
 (E)-2-[[[1-(2-Amino-4-thiazolyl){[(2S,3S)-2-methyl-4-oxo-  
 1-sulfo-3-azetidiny]carbamoyl}methylene]amino]oxy)-  
 2-methylpropionic acid.  
 $C_{13}H_{17}N_5O_8S_2$  435.43  
 USP Endotoxin RS

## Aztreonam Injection

### DEFINITION

Aztreonam Injection is a sterile solution of Aztreonam and Arginine and a suitable osmolality adjusting substance in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ).

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (75:25).

**System suitability solution:** 0.2 mg/mL of each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*

**Sample solution:** Equivalent to 0.2 mg/mL of aztreonam from Injection in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm

**Pre-column:** 4.6-mm  $\times$  5- to 10-cm saturator precolumn containing packing L27

**Column:** 4-mm  $\times$  25-cm analytical column containing packing L20

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for aztreonam and open ring aztreonam are 0.8 and 1.0, respectively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between aztreonam and open ring aztreonam

**Column efficiency:** NLT 1000 theoretical plates determined from the aztreonam peak

**Tailing factor:** NMT 2.0 for the aztreonam peak

**Relative standard deviation:** NMT 2.0% for the aztreonam peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of aztreonam from the *Sample solution*

$r_S$  = peak response of aztreonam from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aztreonam in the *Sample solution* (mg/mL)

$P$  = potency of aztreonam in USP Aztreonam RS ( $\mu$ g/mg)

$F$  = conversion factor (0.001 mg/ $\mu$ g)

**Acceptance criteria:** 90.0%–120.0%

### SPECIFIC TESTS

- PYROGEN TEST** <151>: It meets the requirements, the test dose being a measured volume of Injection sufficient to provide 50 mg/kg of aztreonam.
- STERILITY TESTS** <71>: It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.
- pH** <791>: 4.5–7.5
- PARTICULATE MATTER IN INJECTIONS** <788>: It meets the requirements for small-volume injections.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve as described under *Injections* <1>, *Containers for Sterile Solids*. Maintain in the frozen state.
- LABELING:** It meets the requirements for *Injections* <1>, *Labels and Labeling*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- USP REFERENCE STANDARDS** <11>  
 USP L-Arginine RS  
 USP Aztreonam RS  
 USP Open Ring Aztreonam RS  
 $C_{13}H_{19}N_5O_9S_2$  453.45

## Aztreonam for Injection

### DEFINITION

Aztreonam for Injection is a dry mixture of sterile Aztreonam and Arginine. It contains NLT 90.0% and NMT 105.0% of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ), calculated on the anhydrous and arginine-free basis. Each container contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ).

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (75:25)

**System suitability solution:** 0.2 mg/mL each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*

**Sample solution 1:** Equivalent to 0.2 mg/mL of aztreonam in *Mobile phase* from Aztreonam for Injection. Weigh 1 container of Aztreonam for Injection, transfer the contents to a suitable container and dilute with *Mobile phase* to the appropriate volume. Weigh the empty container and calculate the weight, in mg, of Aztreonam for Injection used.



**Sample solution 2:** Equivalent to 0.2 mg/mL of aztreonam from Aztreonam for Injection constituted as directed below and diluted with *Mobile phase*. Where the vial has a capacity of less than 100 mL, constitute with water using the volume of solvent specified in the labeling.

Where the vial capacity is equal to or greater than 100 mL, constitute with 10 mL of water and dilute the entire withdrawable contents of the container with *Mobile phase* to obtain the final concentration.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm

**Pre-column:** 4.6-mm × 5- to 10-cm saturator precolumn containing packing L27

**Column:** 4-mm × 25-cm analytical column containing packing L20

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for aztreonam and open ring aztreonam are about 0.8 and 1.0, respectively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between aztreonam and open ring aztreonam

**Column efficiency:** NLT 1000 theoretical plates determined from the aztreonam peak

**Tailing factor:** NMT 2.0 for the aztreonam peak

**Relative standard deviation:** NMT 2.0% for the aztreonam peak

#### Analysis

**Samples:** *Standard solution*, *Sample solution 1*, and *Sample solution 2*

Calculate the percentage of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response for aztreonam from *Sample solution 1*

$r_S$  = peak response for aztreonam from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aztreonam for Injection in *Sample solution 1* (mg/mL), corrected for water and arginine content (see *Content of Arginine*)

$P$  = potency of aztreonam in USP Aztreonam RS (µg/mg)

$F$  = conversion factor (0.001 mg/µg)

**Acceptance criteria:** 90.0%–105.0% on the anhydrous and arginine-free basis

Calculate the percentage of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in each container of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response for aztreonam from *Sample solution 2*

$r_S$  = peak response for aztreonam from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL).

$C_U$  = nominal concentration of aztreonam in *Sample solution 2* (mg/mL)

$P$  = potency of aztreonam in USP Aztreonam RS (µg/mg)

$F$  = conversion factor (0.001 mg/µg)

**Acceptance criteria:** 90.0%–120.0% of the labeled content of aztreonam

#### OTHER COMPONENTS

• **CONTENT OF ARGININE:** Use the result of this test to calculate, on the anhydrous and arginine-free basis, the *Assay* result from *Sample solution 1*, obtained as directed in the *Assay*.

**Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution 1, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution 1*

Calculate the percentage of arginine ( $C_6H_{14}N_4O_2$ ) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for arginine from *Sample solution 1*

$r_S$  = peak response for arginine from the *Standard solution*

$C_S$  = concentration of USP L-Arginine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aztreonam for Injection in *Sample solution 1*

#### PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements

#### SPECIFIC TESTS

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* <1>, *Constituted Solutions*.

• **BACTERIAL ENDOTOXINS TEST** <85>: It contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.

• **STERILITY TESTS** <71>: It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined*, *Membrane Filtration*.

• **pH** <791>

**Sample solution:** 100 mg/mL of aztreonam

**Acceptance criteria:** 4.5–7.5

• **WATER DETERMINATION, Method I** <921>: NMT 2.0%

• **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** It meets the requirements for *Injections* <1>, *Labeling*.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Injections* <1>, *Containers for Sterile Solids*.

• **USP REFERENCE STANDARDS** <11>

USP L-Arginine RS

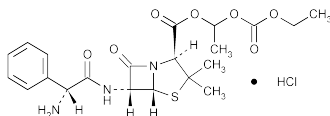
USP Aztreonam RS

USP Endotoxin RS

USP Open Ring Aztreonam RS

$C_{13}H_{19}N_5O_9S_2$  453.45

## Bacampicillin Hydrochloride



$C_{21}H_{27}N_3O_7S \cdot HCl$  501.98

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, 1-[(ethoxycarbonyl)oxyethyl ester, monohydrochloride, [2S-[2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ (S\*)]]]- (2S,5R,6R)-6-[(R)-(2-Amino-2-phenylacetamido)]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid ester with ethyl 1-hydroxyethyl carbonate, monohydrochloride [37661-08-8].

» Bacampicillin Hydrochloride has a potency of not less than 623  $\mu$ g and not more than 727  $\mu$ g of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Bacampicillin Hydrochloride RS

**Identification**—Prepare a test solution of it in alcohol containing 2 mg per mL. Prepare a Standard solution of USP Bacampicillin Hydrochloride RS in alcohol containing 2 mg per mL. Apply two 5- $\mu$ L portions of the test solution 4.0 cm apart to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* (621)). After the spots dry, apply two 5- $\mu$ L portions of the Standard solution, one midway between the test solution spots and the other to one of the test solution spots. Allow the spots to dry, place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, chloroform, and alcohol (10:1:1). When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, and allow to dry. Spray the plate with a spray reagent containing 1 g of ninhydrin and 1 mL of pyridine in each 100 mL of solution in butyl alcohol, and heat at 100° for 10 minutes: bacampicillin appears as a purple spot, and the  $R_F$  values of the spots from the test solution and from the combined test solution and Standard solution, respectively, correspond to the  $R_F$  value of the spot obtained from the Standard solution.

**pH** (791): between 3.0 and 4.5, in a solution containing 20 mg per mL.

**Water**, Method I (921): not more than 1.0%.

**Dimethylaniline** (223): meets the requirement, the *Test Preparation* being prepared using 2 N sodium hydroxide instead of 1 N sodium hydroxide.

**Assay**—

*Mobile phase*—To 500 mL of 0.02 M dibasic sodium phosphate add portions of 0.02 M monobasic sodium phosphate until a pH of  $6.8 \pm 0.05$  is reached. Prepare a suitable filtered mixture of this pH 6.8 buffer and acetonitrile (500:500). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Prepare a solution of USP Bacampicillin Hydrochloride RS, accurately weighed, in water having a known concentration of about 0.8 mg per mL, sonicating for about 20 minutes to achieve complete dissolution. Pass through a filter of 0.5- $\mu$ m or finer porosity.

*Assay preparation*—Transfer about 80 mg of Bacampicillin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, add 90 mL of water, and sonicate for about 20 minutes. Dilute with water to volume, mix, and pass through a filter of 0.5- $\mu$ m or finer porosity.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 3000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in  $\mu$ g, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) equivalent in each mg of the Bacampicillin Hydrochloride taken by the formula:

$$(349.41/501.99)(100,000C/W)(r_U/r_S)$$

in which 349.41 and 501.99 are the molecular weights of anhydrous ampicillin and bacampicillin hydrochloride, respectively, C is the concentration, in mg per mL, of USP Bacampicillin Hydrochloride RS in the *Standard preparation*, W is the weight, in mg, of the portion of Bacampicillin Hydrochloride taken, and  $r_U$  and  $r_S$  are the bacampicillin peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bacampicillin Hydrochloride for Oral Suspension

» Bacampicillin Hydrochloride for Oral Suspension contains an amount of Bacampicillin Hydrochloride equivalent to not less than 90.0 percent and not more than 125.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) when constituted as directed. It contains one or more suitable buffers, colors, flavors, suspending agents, and sweetening ingredients.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ampicillin RS

USP Bacampicillin Hydrochloride RS

**Identification**—Constitute Bacampicillin Hydrochloride for Oral Suspension as directed in the labeling. Transfer a portion of the resulting suspension, equivalent to about 140 mg of ampicillin, to a 100-mL volumetric flask, add 70 mL of alcohol, shake by mechanical means for 30 minutes, dilute with alcohol to volume, and mix: the solution so obtained responds to the *Identification* test under *Bacampicillin Hydrochloride*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 6.5 and 8.0, in the suspension constituted as directed in the labeling.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—

*Standard preparation*—Using USP Ampicillin RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

*Assay preparation*—Transfer an accurately measured volume of Bacampicillin Hydrochloride for Oral Suspension,

constituted as directed in the labeling and free from bubbles, equivalent to about 87.5 mg of ampicillin, to a 250-mL volumetric flask. Add 200 mL of a solvent mixture consisting of alcohol and 0.1 M phosphoric acid (4:1). Shake by mechanical means for 30 minutes, dilute with the same solvent mixture to volume, and mix. Centrifuge a portion of the resulting suspension. Pipet 4.0 mL of the clear solution so obtained into each of two glass-stoppered, 125-mL conical flasks.

**Procedure**—Proceed as directed for *Procedure under Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of  $C_{16}H_{19}N_3O_4S$  in each mL of the constituted Bacampicillin Hydrochloride for Oral Suspension taken by the formula:

$$(0.0625F)(B - I) / V$$

in which  $V$  is the volume, in mL, of constituted Bacampicillin Hydrochloride for Oral Suspension taken, and the other terms are as defined therein.

## Bacampicillin Hydrochloride Tablets

» Bacampicillin Hydrochloride Tablets contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ampicillin RS

USP Bacampicillin Hydrochloride RS

**Identification**—To a portion of powdered Tablets add alcohol to obtain a solution containing the equivalent of 2 mg of ampicillin per mL: the solution so obtained responds to the *Identification* test under *Bacampicillin Hydrochloride*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 30 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ampicillin RS in water to obtain a solution having a known concentration of about 0.3 mg per mL.

**Procedure**—Determine the amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) dissolved as directed for *Procedure* in the section *Antibiotics—Hydroxylamine Assay* under *Automated Methods of Analysis* (16).

**Tolerances**—Not less than 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 2.5%.

**Assay**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Bacampicillin Hydrochloride*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 56 mg of ampicillin ( $C_{16}H_{19}N_3O_4S$ ), to a 100-mL volumetric flask, add 90 mL of water, and sonicate for about 20 minutes. Dilute with water to volume, mix, and filter through a filter of 0.5- $\mu$ m or finer porosity.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Bacampicillin Hydrochloride*. Calculate the quantity, in

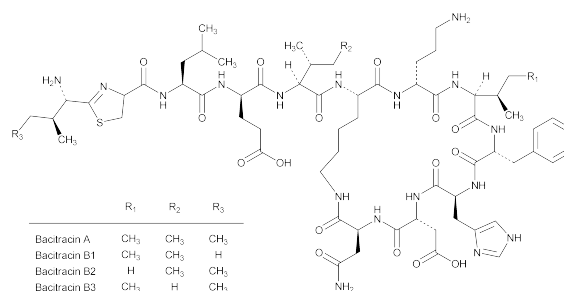
mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) equivalent to the portion of Tablets taken by the formula:

$$(349.41/501.99)(100C)(r_U / r_S)$$

in which the terms are as defined therein.

## Bacitracin

Bacitracin [1405-87-4].



» Bacitracin is a mixture of polypeptides produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis* (Fam. Bacillaceae), the main components being bacitracins A, B1, B2, and B3. It has a potency of not less than 65 Bacitracin Units per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store in a cool place.

**Labeling**—Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units per milligram. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

**USP Reference standards** (11)—

USP Bacitracin Zinc RS

USP Endotoxin RS

**Identification**—

**A:** *Thin-Layer Chromatographic Identification Test* (201BNP): meets the requirements.

**B:** It meets the requirements of the liquid chromatographic procedure in the test for *Composition*.

**pH** (791): between 5.5 and 7.5, in a solution containing 10,000 Bacitracin Units per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Composition**—

**Buffer**—Dissolve 34.8 g of dibasic potassium phosphate in 1 L of water. Adjust with 27.2 g of monobasic potassium phosphate dissolved in 1 L of water to a pH of 6.0.

**Mobile phase**—Prepare a mixture of methanol, water, *Buffer*, and acetonitrile (26:15:5:2), mix well, and degas.

**System suitability solution**—Dissolve an accurately weighed quantity of USP Bacitracin Zinc RS in water, add diluted hydrochloric acid, using about 2% of the final volume, and dilute with water to obtain a solution with a nominal concentration of about 2.0 mg per mL.

**Reporting threshold solution**—Dilute quantitatively, with *Mobile phase*, a suitable volume of *System suitability solution* to obtain a solution having a known concentration of 0.01 mg per mL. This solution is used to determine the reporting threshold.

**Peak identification solution**—Dissolve a suitable quantity of USP Bacitracin Zinc RS in a suitable volume of a 40 g per L solution of edetate disodium (pH adjusted to 7.0 with dilute sodium hydroxide), to obtain a solution having a nominal concentration of about 2.0 mg per mL. Heat in a boiling water bath for 30 minutes. Cool to room temperature.

**Test solution**—Dissolve an accurately weighed quantity of Bacitracin in *Mobile phase* to obtain a solution having a known concentration of about 2.0 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with an absorbance detector and an end-capped, 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is 1.0 mL per minute. Set the wavelength of the detector at 300 nm. Inject about 100 μL of the *Peak identification solution*, and identify the location of bacitracin F, which is a known impurity, using the relative retention time given in *Table 1*. Change the wavelength of the detector and set it to 254 nm. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early eluting peptides (those eluting before the peak due to bacitracin B1) and the impurity, bacitracin F, using the relative retention time values given in *Table 1*. Calculate the peak-to-valley ratio by the formula:

$$H_p / H_v$$

where  $H_p$  is the height above the baseline of the peak due to bacitracin B1 and  $H_v$  is the height above the baseline of the lowest point of the curve separating bacitracin B1 peak from the peak due to bacitracin B2. The peak-to-valley ratio is not less than 1.2.

**Procedure**—Separately inject equal volumes (about 100 μL) of the *Mobile phase*, the *Test solution*, and the *Reporting threshold solution*. Record the chromatograms for about 3 times the retention time of bacitracin A. Identify the peaks using the relative retention time values given in *Table 1*. Measure the peak areas of all peaks in the *Test solution*.

Table 1

Component Name	Relative Retention Time (approximate)
Bacitracin C1	0.5
Bacitracin C2	0.6
Bacitracin C3	0.6
Bacitracin B1	0.7
Bacitracin B2	0.7
Bacitracin B3	0.8
Bacitracin A	1.0
Bacitracin F	2.4

[NOTE—Disregard any peak in the *Test solution* having an area less than the area of bacitracin A in the *Reporting threshold solution*; and disregard any peak observed in the blank run.]

*Total area* in the following calculations is defined as the area of all peaks except the reporting threshold.

**CONTENT OF BACITRACIN A**—

Calculate the percentage of bacitracin A, by the formula:

$$(r_A / \text{Total area}) \times 100$$

where  $r_A$  is the area response from bacitracin A. The bacitracin A content is not less than 40.0% of the *Total area*.

**CONTENT OF ACTIVE BACITRACIN**—

Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) by the formula:

$$((r_A + r_{B1} + r_{B2} + r_{B3}) / \text{Total area}) \times 100$$

where  $r_A$ ,  $r_{B1}$ ,  $r_{B2}$ , and  $r_{B3}$  are the area responses from bacitracin A, B1, B2, and B3, respectively. The sum of bacitracins A, B1, B2, and B3 is not less than 70.0% of the *Total area*.

**LIMIT OF EARLY ELUTING PEPTIDES**—

Calculate the percentage of all peaks eluting before the peak due to bacitracin B1, by the formula:

$$(r_{\text{PreB1}} / \text{Total area}) \times 100$$

where  $r_{\text{PreB1}}$  is the sum of the responses of all peaks eluting before the peak for bacitracin B1. The limit of early eluting peptides (those eluting before the peak due to bacitracin B1) is not more than 20.0%.

**LIMIT OF BACITRACIN F**—

Calculate the percentage of bacitracin F by the formula:

$$100 \times (r_F / r_A)$$

where  $r_F$  is the response of bacitracin F from the *Test solution* and  $r_A$  is the response of bacitracin A from the *Test solution*. The limit of bacitracin F, a known impurity, is not more than 6.0%.

**Other requirements**—Where the label states that Bacitracin is sterile, it meets the requirements for *Sterility Tests* <71> and, where intended for injectable dosage forms, for *Bacterial endotoxins* under *Bacitracin for Injection*. Where the label states that Bacitracin must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Bacitracin for Injection*.

**Assay**—Proceed with Bacitracin as directed under *Antibiotics—Microbial Assays* <81>.

## Bacitracin for Injection

» Bacitracin for Injection has a potency of not less than 50 Bacitracin Units per mg. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of bacitracin.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>, and store in a cool place.

**USP Reference standards** <11>—

USP Bacitracin Zinc RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Thin-layer chromatographic identification test** <201BNP>: meets the requirements.

**Bacterial endotoxins** <85>—It contains not more than 0.01 USP Endotoxin Unit per Bacitracin Unit.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Residue on ignition** <281>: not more than 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Heavy metals, Method II** <231>: not more than 0.003%.

**Other requirements**—It meets the requirements of the tests for *pH* and *Loss on drying* under *Bacitracin*. It meets the

requirements under *Injections* (1) and *Uniformity of Dosage Units* (905).

#### Assay—

**Assay preparation 1**—Constitute 1 container of Bacitracin for Injection as directed in the labeling. Using a suitable hypodermic needle and syringe, withdraw the contents of the container, and dilute quantitatively with *Buffer No. 1* to obtain a solution containing about 100 Bacitracin Units per mL.

**Assay preparation 2** (where the label states the number of Bacitracin Units in a given volume of constituted solution)—Constitute 1 container of Bacitracin for Injection as directed in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Buffer No. 1* to obtain a solution containing about 100 Bacitracin Units per mL.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation*. Add sufficient 0.01 N hydrochloric acid to the *Assay preparation* so that the amount of hydrochloric acid in the *Test Dilution* will be the same as in the median dose level of the Standard, and dilute quantitatively with *Buffer No. 1* to obtain a *Test Dilution* having a bacitracin concentration assumed to be equal to the median dose level of the Standard.

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### Bacitracin Ointment

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» Bacitracin Ointment is Bacitracin in an anhydrous ointment base. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of bacitracin. It may contain a suitable anesthetic.

**Packaging and storage**—Preserve in well-closed containers containing not more than 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.

#### USP Reference standards (11)—

USP Bacitracin Zinc RS

#### Thin-layer chromatographic identification test

(201BNP): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment shaken with about 50 mL of ether in a separator, and extracted with four 20-mL portions of *Buffer No. 1*. Combine the buffer extracts, and dilute with *Buffer No. 1* to an appropriate volume to obtain a stock solution. Add sufficient 0.01 N hydrochloric acid to an accurately measured portion of the stock solution so that the amount of hydrochloric acid in the *Test Dilution* will be the same as in the median dose level of the Standard, and quantitatively dilute with *Buffer No. 1* to obtain a *Test Dilution* having a bacitracin concentration assumed to be equal to the median dose level of the Standard.

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### Bacitracin Ophthalmic Ointment

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» Bacitracin Ophthalmic Ointment is a sterile preparation of Bacitracin in an anhydrous ointment base. It contains not less than 90.0 percent

and not more than 140.0 percent of the labeled amount of bacitracin.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

#### USP Reference standards (11)—

USP Bacitracin Zinc RS

#### Thin-layer chromatographic identification test

(201BNP): meets the requirements.

**Sterility** (71): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Metal particles** (751): It meets the requirements.

**Assay**—Proceed with Ophthalmic Ointment as directed in the *Assay* under *Bacitracin Ointment*.

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### Bacitracin and Polymyxin B Sulfate Topical Aerosol

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» Bacitracin and Polymyxin B Sulfate Topical Aerosol is a suspension of Bacitracin and Polymyxin B Sulfate in a suitable vehicle, packaged in a pressurized container with a suitable inert propellant. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic.

**Packaging and storage**—Preserve in pressurized containers, and avoid exposure to excessive heat.

#### USP Reference standards (11)—

USP Bacitracin Zinc RS

USP Polymyxin B Sulfate RS

[NOTE—Prepare the specimen for the following tests and assays as follows. Maintain the container in the inverted position throughout this procedure. Store the container in a freezer at  $-70^{\circ}$  for 16 to 24 hours. Remove the container from the freezer, promptly puncture the container, and allow the propellant to volatilize. Open the container, and mix the contents.]

#### Thin-layer chromatographic identification test

(201BNP)—A portion of the contents of 1 container prepared as directed above and tested as directed for *Creams, Lotions, and Ointments* meets the requirements.

**Water, Method I** (921): not more than 0.5%, an accurately weighed portion of the contents of 1 container, prepared as directed above, being used, and 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Other requirements**—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

**Assay for bacitracin**—Proceed as directed for bacitracin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of the contents of 1 container, prepared as directed above, equivalent to about 500 USP Bacitracin Units. Transfer to a suitable separator containing about 50 mL of ether, and extract with three 25-mL portions of *Buffer No. 1*. Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer No. 1* to volume, and mix. Add sufficient 0.01 N hydrochloric acid to an accurately measured volume of this solution so that the amount of hydrochloric acid in the *Test Dilution* will be the same as in the median dose level of the Standard, and quantitatively dilute with *Buffer No. 1* to obtain a *Test Dilution* having a

bacitracin concentration assumed to be equal to the median dose level of the Standard.

**Assay for polymyxin B**—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81). Transfer an accurately weighed portion of the contents of 1 container, prepared as directed above, equivalent to about 5000 USP Polymyxin B Units, to a suitable separator containing about 50 mL of ether, and extract with three 25-mL portions of *Buffer No. 6*. Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer No. 6* to volume, and mix. Dilute an accurately measured volume of this solution, quantitatively and stepwise, with *Buffer No. 6* to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

## Soluble Bacitracin Methylenedisalicylate

### DEFINITION

Soluble Bacitracin Methylenedisalicylate is a mixture of Bacitracin Methylenedisalicylate and Sodium Bicarbonate. It has a potency of NLT 8 Bacitracin Units/mg, calculated on the dried basis.

### ASSAY

#### • ANTIBIOTICS—MICROBIAL ASSAYS (81)

**Diluent:** 20 g/L of sodium bicarbonate

**Sample stock solution:** Transfer a suitable amount of Soluble Bacitracin Methylenedisalicylate to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.

**Test dilution:** To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid, and dilute with *Buffer No. 1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]

**Analysis:** Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* (81).

**Acceptance criteria:** NLT 8 Bacitracin Units/mg on the dried basis

### SPECIFIC TESTS

- **PH (791):** 8.0–9.5, in a 25 mg/mL solution
- **LOSS ON DRYING (731):** Dry 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS

## Bacitracin Methylenedisalicylate Soluble Powder

### DEFINITION

Bacitracin Methylenedisalicylate Soluble Powder contains NLT 90.0% and NMT 120.0% of the labeled amount of bacitracin.

### ASSAY

#### • ANTIBIOTICS—MICROBIAL ASSAYS (81)

**Diluent:** 20 g/L of sodium bicarbonate

**Sample stock solution:** Transfer a suitable amount of Bacitracin Methylenedisalicylate Soluble Powder to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.

**Test dilution:** To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid and dilute with *Buffer No. 1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]

**Analysis:** Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* (81).

**Acceptance criteria:** 90.0%–120.0%

### SPECIFIC TESTS

- **PH (791):** 8.0–9.5 in a 50 mg/mL solution
- **LOSS ON DRYING (731):** Dry 100 mg in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.
- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS

## Bacitracin Zinc

Bacitracins, zinc complex.

Bacitracins zinc complex [1405-89-6].

» Bacitracin Zinc is the zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides, the main components being bacitracins A, B1, B2, and B3. It has a potency of not less than 65 Bacitracin Units per mg, calculated on the dried basis. It contains not less than 4.0 percent and not more than 6.0 percent of zinc (Zn), calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store in a cool place.

**Labeling**—Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only. Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units per milligram. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards (11)**—

USP Bacitracin Zinc RS

**Identification**—

**A:** *Thin-Layer Chromatographic Identification Test* (201BNP): meets the requirements.

**B:** It meets the requirements of the liquid chromatographic procedure in the test for *Composition*.

**Sterility (71)**—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be*

*Examined*, except to use *Fluid A* to each L of which has been added 20 g of edetate disodium.

**pH** (791): between 6.0 and 7.5, in a (saturated) solution containing approximately 100 mg per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Zinc content**—[NOTE—The *Standard preparations* and the *Test preparation* may be quantitatively diluted with 0.001 N hydrochloric acid, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

*Standard preparations*—Transfer 3.11 g of zinc oxide, accurately weighed, to a 250-mL volumetric flask, add 80 mL of 1 N hydrochloric acid, warm to dissolve, cool, dilute with water to volume, and mix. This solution contains 10 mg of zinc per mL. Further dilute this solution with 0.001 N hydrochloric acid to obtain *Standard preparations* containing 0.5, 1.5, and 2.5 µg of zinc per mL, respectively.

*Test preparation*—Transfer about 200 mg of Bacitracin Zinc, accurately weighed, to a 100-mL volumetric flask. Dissolve in 0.01 N hydrochloric acid, dilute with the same solvent to volume, and mix. Pipet 2 mL of this solution into a 200-mL volumetric flask, dilute with 0.001 N hydrochloric acid to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the zinc resonance line of 213.8 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)), equipped with a zinc hollow-cathode lamp and an air-acetylene flame, using 0.001 N hydrochloric acid as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of zinc in the *Test preparation*. Calculate the content of zinc, in percent, in the portion of Bacitracin Zinc taken by the formula:

$$1000C / W$$

in which *C* is the concentration in µg per mL, of zinc in the *Test preparation*; and *W* is the weight, in mg, of the portion of Bacitracin Zinc taken.

#### Composition—

*Buffer*—Dissolve 34.8 g of potassium phosphate, dibasic, in 1 L of water. Adjust with 27.2 g of potassium phosphate, monobasic, dissolved in 1 L of water, to a pH of 6.0.

*Mobile Phase*—Prepare a mixture of methanol, water, *Buffer*, and acetonitrile (26:15:5:2). Mix well, and degas.

*Diluent*—Dissolve 40 g of edetate disodium in 1 L of water. Adjust with dilute sodium hydroxide to a pH of 7.0.

*System suitability solution*—Dissolve an accurately weighed quantity of USP Bacitracin Zinc RS in *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL.

*Reporting threshold solution*—Dilute quantitatively, with water, a suitable volume of *System suitability solution* to obtain a solution with a known concentration of 0.01 mg per mL.

*Peak identification solution*—Dissolve a weighed quantity of USP Bacitracin Zinc RS in a suitable volume of *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL. Heat in boiling water bath for 30 minutes. Cool to room temperature.

*Test solution*—Dissolve an accurately weighed quantity of Bacitracin Zinc in *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with an absorbance detector and an end-capped 4.6- × 250-mm column that con-

tains 5-µm packing L1. The flow rate is 1.0 mL per minute. Set the wavelength of the detector at 300 nm. Inject about 100 µL of the *Peak identification solution*, and identify the location of bacitracin F, which is a known impurity, using the relative retention time shown in *Table 1*.

Table 1

Component Name	Relative Retention Time (approximate)
Bacitracin C1	0.5
Bacitracin C2	0.6
Bacitracin C3	0.6
Bacitracin B1	0.7
Bacitracin B2	0.7
Bacitracin B3	0.8
Bacitracin A	1.0
Bacitracin F	2.4

Change the wavelength of the detector and set it to 254 nm. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2 and B3), early eluting peptides (those eluting before the peak due to bacitracin B1) and the impurity, bacitracin F, using the relative retention time values given in *Table 1*. Calculate the peak-to-valley ratio using the formula:

$$H_p / H_v$$

in which *H<sub>p</sub>* is the height above the baseline of the peak due to bacitracin B1; and *H<sub>v</sub>* is the height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the peak due to bacitracin B2. The peak-to-valley ratio is not less than 1.2.

*Procedure*—Separately inject equal volumes (100 µL) of *Diluent*, *Test solution*, and *Reporting threshold solution*. Record the chromatograms for about three times the retention time of bacitracin A. Identify the peaks using the relative retention times shown in *Table 1*. Measure the peak areas of all peaks in the *Test solution*. [NOTE—Disregard any peak in the *Test solution* having an area less than the area of the bacitracin A peak in the *Reporting threshold solution*; disregard any peak observed in the *Diluent*.]

NOTE—*Total area* in the following calculations is defined as the area of all peaks except the reporting threshold.

CONTENT OF BACITRACIN A—Calculate the percentage of bacitracin A using the formula:

$$(r_A / \text{Total area}) \times 100$$

in which *r<sub>A</sub>* is the area response from bacitracin A. Bacitracin A content is not less than 40.0% of the *Total area*.

CONTENT OF ACTIVE BACITRACIN—Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) using the formula:

$$(r_A + r_{B1} + r_{B2} + r_{B3} / \text{Total area}) \times 100$$

in which *r<sub>A</sub>*, *r<sub>B1</sub>*, *r<sub>B2</sub>*, and *r<sub>B3</sub>* are the area responses from bacitracin A, B1, B2, and B3, respectively. The sum of bacitracin A, B1, B2, and B3 is not less than 70.0% of the *Total area*.

LIMIT OF EARLY ELUTING PEPTIDES—Calculate the percentage of all peaks eluting before the peak due to bacitracin B1 using the formula:

$$(r_{\text{preB1}} / \text{Total area}) \times 100$$

in which *r<sub>preB1</sub>* is the sum of the responses of all peaks eluting before the peak for bacitracin B1. The limit of early eluting peptides (those eluting before the peak due to bacitracin B1) is not more than 20.0%.

LIMIT OF BACITRACIN F—Calculate the percentage of bacitracin F using the formula:

$$100 \times (r_F / r_A)$$

in which  $r_F$  is the response of bacitracin F from the *Test solution*; and  $r_A$  is the response of bacitracin A from the *Test solution*. The limit of bacitracin F, a known impurity, is not more than 6.0%.

**Assay**—Proceed with Bacitracin Zinc as directed under *Antibiotics—Microbial Assays* (81).

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### Bacitracin Zinc Ointment

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» Bacitracin Zinc Ointment is Bacitracin Zinc in an anhydrous ointment base. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of bacitracin.

**Packaging and storage**—Preserve in well-closed containers containing not more than 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.

**USP Reference standards** (11)—

USP Bacitracin Zinc RS

**Thin-layer chromatographic identification test**

(201BNP): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment shaken with about 50 mL of ether in a separator, and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer No. 1* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard, adding additional hydrochloric acid to each test dilution of the Standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.

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### Bacitracin Zinc Soluble Powder

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» Bacitracin Zinc Soluble Powder is a mixture of Bacitracin Zinc and zinc proteinates. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bacitracin.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.

**USP Reference standards** (11)—

USP Bacitracin Zinc RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 5.0% of its weight.

**Zinc content**—Using Powder, proceed as directed for *Zinc content* under *Bacitracin Zinc*. Calculate the zinc content, in

g, in relation to each 42,000 Bacitracin Units in the specimen taken by the formula:

$$280,000C / WA$$

in which  $A$  is the bacitracin content of the specimen, in Bacitracin Units per g, and the other terms are as defined therein: it contains not more than 2.0 g for each 42,000 Bacitracin Units.

**Assay**—Dissolve an accurately weighed quantity of Powder quantitatively in 0.01 N hydrochloric acid to obtain a stock solution containing about 100 Bacitracin Units per mL. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively and stepwise with *Buffer No. 1* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. In preparing each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.

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### Bacitracin Zinc and Polymyxin B Sulfate Ointment

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» Bacitracin Zinc and Polymyxin B Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Bacitracin Zinc RS

USP Polymyxin B Sulfate RS

**Thin-layer chromatographic identification test**

(201BNP): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay for bacitracin**—Proceed with Ointment as directed in the *Assay* under *Bacitracin Zinc Ointment*.

**Assay for polymyxin B**—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment shaken with about 50 mL of ether in a separator, and extracted with four 20-mL portions of *Buffer No. 6*. Combine the aqueous extracts, and dilute with *Buffer No. 6* to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer No. 6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

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### Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment

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» Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of bacitracin and polymyxin B.



**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Bacitracin Zinc RS

USP Polymyxin B Sulfate RS

**Thin-layer chromatographic identification test**

(201BNP): meets the requirements.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Minimum fill** (755): meets the requirements.

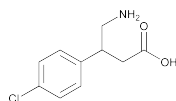
**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Metal particles** (751): meets the requirements.

**Assay for bacitracin**—Proceed with Ophthalmic Ointment as directed in the Assay under *Bacitracin Zinc Ointment*.

**Assay for polymyxin B**—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ophthalmic Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of *Buffer No. 6*. Combine the aqueous extracts, and dilute with *Buffer No. 6* to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer No. 6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Baclofen



$C_{10}H_{12}ClNO_2$  213.66

Butanoic acid, 4-amino-3-(4-chlorophenyl)-

$\beta$ -(Aminomethyl)-*p*-chlorohydrocinnamic acid [1134-47-0].

» Baclofen contains not less than 99.0 percent and not more than 101.0 percent of  $C_{10}H_{12}ClNO_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Baclofen RS

USP Baclofen Related Compound A RS  
4-(4-Chlorophenyl)-2-pyrrolidinone.

$C_{10}H_{10}ClNO$  195.65

**Identification, Infrared Absorption** (197M).

**Water, Method I** (921): not more than 3.0%.

**Residue on ignition** (281): not more than 0.3%.

**Heavy metals, Method II** (231): 0.001%.

**Related compounds**—[Caution: Avoid contact with *o*-tolidine when performing this test, and conduct the test in a well-ventilated hood.]

**Detection reagent**—Dissolve 200 mg of *o*-tolidine in 2.0 mL of glacial acetic acid with the aid of a hot water bath. Dilute with water to 100.0 mL, and filter. Mix one volume of this solution with an equal volume of potassium iodide solution (0.83 in 100).

**Diluting solution**—Prepare a mixture of alcohol and glacial acetic acid (4:1).

**Standard preparations**—Dissolve USP Baclofen RS in *Diluting solution* to obtain a *Standard stock solution* having a known concentration of 0.1 mg per mL. Dilute portions of

this *Standard stock solution* quantitatively with *Diluting solution* to obtain solutions having concentrations of 0.05 mg per mL (*Standard preparation A*) and 0.03 mg per mL (*Standard preparation B*), respectively.

**Identification preparation**—Dissolve USP Baclofen Related Compound A RS in *Diluting solution* to obtain a solution having a known concentration of 0.1 mg per mL.

**Test preparation**—Prepare a solution in *Diluting solution* containing 10.0 mg of Baclofen per mL.

**Procedure**—Apply separately 10  $\mu$ L of the *Test preparation*, *Identification preparation*, *Standard preparation A*, and *Standard preparation B* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (4:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry under a current of warm air. Transfer the dry plate to another chromatographic chamber containing a beaker with 1 g of potassium permanganate. Add 10 mL of dilute hydrochloric acid (4 in 10) to the beaker. Cover the chamber, and allow the chamber to become saturated with chlorine gas. Expose the plate to the chlorine gas for 8 minutes. Remove the plate and expose to the air for 2 minutes, then spray with freshly prepared *Detection reagent*: the intensity of the secondary spot from the *Test preparation*, corresponding in  $R_f$  value to the principal spot from the *Identification preparation*, is not greater than that of the principal spot from the *Identification preparation* (1.0%), and no other secondary spot from the *Test preparation* is greater in intensity than the principal spot from *Standard preparation A* (0.5%). The sum of all secondary spots from the *Test preparation* is not more than 2.0%.

**Assay**—Dissolve about 200 mg of Baclofen, accurately weighed, in a suitable volume of glacial acetic acid, sufficient to immerse the electrodes. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a saturated solution of lithium chloride in glacial acetic acid (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.37 mg of  $C_{10}H_{12}ClNO_2$ .

## Baclofen Oral Suspension

### DEFINITION

Baclofen Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of baclofen ( $C_{10}H_{12}ClNO_2$ ). Prepare Baclofen Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Baclofen	500 mg
Vehicle: a mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF (1:1), a sufficient quantity to make	100 mL

If using Baclofen Tablets, place the Tablets in a suitable mortar and comminute to a fine powder, or add *Baclofen* powder. Add 5 mL of the *Vehicle* to wet the powder, and triturate the powder to form a fine paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer, stepwise and quantitatively, the contents of the mortar to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile and 0.05 M monobasic sodium phosphate (20:80). Adjust with phosphoric acid to a pH of 3.5.

**Standard solution:** 5 µg/mL of USP Baclofen RS in water

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 0.5 mL of Oral Suspension from each bottle to a 500-mL volumetric flask, dilute with water to volume to obtain a concentration of 5 µg/mL, and pass through a 0.22-µm polyvinylidene fluoride (PVDF) filter.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 15 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time of baclofen is about 5.5 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of baclofen (C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of baclofen in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of baclofen in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH <791>:** 4.2–5.2

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a cold place.

**• LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** NMT 35 days after the day on which it was compounded

**• USP REFERENCE STANDARDS <11>**

USP Baclofen RS

**Baclofen Tablets**

» Baclofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub>.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards <11>—**

USP Baclofen RS

USP Baclofen Related Compound A RS

4-(4-Chlorophenyl)-2-pyrrolidinone.

C<sub>10</sub>H<sub>10</sub>ClNO 195.65

**Identification—**

**A:** Transfer a portion of powdered Tablets, equivalent to about 50 mg of baclofen, to a glass-stoppered 40-mL centrifuge tube. Add 10.0 mL of a mixture of dehydrated alcohol and glacial acetic acid (4:1), shake by mechanical means for 30 minutes, and centrifuge. Apply 20 µL of this solution and 20 µL of a Standard solution containing 5 mg of USP Baclofen RS per mL in a mixture of dehydrated alcohol and glacial acetic acid (4:1) to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a chromatographic chamber (see *Chromatography* <621>) containing a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (4:1:1), and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry in a current of warm air. Spray with a detecting reagent consisting of 0.4 g of ninhydrin in 95 mL of butyl alcohol and 5 mL of dilute glacial acetic acid (1 in 10) until the plate is slightly wet. Place the plate in an oven maintained at 100° for 10 minutes: the  $R_f$  value of the principal orange-red spot obtained from the solution from the Tablets corresponds to that obtained from the Standard solution.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution, Procedure for a Pooled Sample <711>—**

**Medium:** 0.01 N hydrochloric acid; 500 mL for Tablets containing 10 mg or less of drug and 1000 mL for Tablets containing more than 10 mg of drug.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub> dissolved by employing the following method.

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay*.

**Procedure**—Inject an accurately measured volume (about 190 µL) of a filtered portion of the solution under test into the chromatograph by means of a microsyringe or a sampling valve, record the chromatogram, and measure the response for the major peak. Calculate the quantity of C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub> dissolved in comparison with a Standard solution having a known concentration of USP Baclofen RS in the same *Medium* and similarly chromatographed.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub> is dissolved in 30 minutes.

**Uniformity of dosage units <905>:** meet the requirements.

**Related compounds—**

**Diluting solution, Mobile phase, and Chromatographic system**—Proceed as directed in the *Assay*.

**Standard preparation**—Transfer about 10 mg of USP Baclofen Related Compound A RS, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

**Test preparation**—Use the *Assay preparation*.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, and proceed as directed in the *Procedure* under *Assay*. Calculate the percentage of baclofen related compound A in the portion of Tablets taken by the formula:

$$1000(C/W)(r_U/r_S)$$

in which C is the concentration of USP Baclofen Related Compound A RS, in mg per mL, in the *Standard preparation*; W is the weight of baclofen in the *Test preparation*; and  $r_U$

and  $r_s$  are the peak responses due to baclofen related compound A in the chromatograms of the *Test preparation* and the *Standard preparation*, respectively. Not more than 4.0% is found.

#### Assay—

*Diluting solution*—Transfer 75 mL of methanol and 10 mL of glacial acetic acid to a 250-mL volumetric flask. Dilute with water to volume, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of 0.3 N acetic acid, methanol, and 0.36 N sodium 1-pentane-sulfonate (550:440:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Baclofen RS in *Diluting solution* to obtain a solution having a known concentration of about 4 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of baclofen, to a 50-mL flask. Transfer 10.0 mL of *Diluting solution* to the flask, sonicate to disperse, and shake by mechanical means for 30 minutes. Centrifuge a portion of this solution for 5 minutes, and filter.

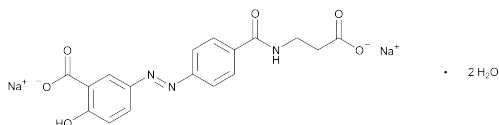
*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 0.6 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and allow the *Assay preparation* to elute for not less than three times the retention time of baclofen. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{10}H_{12}ClNO_2$  in the portion of Tablets taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Baclofen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the baclofen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Balsalazide Disodium



$C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  437.31  
Benzoic acid, 5-[[4-[(2-carboxyethyl)amino]carbonyl]phenyl]azo]-2-hydroxy-, disodium salt, dihydrate, (E)-; (E)-5-[[p-[(2-Carboxyethyl)carbamoyl]phenyl]azo]salicylic acid, disodium salt, dihydrate [150399-21-6].

#### DEFINITION

Balsalazide Disodium contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ , calculated on the as-is basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B. ULTRAVIOLET ABSORPTION** <197U>  
Sample solution: 10 µg/mL in water
- **C. IDENTIFICATION TESTS—GENERAL**, Sodium <191>

#### ASSAY

##### PROCEDURE

Sample: 219 mg

Analysis: Add 80 mL of glacial acetic acid to the *Sample*, sonicate to dissolve, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ .

Acceptance criteria: 98.0%–102.0% on the as-is basis

#### IMPURITIES

##### Inorganic Impurities

- **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

##### Organic Impurities

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when impurities 1, 2, and 3 listed in *Impurity Table 2* may be present.]

##### PROCEDURE 1

**Solution A:** Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of  $6.00 \pm 0.1$ .

**Diluent:** Use *Solution A*.

**Solution B:** Use acetonitrile.

**Standard solution:** 0.5 µg/mL of USP Balsalazide Disodium RS, 0.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*. If needed, a small amount of acetonitrile may be added to facilitate dissolution. [NOTE—USP Balsalazide Related Compound A RS is the disodium salt of (E)-5-[(p-carboxyphenyl)azo]-2-salicylic acid. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]

**Sample solution:** 1 mg/mL of Balsalazide Disodium in *Diluent*

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 238 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 30 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 5 between balsalazide and balsalazide related compound B

**Relative standard deviation:** NMT 5% for each peak

**Tailing factor:** NMT 1.8 for the balsalazide peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for each individual impurity from the *Sample solution*  
 $r_S$  = peak response for the corresponding impurity from the *Standard solution*. [NOTE—For unspecified impurities,  $r_S$  is the peak response for the balsalazide peak from the *Standard solution*.]  
 $C_S$  = concentration of the corresponding impurity in the *Standard solution* (mg/mL). [NOTE—For unspecified impurities,  $C_S$  is the concentration of balsalazide disodium in the *Standard solution*.]  
 $C_U$  = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Reporting level for impurities:** 0.03%

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	0.05
Balsalazide related compound A <sup>a</sup>	0.70	0.05
Balsalazide	1.00	—
Balsalazide related compound B <sup>b</sup>	1.2	0.05
Any other individual unspecified impurity	—	0.05

<sup>a</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.

<sup>b</sup> (E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.

#### PROCEDURE 2

**Solution A:** Prepare 50 mM monobasic potassium phosphate buffer as follows: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 2 N potassium hydroxide solution to a pH of 6.8–7.0. [NOTE—To ensure proper identification of impurity 1, the pH must be maintained between 6.8 and 7.0.]

**Solution B:** Acetonitrile, methanol and *Solution A* (5:1:14)

**Solution C:** Acetonitrile, methanol and *Solution A* (9:1:10)

**Diluent:** Use *Solution B*.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
37	0	100	0
60	0	0	100
75	100	0	0
85	100	0	0

**Standard solution:** 0.075 mg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication to dissolve.]

**Sensitivity solution:** 0.375 µg/mL in *Diluent*, from *Standard solution*

**System suitability solution:** 1.5 mg/mL of USP Balsalazide Disodium RS and 1.5 µg/mL of USP Balsalazide Related Compound A RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Balsalazide Disodium in *Diluent*. [NOTE—Use sonication to dissolve.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Column temperature:** 25°–27°. [NOTE—To ensure proper identification of impurity 1, the column temperature must be maintained between 25° and 27°.]

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 8.5 between balsalazide related compound A and balsalazide, from the *System suitability solution*

**Tailing factor:** NMT 3.4 for the balsalazide peak, from the *System suitability solution*

**Signal-to-noise ratio:** NLT 10, from the *Sensitivity solution*

**Relative standard deviation:** NMT 2.0%, from the *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response for each individual impurity from the *Sample solution*  
 $r_S$  = peak response for balsalazide from the *Standard solution*  
 $C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution*  
 $C_U$  = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Impurity Table 2*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 2*.

**Reporting level for impurities:** 0.03%

**Total impurities:** NMT 0.50%

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-(4-Aminobenzoyl)-β-alanine <sup>a</sup>	0.29	1.8	0.05
Salicylic acid	0.55	1.4	0.05
Balsalazide related compound A <sup>b</sup>	0.88	1.4	0.05
Impurity 1 <sup>c</sup>	0.91	1.9	0.05
Impurity 2 <sup>d</sup>	0.92	1.4	0.05
Impurity 3 <sup>e</sup>	0.94	0.83	0.05

<sup>a</sup> This impurity may be present as two peaks. Use the sum of the two peaks to determine compliance.

<sup>b</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.

<sup>c</sup> (E,E)-3,5-di-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.

<sup>d</sup> (E)-3-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.

<sup>e</sup> (E,E)-5-[(2-[4-(2-Carboxyethylcarbamoyl)phenylazo]-4-[2-carboxyethylcarbamoyl])phenylazo]-salicylic acid.

<sup>f</sup> (E)-2-[4-(2-Carboxyethylcarbamoyl)phenoxy]-5-[[4-(2-carboxyethylcarbamoyl)phenylazo]-benzoic acid

<sup>g</sup> (E)-2-Hydroxy-5-[[4-[(3-isopropoxy-3-oxopropyl)amino]carbonyl]phenylazo]benzoic acid

**Impurity Table 2** (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Balsalazide	1.00	—	—
Impurity 4 <sup>f</sup>	1.35	2.1	0.05
Impurity 5 <sup>g</sup>	1.77	0.91	0.05
Any other individual unspecified impurity	—	—	0.05

<sup>a</sup> This impurity may be present as two peaks. Use the sum of the two peaks to determine compliance.

<sup>b</sup> (*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid.

<sup>c</sup> (*E*)-3,5-di-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.

<sup>d</sup> (*E*)-3-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.

<sup>e</sup> (*E*)-5-[(2-[4-(2-Carboxyethylcarbamoyl)phenylazo]-4-[2-carboxyethylcarbamoyl])phenylazo]-salicylic acid.

<sup>f</sup> (*E*)-2-[4-(2-Carboxyethylcarbamoyl)phenoxy]-5-[4-(2-carboxyethylcarbamoyl)phenylazo]-benzoic acid

<sup>g</sup> (*E*)-2-Hydroxy-5-[4-[(3-isopropoxy-3-oxopropyl)amino]carbonyl]phenylazo]benzoic acid

**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method 1a* (921): 7.8%–9.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Test 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** (11)
  - USP Balsalazide Disodium RS
  - USP Balsalazide Related Compound A RS
  - (*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.
   
C<sub>14</sub>H<sub>8</sub>N<sub>2</sub>O<sub>5</sub>Na<sub>2</sub> 330.12
  - USP Balsalazide Related Compound B RS
  - (*E*)-5-[(*m*-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.
   
C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>6</sub> 357.17
  - USP Salicylic Acid RS

**Balsalazide Disodium Capsules****DEFINITION**

Balsalazide Disodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of balsalazide disodium (C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>Na<sub>2</sub>O<sub>6</sub> · 2H<sub>2</sub>O).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION**

Using a 0.2-cm cell, record the UV spectrum of the *Sample solution*, obtained in the *Assay*, in the range of 200–400 nm: it exhibits maxima at about 261 nm and 357 nm.

**ASSAY**• **PROCEDURE**

**Buffer:** Add 5 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of 6.00 ± 0.1.  
**Mobile phase:** Acetonitrile and *Buffer* (1:4)  
**Diluent:** Water  
**Standard solution:** 60 µg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication as necessary.]  
**Sample stock solution:** Transfer an equivalent to 150 mg of balsalazide disodium, from the Capsules con-

tents, to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.

**Sample solution:** 60 µg/mL of balsalazide disodium, from the *Sample stock solution*, in *Diluent*. Pass a portion of this solution through a suitable filter, discarding the first 3 mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 10,000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Sample:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>Na<sub>2</sub>O<sub>6</sub> · 2H<sub>2</sub>O in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of balsalazide disodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****Organic Impurities**• **PROCEDURE**

**Buffer:** Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of 6.00 ± 0.1.

**Diluent:** Water

**Solution A:** *Buffer*

**Solution B:** Acetonitrile

**Sample solution:** Transfer an amount of finely crushed powder equivalent to 100 mg of balsalazide disodium to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size.

**Standard solution:** 1.0 µg/mL of USP Balsalazide Disodium RS in *Diluent*

**System suitability solution:** 1.0 µg/mL of USP Balsalazide Disodium RS, 1.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 238 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 45°**Flow rate:** 1 mL/min**Injection size:** 30 μL**System suitability****Samples:** *Standard solution* and *System suitability solution***Suitability requirements****Resolution:** NLT 5 between balsalazide and balsalazide related compound B, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Tailing factor:** NMT 1.5, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response for each individual impurity from the *Sample solution* $r_S$  = peak response for the balsalazide peak from the *Standard solution* $C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of balsalazide disodium in the *Sample solution*, based on the label claim (mg/mL) $F$  = relative response factor (see *Impurity Table 1*)**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Reporting level for impurities:** 0.05%**Total impurities:** NMT 1.0%. [NOTE— When reporting results for *Individual impurities* and *Total impurities*, disregard peaks corresponding to salicylic acid and balsalazide related compound B, as these impurities are controlled in the drug substance only.]**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	—	—
Balsalazide related compound A <sup>a</sup>	0.70	1.3	0.15
Balsalazide	1.00	—	—
Balsalazide related compound B <sup>b</sup>	1.2	—	—
Any other individual unspecified impurity	—	1.0	0.10

<sup>a</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.<sup>b</sup> (E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.**PERFORMANCE TESTS****• DISSOLUTION <711>****Medium:** pH 6.8 phosphate buffer; 900 mL**Apparatus 2:** 50 rpm, with stainless steel wire helix sinkers**Time:** 30 min**Detector:** UV 357 nm, with background correction at 590 nm**Path length:** 0.02-cm flow cell**Blank:** *Medium***Standard solution:** 0.83 mg/mL of USP Balsalazide Disodium RS in *Medium***Sample solution:** Pass a portion of the solution under test through a suitable filter of 20-μm pore size.**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL) $V$  = volume of *Medium* (mL), 900 $L$  = Capsule label claim (mg)**Tolerances:** NLT 70% (Q) of the labeled amount of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS <11>**

USP Balsalazide Disodium RS

USP Balsalazide Related Compound A RS

(E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.

 $C_{14}H_8N_2O_5Na_2$  330.12

USP Balsalazide Related Compound B RS

(E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.

 $C_{17}H_{13}N_3O_6$  357.17

USP Salicylic Acid RS

**Adhesive Bandage**

» Adhesive Bandage consists of a compress of four layers of Type I Absorbent Gauze, or other suitable material, affixed to a film or fabric coated with a pressure-sensitive adhesive substance. It is sterile. The compress may contain a suitable antimicrobial agent and may contain one or more suitable colors. The adhesive surface is protected by a suitable removable covering.

**Packaging and storage**—Package Adhesive Bandage that does not exceed 15 cm (6 inches) in width individually in such manner that sterility is maintained until the individual package is opened. Package individual packages in a second protective container.

**Labeling**—The label of the second protective container bears a statement that the contents may not be sterile if the individual package has been damaged or previously opened, and it bears the names of any added antimicrobial agents. Each individual package is labeled to indicate the dimensions of the compress and the name of the manufacturer, packer, or distributor, and each protective container indicates also the address of the manufacturer, packer, or distributor.

**Sterility <71>:** meets the requirements.

## Gauze Bandage

» Gauze Bandage is Type I Absorbent Gauze. Its length is not less than 98.0 percent of that declared on the label, and its average width is not more than 1.6 mm less than the declared width. It contains no dye or other additives.

**Packaging and storage**—Gauze Bandage that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.

**Labeling**—The width and length of the Bandage, the number of pieces contained, and the name of the manufacturer, packer, or distributor, are stated on the package. The designation “non-sterilized” or “not sterilized” appears prominently on the package unless the Gauze Bandage has been rendered sterile, in which case it may be labeled to indicate that it is sterile and that the contents may not be sterile if the package bears evidence of damage or if the package has been previously opened.

**NOTE**—Before determining the thread count, dimensions, and weight, hold the Bandage, unrolled, for not less than 4 hours in a standard atmosphere of  $65 \pm 2\%$  relative humidity at  $21 \pm 1.1^\circ \text{C}$  ( $70 \pm 2^\circ \text{F}$ ).

**Thread count**—Count the number of warp and filling threads of it in areas of 1.27 cm ( $1/2$  inch) square at 5 points evenly spread along the center line of the Bandage, no point being within 30.5 cm (12 inches) of either end of the Bandage, and calculate the average number of threads per 2.54 cm (1 inch) in each direction. A variation of not more than 3 threads per inch is allowed in either warp or filling, provided that the combined variations do not exceed 5 threads per square inch.

**Width**—Measure its width at each of the 5 points selected for the determination of the thread count: the average of 5 measurements is not more than 1.6 mm ( $1/16$  inch) less than the labeled width of the Bandage.

**Length**—Measure the length of the unrolled Gauze Bandage, smoothed without tension, along the center line of the Gauze Bandage: the length is not less than 98.0% of the labeled length of the Bandage.

**Weight**—Weigh the entire Bandage: the calculated weight in g per 0.894 square meter (1 linear yard *Type I gauze*), using the measurements obtained as described in the two paragraphs just preceding, is not less than 39.2 g.

**Absorbency**—Hold a rolled Gauze Bandage horizontal to and almost in contact with the surface of water at  $25^\circ$ , and allow it to drop lightly upon the water: complete submersion takes place in not more than 30 seconds.

**Sterility** (71): Gauze Bandage that has been rendered sterile meets the requirements.

**Other requirements**—It meets the requirements of the tests for *Ignited residue*, *Acid or alkali*, and *Dextrin or starch*, in *water extract*, *Residue on ignition*, *Fatty matter*, and *Alcohol-soluble dyes* under *Absorbent Gauze*.

## Barium Hydroxide Lime

### DEFINITION

Barium Hydroxide Lime is a mixture of barium hydroxide octahydrate and Calcium Hydroxide. It may also contain Potassium Hydroxide and may contain an indicator that is inert toward anesthetic gases such as Ether, Cyclopropane, and Nitrous Oxide and that changes color when

the Barium Hydroxide Lime no longer can absorb carbon dioxide.

[**CAUTION**—Because Barium Hydroxide Lime contains a soluble form of barium, it is toxic if swallowed.]

### IDENTIFICATION

- **A.** **Analysis:** Place a granule of it on a piece of moistened red litmus paper.  
**Acceptance criteria:** The paper turns blue immediately.
- **B. IDENTIFICATION TESTS—GENERAL, Barium, Calcium, and Potassium (191)**  
**Sample solution:** 100 mg/mL in 6 N acetic acid  
**Acceptance criteria:** Meets the requirements for *Barium* and for *Calcium*, and it may meet the requirement of the flame test for *Potassium*.

### SPECIFIC TESTS

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING (786)**  
**Sample:** 100 g  
**Analysis:** Screen the *Sample* for 5 min as directed in the chapter, using a mechanical shaker.  
**Acceptance criteria:** It passes completely through a No. 2 standard-mesh sieve, and NMT 2.0% passes through a No. 40 standard-mesh sieve. NMT 7.0% is retained on the coarse-mesh sieve, and NMT 15.0% passes through the fine-mesh sieve designated on the label.
- **LOSS ON DRYING (731)**  
**Sample:** 10 g  
**Analysis:** Weigh the *Sample* in a tared weighing bottle, and dry at  $105^\circ$  for 2 h.  
**Acceptance criteria:** 11.0%–16.0%
- **HARDNESS**  
**Sample:** 200 g  
**Analysis:** Screen the *Sample* on a mechanical sieve shaker (see *Particle Size Distribution Estimation by Analytical Sieving (786)*) having a frequency of oscillation of  $285 \pm 3$  cycles/min, for 3 min, to remove granules coarser than 4-mesh and finer than 8-mesh. Weigh 50 g of the granules retained on the screen, and place them in a hardness pan of the following description: the hardness pan has a diameter of 200 mm and a concave brass bottom, and the bottom of the pan is 7.9 mm thick at the circumference and 3.2 mm thick at the center and has an inside spherical radius of curvature of 109 cm. Add 15 steel balls of 7.9-mm diameter, and shake on a mechanical sieve shaker for 30 min. Remove the steel balls, brush the contents of the hardness pan onto a sieve of the fine-mesh size designated on the label, shake for 3 min on the mechanical sieve shaker, and weigh.  
**Acceptance criteria:** The percentage of Barium Hydroxide Lime retained on the screen is NLT 75.0% and represents the hardness.
- **CARBON DIOXIDE ABSORBENCY**  
**Analysis:** Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and weigh the tube and the contents. Into the other arm of the tube, place 9.5–10.5 g of Barium Hydroxide Lime, and again weigh. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with Barium Hydroxide Lime to a calcium chloride drying tube, which in turn is connected to a suitable source of supply of carbon dioxide. Pass the carbon dioxide through the U-tube at a rate of 75 mL/min for 20 min, timed. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh.  
**Acceptance criteria:** The increase in weight is NLT 19.0% of the weight of Barium Hydroxide Lime used for the test.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** If an indicator has been added, the name and color change of such indicator are stated on the container label. The container label indicates also the mesh size in terms of standard-mesh sieve sizes (see *Powder Fineness* <811>).

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**Barium Sulfate**

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BaSO<sub>4</sub> 233.39  
Sulfuric acid, barium salt (1:1);  
Barium sulfate (1:1) [7727-43-7].

**DEFINITION**

Barium Sulfate contains NLT 97.5% and NMT 100.5% of barium sulfate (BaSO<sub>4</sub>).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>  
**Sample solution:** Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter.  
**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Barium** <191>  
**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.  
**Acceptance criteria:** The solution meets the requirements.

**ASSAY****• PROCEDURE**

**Sample:** 0.58–0.62 g, weighed in a tared platinum crucible

**Analysis:** Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL

of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate (BaSO<sub>4</sub>).

**Acceptance criteria:** 97.5%–100.5%

**IMPURITIES****• HEAVY METALS** <231>

**Sample solution:** Boil 4.0 g with a mixture of 2 mL of glacial acetic acid and 48 mL of water for 10 min. Dilute with water to 50 mL, filter, and use 25 mL of the filtrate.

**Acceptance criteria:** NMT 10 ppm

**• LIMIT OF SULFIDE**

**Sample solution:** Transfer 10 g to a 500-mL conical flask. Add 100 mL of 0.3 N hydrochloric acid.

**Control solution:** 100 mL of 0.3 N hydrochloric acid containing 5 µg of sulfide in a 500-mL conical flask

**Analysis:** Cover the mouth of both conical flasks with a circle of filter paper that has been moistened at the area over the mouth of the flask with 0.15 mL of lead acetate TS, the paper being held in place with a string tied around the neck of the flask. Boil each mixture gently for 10 min, taking care to avoid spattering the paper.

**Acceptance criteria:** NMT 0.5 µg/g; any darkening of the paper by the *Sample solution* is not greater than that produced by the similarly treated *Control solution*.

**• LIMIT OF ACID-SOLUBLE SUBSTANCES**

**Sample solution:** Cool the mixture obtained in the test for *Limit of Sulfide*, add water to restore approximately the original volume, and filter it through paper that previously has been washed with a mixture of 10 mL of 3 N hydrochloric acid and 90 mL of water, returning the first portions, if necessary, to obtain a clear filtrate.

**Analysis:** Evaporate 50 mL of the filtrate on a steam bath to dryness, and add 2 drops of hydrochloric acid and 10 mL of hot water. Filter again through acid-washed paper, prepared as directed above. Wash the filter with 10 mL of hot water, and evaporate the combined filtrate and washings in a tared dish on a steam bath to dryness. Dry the residue at 105° for 1 h.

**Acceptance criteria:** NMT 0.3%; the residue weighs NMT 15 mg.

**• LIMIT OF SOLUBLE BARIUM SALTS**

**Sample:** The residue obtained in the test for *Limit of Acid-Soluble Substances*

**Control:** 10 mL of water containing 0.5 mL of 2 N sulfuric acid and 50 µg of barium

**Analysis:** Treat the *Sample* with 10 mL of water, pass the solution through a filter previously washed with 100 mL of 0.3 N hydrochloric acid, and add 0.5 mL of 2 N sulfuric acid.

**Acceptance criteria:** NMT 0.001%; any turbidity formed in the *Sample* within 30 min is NMT that produced in the similarly treated *Control*.

**SPECIFIC TESTS**

- **PH** <791>: 3.5–10.0, in a 10% (w/w) aqueous suspension

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

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**Barium Sulfate Paste**

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**DEFINITION**

Barium Sulfate Paste is a semisolid formulation of finely divided particles of Barium Sulfate in a suitable base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate (BaSO<sub>4</sub>). It may contain one or more



suitable colors, flavors, suspending or dispersing agents, and preservatives.

## IDENTIFICATION

### • A. IDENTIFICATION TESTS—GENERAL, Sulfate <191>

**Sample:** Ignite a quantity of Paste equivalent to 0.5 g of barium sulfate to constant weight.

**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.

### • B. IDENTIFICATION TESTS—GENERAL, Barium <191>

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.

**Acceptance criteria:** The solution meets the requirements.

## ASSAY

### • PROCEDURE

**Sample:** Barium Sulfate Paste, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible

**Analysis:** Ignite the *Sample* over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and di-

gest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

## SPECIFIC TESTS

### • MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>

**For products labeled for oral administration only:**

The total aerobic microbial count does not exceed  $10^2$  cfu/g. The total combined molds and yeasts count does not exceed  $10^1$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

**For products labeled for oral administration and rectal administration:**

The total aerobic microbial count does not exceed  $10^2$  cfu/g. The total combined molds and yeasts count does not exceed  $10^1$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

**For products labeled for rectal administration only:**

The total aerobic microbial count does not exceed  $10^3$  cfu/g. The total combined molds and yeasts count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

### • pH <791>: 3.0–10.0

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from freezing and from excessive heat.

## Barium Sulfate Suspension

### DEFINITION

Barium Sulfate Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ). It contains suitable dispersing and/or suspending agents so that when mixed as directed in the labeling, it yields a uniformly dispersed suspension. It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Sulfate <191>

**Sample:** Shake the Suspension, and transfer a volume equivalent to 0.5 g of barium sulfate to a suitable container. Ignite to constant weight.

**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.

#### • B. IDENTIFICATION TESTS—GENERAL, Barium <191>

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.

**Acceptance criteria:** The solution meets the requirements.

**ASSAY****• PROCEDURE**

**Sample:** A volume of Suspension, previously well shaken in its original container, equivalent to 0.60 g of barium sulfate, in a tared platinum crucible

**Analysis:** Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total bacterial count does not exceed  $10^2$  cfu/mL, the total combined molds and yeasts count does not exceed  $10^1$  cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

- pH (791):** 3.5–10.0

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

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**Barium Sulfate for Suspension**


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**DEFINITION**

Barium Sulfate for Suspension is a dry mixture of Barium Sulfate and one or more suitable dispersing and/or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ). It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

**IDENTIFICATION**

- A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)**

**Sample:** Ignite 1 g to constant weight.

**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter.

**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.

- B. IDENTIFICATION TESTS—GENERAL, Barium (191)**

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification* test A in 6 N acetic acid.

**Acceptance criteria:** The solution meets the requirements.

**ASSAY****• PROCEDURE**

**Sample:** Barium Sulfate for Suspension, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible

**Analysis:** Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731)  
Analysis: Dry at 105° for 4 h.  
Acceptance criteria: NMT 1.0%
- **pH** (791): 3.5–10.0, in a 60% (w/w) aqueous suspension, or constituted for its intended use as directed in the labeling

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

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### Barium Sulfate Tablets

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#### DEFINITION

Barium Sulfate Tablets are flat-sided disks between 11.5 mm and 13.5 mm in diameter and contain NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)  
Sample: A portion of powdered Tablets equivalent to 0.6 g of barium sulfate  
Analysis: Mix the Sample with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.  
Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Barium** (191)  
Sample solution: Dissolve a portion of the well-washed residue from Identification test A in 6 N acetic acid.  
Acceptance criteria: The solution meets the requirements.

#### ASSAY

- **PROCEDURE**  
Sample: A portion of powdered Tablets, equivalent to 0.6 g of barium sulfate, weighed in a tared platinum crucible  
Analysis: Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a

400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701): NLT 10 min and NMT 30 min
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

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### BCG Live

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» BCG Live (intravesical) for immunotherapy is a freeze-dried preparation of attenuated live bacteria derived from a culture of *Bacillus Calmette-Guérin* (*Mycobacterium bovis*, var. BCG) and used intravesically in the treatment of carcinoma *in situ* and papilloma tumors of the urinary bladder. The bacteria are grown in a medium that does not contain substances known to cause toxic or allergic reactions in human beings or to cause the bacteria to become virulent for guinea pigs. The culture is harvested and formulated to contain one or more excipients. The freeze-dried preparation is reconstituted and further diluted aseptically with a sterile diluent for use. A reconstituted dose contains  $1.0\text{--}19.2 \times 10^8$  colony-forming units (cfu). BCG Live does not contain a preservative.

**Packaging and storage**—BCG Live is sensitive to light and therefore must be preserved and stored in a glass con-

tainer where it is protected from direct light at a temperature between 2° and 8°.

**Expiration date**—The product is stable for 3 years when stored between 2° and 8°.

**Labeling**—Label it to indicate the dry weight of bacteria in a vial, cfu per dose, the storage conditions, the expiration date, and that it is not to be used after the expiration date given on the package. Label it to state that it should be protected from light and that it should be used immediately after reconstitution/dilution. Label it to indicate that it is “for intravesical use”.

**Identification**—BCG Live is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property. Alternatively, validated molecular biology techniques may be used.

**General safety**—It meets the requirements as set forth for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88), modified as follows. Guinea pigs are injected intraperitoneally with 3.0 mL of the reconstituted product.

**Sterility** (71)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*.

#### **Virulent mycobacteria—**

**Test suspension**—Reconstitute the freeze-dried BCG Live as per the manufacturer's instructions for human use with the diluent recommended by the manufacturer, and dilute aseptically to about 2 mg per mL with sterile BCG diluent.

**Procedure**—Randomly select not fewer than six guinea pigs of the same sex, each weighing 250 to 300 g. Inject each animal with a total of at least 4 mg of the *Test suspension* intramuscularly or subcutaneously in the rear left internal thigh, and observe them for a period of 6 weeks. Note the number of animals that survive at the end of the observation period, and then sacrifice them. Perform autopsies of all animals postmortem to examine them for evidence of tuberculous infections, particularly at the popliteal and inguinal lymph nodes, liver, spleen, pancreas, and lungs, as well as at the injection site. If any abnormalities are found, perform a histological examination using standard and Acid-Fast staining techniques to detect Acid-Fast organisms. The product complies with the test if none of the animals show signs of tuberculosis and not more than one-third of the animals die during the observation period.

#### **Skin reactivity—**

**Test suspensions**—Using the same diluent and the *Test suspension* prepared as directed in the test for *Virulent mycobacteria*, further dilute aseptically by making three serial tenfold dilutions.

**Procedure**—Randomly select two guinea pigs (male or female), each weighing 250 to 300 g. Inject 0.1 mL of each of the four suspensions intradermally at different sites on the back of each animal. After 4 weeks, the animals are shaved so that the injection sites and any reactions are made clearly visible. The diameters of the reactions are measured, and the presence of necrosis or nodules are noted. The reaction for the largest dose is between 4 and 10 mm and the smallest dose induces a nodule less than or equal to 4 mm. Each animal gains weight during the observation period.

#### **Tuberculin sensitivity—**

**Tuberculin solution**—Use tuberculin, purified protein derivative, to prepare a solution containing 25 U.S. Tuberculin Units per 0.1 mL. Dilute aseptically, if necessary, with sterile 0.9% sodium chloride solution.

**Procedure**—Use the same animals on which the *Skin reactivity* test is performed. After the *Skin reactivity* test is completed, inject each animal intradermally on the back with 0.1 mL of the *Tuberculin solution*, and observe after 18 to 24 hours. An erythematous reaction of not less than 10 mm in diameter is measured on each animal.

**Residual moisture**: Not greater than the limit approved for the particular product, determined by a suitable validated method. Limits vary in accordance with the method.

**Viability**—Determine the potencies of BCG Live using not less than 5 containers before freeze-drying and an equal number of containers after freeze-drying, following the procedure described under *Potency*, except use the suspension before freeze-drying as is. The loss in viability due to freeze-drying is not more than 90%.

**Potency**—Determine the number of viable units per mL by viable count on solid medium using a method suitable for the product to be examined. Alternatively, a validated biochemical method may be used.

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## **BCG Vaccine**

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» BCG Vaccine conforms to the regulations of the FDA concerning biologics (see *Biologics* (1041)). It is a dried, living culture of the bacillus Calmette-Guérin strain of *Mycobacterium tuberculosis* var. *bovis*, grown in a suitable medium from a seed strain of known history that has been maintained to preserve its capacity for conferring immunity. It contains an amount of viable bacteria such that inoculation, in the recommended dose, of tuberculin-negative persons results in an acceptable tuberculin conversion rate. It is free from other organisms, and contains a suitable stabilizer. It contains no antimicrobial agent.

**NOTE**—Use the Vaccine immediately after its constitution, and discard any unused portion after 2 hours.

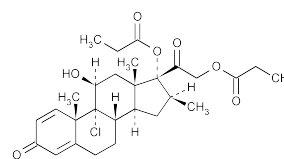
**Packaging and storage**—Preserve in hermetic containers, preferably of Type I glass, at a temperature between 2° and 8°.

**Expiration date**—The expiration date is not later than 6 months after date of issue, or not later than 1 year after date of issue if stored at a temperature below 5°.

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## **Beclomethasone Dipropionate**

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$C_{28}H_{37}ClO_7$  521.04

Pregna-1,4-diene-3,20-dione, 9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11 $\beta$ ,16 $\beta$ )-, 9-Chloro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5534-09-8].  
Monohydrate 539.07.

» Beclomethasone Dipropionate is anhydrous or contains one molecule of water of hydration. It contains not less than 97.0 percent and not more than 103.0 percent of  $C_{28}H_{37}ClO_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Beclomethasone Dipropionate RS  
USP Testosterone Propionate RS

**Identification**, *Infrared Absorption* (197M).

**Specific rotation** (781S): between +88° and +94°.

*Test solution*: 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 3 hours: the anhydrous form loses not more than 0.5% of its weight; the monohydrate form loses between 2.8% and 3.8% of its weight.

**Residue on ignition** (281): not more than 0.1%.

#### Assay—

*Mobile phase*—Prepare a suitable degassed solution of 3 volumes of acetonitrile in 2 volumes of water, such that the retention time of beclomethasone dipropionate is approximately 6 minutes and that of testosterone propionate is approximately 10 minutes.

*Internal standard solution*—Dissolve a suitable quantity of USP Testosterone Propionate RS, accurately weighed, in methanol to obtain a solution having a concentration of about 1.2 mg per mL.

*Standard preparation*—Dissolve a suitable quantity of USP Beclomethasone Dipropionate RS, accurately weighed, in methanol to obtain a solution having a concentration of about 1.4 mg per mL. Transfer 4.0 mL of this solution to a suitable vial, and add 4.0 mL of *Internal standard solution* to obtain a solution having a known concentration of about 0.7 mg per mL with respect to the Reference Standard and 0.6 mg per mL with respect to the internal standard.

*Assay preparation*—Weigh accurately about 70 mg of Beclomethasone Dipropionate, transfer to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a suitable vial, and add 4.0 mL of *Internal standard solution*.

*Procedure*—Separately inject equal volumes (between 5  $\mu$ L and 25  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into a high-performance liquid chromatograph (see *Chromatography* (621)) operated at room temperature, adjusting the specimen size and other operating parameters such that the peak obtained with the internal standard in the *Standard preparation* is about 0.6 to 0.9 full-scale. Typically, the apparatus is fitted with a 4-mm  $\times$  30-cm column that contains packing L1 and is equipped with an UV detector capable of monitoring absorption at 254 nm, a suitable recorder, and a pump capable of operating at a column pressure of up to 3500 psi. In a suitable chromatogram, the coefficient of variation for five replicate injections of the *Standard preparation* is not more than 3.0%. Calculate the quantity, in mg, of  $C_{28}H_{37}ClO_7$  in the portion of Beclomethasone Dipropionate taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Beclomethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of beclomethasone dipropionate to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Belladonna Leaf

» Belladonna Leaf consists of the dried leaf and flowering or fruiting top of *Atropa belladonna* Linné or of its variety *acuminata* Royle ex Lindley (Fam. Solanaceae). Belladonna Leaf yields not less

than 0.35 percent of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in well-closed containers and avoid long exposure to direct sunlight. Preserve powdered Belladonna Leaf in light-resistant containers.

**USP Reference standards** (11)—  
USP Atropine Sulfate RS  
USP Homatropine Hydrobromide RS  
USP Scopolamine Hydrobromide RS

#### Botanic characteristics—

*Belladonna Leaf*—Usually partly matted together, crumpled or broken leaves, together with some smaller stems and a number of flowers and fruits. The leaves are thin and brittle, mostly light green to moderate olive-green. The lamina is mostly from 5 to 25 cm in length and from 4 to 12 cm in width and possesses an ovate-lanceolate to broadly ovate outline, an acute to acuminate apex, an entire margin, an acute to somewhat decurrent base and slightly hairy surface, the hairs being more abundant along the veins; when broken transversely, it shows numerous light-colored dots (crystal cells) visible with a lens. The petiole is slender and usually up to 4 cm in length. The flowers possess a campanulate corolla with 5 small, reflexed lobes, purplish to yellowish purple, becoming faded to brown or dusky yellow or yellow, a green, 5-lobed calyx, 5 epipetalous stamens, and a superior, bilocular ovary with numerous ovules. The fruit is subglobular, dark yellow to yellowish brown to dusky red or black, up to about 12 mm in width and sometimes subtended by the persistent calyx and containing numerous flattened, somewhat reniform seeds, the latter up to about 2 mm in width. The stems are more or less flattened and hollow and finely hairy when young.

*Histology*—*Leaf*: The epidermis of the lamina possesses wavy anticlinal walls and a distinctly striated cuticle. Stomata are more numerous in the lower epidermis and are surrounded by 3 or 4 neighboring cells, one of which is smaller than the others. The nonglandular hairs are uniseriate and up to 6-celled. Short club-shaped glandular hairs with a 1-celled stalk and multicellular head and long glandular hairs with a uniseriate stalk and unicellular head occur on both epidermises. The mesophyll consists of a single layer of palisade parenchyma beneath which occurs spongy parenchyma, the latter with scattered cells filled with microcrystals. The midrib contains an arc of bicollateral bundles, collenchyma beneath upper epidermis, and scattered parenchyma cells with microcrystals. *Stem*: The stem shows an epidermis with striated cuticle and few hairs, a distinct endodermis, small strands of long, thin-walled, slightly lignified pericyclic fibers, and a circle of bicollateral bundles. The parenchyma of the cortex and pith is interspersed with crystal cells. *Flower*: The calyx possesses numerous glandular hairs with uniseriate stalks and 1- to 3-celled glandular heads. The corolla shows a papillose inner epidermis and an outer epidermis with glandular hairs similar to those of the calyx. The pollen grains, when mounted in chloral hydrate solution, are subspherical, about 40  $\mu$ m in diameter, tricolpate, having 3 germinal furrows and rows of pits between the ridges on the exine. *Fruit*: The epicarp exhibits polygonal epidermal cells with a striated cuticle and stomata. The mesocarp consists of large pulp cells some of which contain rosette aggregate crystals of calcium oxalate. *Seed*: The seed is characterized by an epidermis of large, wavy-walled cells with prominent ridges over the anticlinal walls.

*Powdered Belladonna Leaf*—Light olive-brown to moderate olive-green in color. The following are among the elements of identification: the separate microcrystals, the dark gray crystal cells, the cuticular striping of the epidermal cells, the vessels with ellipsoidal bordered pits, the fibers of the stem, and occasional hairs and pollen grains. Rosette aggregates of calcium oxalate and fragments of the seed occur when the drug contains belladonna fruits. Examine Belladonna

Leaf for hairs having a papillose cuticle and for raphides of calcium oxalate: their presence indicates adulteration.

**Acid-insoluble ash** (561): not more than 3.0%.

**Belladonna stems**—The proportion of belladonna stems over 10 mm in diameter does not exceed 3.0%.

**Assay—**

*pH 9.5 Phosphate buffer, Internal standard solution, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and System suitability*—Proceed as directed in the Assay under *Belladonna Extract*.

*Assay preparation*—Moisten 10 g, previously reduced to a moderately coarse powder and accurately weighed, with a mixture of 8 mL of ammonium hydroxide, 10 mL of alcohol, and 20 mL of ether, and extract the alkaloids by either of the methods given in the following two paragraphs. If necessary, reduce the volume of the extract to 100 mL by evaporation on a steam bath.

*I*—Place the moistened drug in a continuous-extraction thimble, and allow maceration to proceed overnight, then extract with ether for 3 hours, or longer if necessary to effect complete extraction.

*II*—Place the moistened drug in a small percolator, and allow maceration to proceed overnight. Percolate slowly with a mixture of 3 volumes of ether and 1 volume of chloroform. Continue the percolation until the residue from 3 to 4 mL of percolate last passed, when dissolved in dilute sulfuric acid (1 in 70) and treated with mercuric iodide TS, shows not more than a faint turbidity.

Transfer the extract to a separator with the aid of ether. Extract with five 15-mL portions of dilute sulfuric acid (1 in 70), filtering each portion drawn off into a 100-mL volumetric flask. Wash the filter with dilute sulfuric acid (1 in 70), and collect the washings in the flask. Add dilute sulfuric acid (1 in 70) to volume, and mix. Dilute 20.0 mL of the resulting solution with the same dilute acid to 100.0 mL.

Pipet 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a *mixed solvent* consisting of chloroform-isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of *pH 9.5 Phosphate buffer* and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Sodium Sulfate, Anhydrous*, in the section *Reagent Specifications*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

*Procedure*—Record from the *Standard curves* the quantities, in mg, of atropine and scopolamine in the weight of the specimen taken. Proceed as directed for *Procedure* in the Assay under *Belladonna Extract*, through the next-to-the-last sentence. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids in the portion of Belladonna Leaf taken.

## Belladonna Extract

» Belladonna Extract contains, in each 100 g, not less than 1.15 g and not more than 1.35 g of the alkaloids of belladonna leaf.

**PILULAR BELLADONNA EXTRACT**

Prepare the extract by percolating 1000 g of Belladonna Leaf, using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Macerate the drug for 16 hours, and then percolate it at a moderate rate. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a pilular consistency, and adjust the remaining extract, after assaying, by dilution with liquid glucose so that the finished Extract will contain, in each 100 g, 1.25 g of the alkaloids of belladonna leaf.

**POWDERED BELLADONNA EXTRACT**

Prepare the extract by percolating 1000 g of Belladonna Leaf, using alcohol as the menstruum. Macerate the drug for 16 hours, and then percolate it slowly. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a soft extract, add 50 g of dry starch, and continue the evaporation, at the same temperature, until the product is dry. Powder the residue. The extract may be deprived of its fat by treating either the soft extract first obtained, or the dry and powdered extract, as directed under *Extracts* (see *Pharmaceutical Dosage Forms* <1151>). Assay the powdered residue, and add sufficient starch, previously dried at 100°, to obtain a finished Extract containing 1.25 g of the alkaloids of belladonna leaf in each 100 g. Mix the powders, and pass the Extract through a fine sieve.

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 30°.

**USP Reference standards** <11>—

USP Atropine Sulfate RS

USP Homatropine Hydrobromide RS

USP Scopolamine Hydrobromide RS

**Assay—**

*pH 9.5 Phosphate buffer*—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.5, determined electrometrically, by the addition of 3 N hydrochloric acid or sodium hydroxide, with mixing.

*Internal standard solution*—Dissolve about 40 mg of USP Homatropine Hydrobromide RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add the same dilute acid to volume, and mix. Prepare fresh on the day of use.

*Standard preparation*—Dissolve about 10 mg of USP Scopolamine Hydrobromide RS, accurately weighed, in about 5 mL of dilute sulfuric acid (1 in 350) in a 10-mL volumetric flask, add the same dilute acid to volume, and mix (*Solution A*). Dissolve about 20 mg of USP Atropine Sulfate RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add 2.0 mL of *Solution A*, and mix. Add dilute sulfuric acid (1 in 350) to volume, and mix. Prepare fresh on the day of use.

*Extraction blank*—Place about 10 mL of dilute sulfuric acid (1 in 350) in a 60-mL separator. Proceed as directed under

*Assay preparation*, beginning with "then add 15 mL of chloroform." The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.

*Assay preparation*—Weigh accurately about 0.5 g of Extract, transfer to a 125-mL conical flask, and add 40 mL of dilute sulfuric acid (1 in 350). Heat to a temperature not above 45°, and stir to hasten solution. Filter the solution through filter paper into a 100-mL volumetric flask. Wash the flask and the filter with two 20-mL portions of warmed dilute sulfuric acid (1 in 350), and collect the washings in the 100-mL volumetric flask. Add dilute sulfuric acid (1 in 350) to volume, and mix.

Pipet 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a *mixed solvent* consisting of chloroform and isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of pH 9.5 Phosphate buffer and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Suitability for alkaloid assays* under *Sodium Sulfate, Anhydrous*, in the section *Reagents, Indicators, and Solutions*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

*Standard curve*—Prepare three *Standard solutions* as follows. Pipet into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard preparation*, and add 9.0, 8.0, and 7.0 mL, respectively, of dilute sulfuric acid (1 in 350). Proceed as directed under *Assay preparation*, beginning with "add 1.0 mL of *Internal standard solution*."

*Chromatographic system*—Under typical condition, the instrument contains a 1.2-m × 4-mm glass column packed with 3% G3 on S1AB. The column may be cured and conditioned as specified under *Gas Chromatography* (see *Chromatography* <621>). The column is maintained at a temperature of about 215°, and the injection port and detector block at about 240°, and dry helium is used as a carrier gas at a flow rate of about 65 mL per minute.

*System suitability*—Chromatograph six to ten injections of the *Assay preparation*, and record peak areas as directed for *Procedure*. The analytical system is suitable for conducting this assay if the relative standard deviation for the ratio,  $R_A$ , calculated by the formula:

$$100 \times (\text{standard deviation} / \text{mean ratio})$$

does not exceed 2.0%; the resolution,  $R$ , between  $a_H$  and  $a_A$  is not less than 3; and the tailing factor (the sum of the distances from peak center to the leading edge and to the trailing edge divided by twice the distance from peak center to the leading edge), measured at 5% of the peak height of  $a_A$ , does not exceed 2.0.

*Procedure*—Inject a portion (about 5  $\mu$ L) of each *Standard solution* into a suitable gas chromatograph equipped with a flame-ionization detector. Measure the areas,  $a_A$ ,  $a_H$ , and  $a_S$ , of the atropine, homatropine, and scopolamine peaks, re-

spectively, in each chromatogram, and calculate the ratios  $A_A$  and  $A_S$  by the formulas:

$$a_A / a_H \text{ and } a_S / a_H.$$

Plot the *Standard curves* of the values of  $R_A$  and  $R_S$  against the amounts, in mg, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of the *Assay preparation* into the chromatograph, obtain the chromatogram area ratios, measure the peak areas, and calculate the area ratios, as with the *Standard solutions*. Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the volume of specimen taken. Add the quantity, in mg, of atropine and scopolamine, and multiply by 10 to obtain the weight, in mg, of alkaloids in the portion of Extract taken.

## Belladonna Extract Tablets

» Belladonna Extract Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Atropine Sulfate RS

USP Homatropine Hydrobromide RS

USP Scopolamine Hydrobromide RS

**Identification**—Macerate a quantity of powdered Tablets, equivalent to about 5 mg of the alkaloids of belladonna extract, with 20 mL of water, and transfer to a separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract the alkaloids with 50 mL of chloroform. Filter the chloroform layer, divide it into two equal portions, and evaporate to dryness: the residue responds to the following tests.

**A:** To one portion of the dry residue add 2 drops of nitric acid, evaporate on a steam bath to dryness, and add a few drops of alcoholic potassium hydroxide TS: a violet color is produced.

**B:** Dissolve the other portion of the residue in 1 mL of dilute hydrochloric acid (1 in 120), and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool: a lusterless precipitate is produced.

**Disintegration** <701>: 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

pH 9.5 Phosphate buffer, *Internal standard solution*, *Standard preparation*, *Extraction blank*, *Standard curve*, *Chromatographic system*, and *System suitability*—Proceed as directed in the Assay under *Belladonna Extract*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600  $\mu$ g of atropine and scopolamine, to a 60-mL separator, add 10.0 mL of dilute sulfuric acid (1 in 350), and sonicate to dissolve as much as possible of the specimen. Proceed as directed for *Assay preparation* in the Assay under *Belladonna Leaf*, beginning with "add 1.0 mL of *Internal standard solution*."

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Belladonna Extract*. Record from the *Standard curves*

the quantities, in mg, of atropine and scopolamine in the weight of specimen taken.

## Belladonna Tincture

» Belladonna Tincture yields, from each 100 mL, not less than 27 mg and not more than 33 mg of the alkaloids of belladonna leaf.

Belladonna Leaf, in moderately coarse powder .....	100 g
To make about .....	1000 mL

Prepare a tincture by *Process P* as modified for assayed *Tinctures* (see *Pharmaceutical Dosage Forms* (1151)), using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Finally adjust the Tincture to contain, in each 100 mL, 30 mg of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

### USP Reference standards (11)—

USP Atropine Sulfate RS

USP Homatropine Hydrobromide RS

USP Scopolamine Hydrobromide RS

**Alcohol content**, *Method II* (611): between 65.0% and 70.0% of C<sub>2</sub>H<sub>5</sub>OH, determined by the gas-liquid chromatographic procedure, acetone being used as the internal standard.

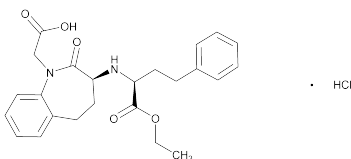
### Assay—

pH 9.5 Phosphate buffer, Internal standard solution, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and System suitability—Proceed as directed in the Assay under *Belladonna Extract*.

**Assay preparation**—Proceed with Tincture as directed in the Assay under *Belladonna Leaf*, but pipet 2 mL of Tincture (in place of "10 mL of this solution") into a 60-mL separator containing 10 mL of dilute sulfuric acid (1 in 350).

**Procedure**—Proceed as directed in the Assay under *Belladonna Leaf*. Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the specimen. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids per 100 mL.

## Benazepril Hydrochloride



C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> · HCl 460.95

1*H*-1-Benzazepine-1-acetic acid, 3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-, monohydrochloride, [*S*-(*R*\*,*R*\*)]-.

(3*S*)-3-[[1-(1*S*)-1-Carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, 3-ethyl ester, monohydrochloride [86541-74-4].

» Benazepril Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> · HCl, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, and store at a temperature below 30°, preferably between 15° and 30°.

### USP Reference standards (11)—

USP Benazepril Hydrochloride RS

USP Benazepril Related Compound A RS

(3*R*) 3-[[1-(1*R*) 1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride.

C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> · HCl 460.95

USP Benazepril Related Compound B RS

(3*S*) 3-[[1-(1*R*) 1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride.

C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> · HCl 460.95

USP Benazepril Related Compound C RS

3-(1-Carboxy-3-phenyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.

C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub> 396.44

USP Benazepril Related Compound D RS

(3-(1-Ethoxycarbonyl-3-cyclohexyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid, monohydrochloride.

C<sub>24</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub> · HCl 467.00

USP Benazepril Related Compound E RS

3-Amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.

USP Benazepril Related Compound F RS

*tert*-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.

USP Benazepril Related Compound G RS

(3-(1-Ethoxycarbonyl-3-phenyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid ethyl ester.

### Identification—

**A: Infrared Absorption** (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**C:** It responds to the test for *Chloride* (191).

**Absorbance of solution**—The absorbance of a 1 in 100 solution of it in methanol, determined in a 1-cm cell at 420 nm, is not more than 0.015, methanol being used as the blank.

### Absorptivity—

**Test preparation**—Dissolve an accurately weighed quantity of Benazepril Hydrochloride in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.025 mg per mL.

**Procedure**—Proceed as directed under *Spectrophotometry and Light-Scattering* (851), and measure the absorbance at 238 nm: the absorptivity is between 21.0 and 23.2.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281)—Ignite at 600°. Not more than 0.1% residue is found.

**Heavy metals**, *Method II* (231): 0.001%.

### Related compounds—

TEST 1 (FOR BENAZEPRIL RELATED COMPOUND A)—

pH 6.0 Phosphate buffer—Dissolve 9.66 g of monobasic potassium phosphate and 2.68 g of dibasic sodium phos-



phate, heptahydrate in about 900 mL of water, and dilute with water to 1000 mL.

**Mobile phase**—Prepare a filtered and degassed mixture of pH 6.0 Phosphate buffer and methanol (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 1.0 mg per mL and 0.005 mg per mL, respectively.

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Benazepril Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Standard solution**—Dilute a suitable portion of *Standard stock solution*, accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 5 µg per mL.

**Dilute standard solution**—Dilute a suitable portion of *Standard stock solution*, accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

**Test solution**—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.0-mm × 10-cm column that contains packing L41. The flow rate is about 0.9 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between benazepril hydrochloride and benazepril related compound A is not less than 2.0. Chromatograph the *Dilute standard solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio is not less than 10:1. Chromatograph the *Standard solution*: the relative standard deviation for replicate injections determined from the benazepril related compound A peak is not more than 10%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area for the benazepril related compound A peak. Calculate the percentage of benazepril related compound A in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Benazepril Related Compound A RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Benazepril Hydrochloride in the *Test solution*;  $r_U$  is the peak response for benazepril related compound A obtained from the *Test solution*; and  $r_S$  is the peak response for benazepril related compound A obtained from the *Standard solution*: The limit of benazepril related compound A is given in the table below.

Benazepril Related Compound	Relative Retention Time	Limit (%)
A <sup>1</sup>	2.3	0.1

<sup>1</sup>[(3R)-3-[[[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride]

TEST 2 (FOR BENAZEPRIL RELATED COMPOUNDS B, C, D, E, F, AND G)—

**Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed in the Assay.

**Standard solution**—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS, USP Benazepril Related Compound B RS, USP Benazepril Related Compound C RS, USP Benazepril Related Compound D RS, USP Benazepril Related Compound E RS, USP Benazepril Related Compound F RS, and USP Benazepril Related Compound G RS in *Mobile phase* to obtain a solution having known concentrations of about 1 µg of USP Benazepril Hydrochloride RS per mL and 10 µg of each related compound per mL.

**Test solution**—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of benazepril related compounds in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of benazepril hydrochloride in the *Test solution*;  $r_U$  is the peak response for the relevant benazepril related compound obtained from the *Test solution*; and  $r_S$  is the peak response for the relevant benazepril related compound obtained from the *Standard solution* (see *Table 1* for values).

Table 1

Benazepril Related Compound	Relative Retention Time	Limit (%)
E <sup>1</sup>	0.4	0.2
F <sup>2</sup>	0.5	0.2
C <sup>3</sup>	0.6	0.3
B <sup>4</sup>	1.5	0.5
D <sup>5</sup>	1.7	0.2
G <sup>6</sup>	2.0	0.2

<sup>1</sup>3-Amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>2</sup>t-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>3</sup>3-(1-Carboxy-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>4</sup>Mixture of diastereoisomers (3-(1-ethoxycarbonyl-3-phenyl-(1R)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid and (3-(1-ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3R)-benzazepine)-1-acetic acid

<sup>5</sup>3-(1-Ethoxycarbonyl-3-cyclohexyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid monohydrochloride

<sup>6</sup>3-(1-Ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid ethyl ester

In addition to not exceeding the limits for benazepril related compounds in *Table 1*, not more than 0.1% of any other single impurity is found; [NOTE—For calculating any other single unspecified impurity,  $C_S$  is the concentration of the USP Benazepril Hydrochloride RS in the *Standard solution*.] and not more than 2.0% of total impurities (excluding benazepril related compound A from *Test 1*) is found.

#### Assay—

**Tetrabutylammonium bromide solution**—Dissolve 0.81 g of tetrabutylammonium bromide in 360 mL of water containing 0.2 mL of glacial acetic acid.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Tetrabutylammonium bromide solution* (64:36).

Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound B RS in *Mobile phase* to obtain a solution having known concentrations of about 0.4 mg of each per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Benazepril Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer about 10.0 mL of the *Test solution* (from either *Test 1* or *Test 2*), prepared as directed in the test for *Related compounds*, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 3-cm guard column that contains packing L1 connected to a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between benazepril hydrochloride and benazepril related compound B is not less than 1.7; and the relative standard deviation for replicate injections determined from benazepril hydrochloride and benazepril related compound B is not more than 2.0% for each.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of  $C_{24}H_{28}N_2O_5 \cdot HCl$  in the portion of Benazepril Hydrochloride taken by the formula:

$$250C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Benazepril Hydrochloride Tablets

» Benazepril Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

### USP Reference standards <11>—

USP Benazepril Hydrochloride RS

USP Benazepril Related Compound B RS

(3S) 3-[[[(1R) 1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride.

$C_{24}H_{28}N_2O_5 \cdot HCl$  460.95

USP Benazepril Related Compound C RS

3-(1-Carboxy-3-phenyl-1(S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid.

$C_{22}H_{24}N_2O_5$  396.44

### Identification—

**A: Thin-Layer Chromatographic Identification Test** <201>—

**Test solution**—Finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of benazepril hydrochloride, to a

50-mL volumetric flask. Add about 30 mL of methanol, and shake by mechanical means for 15 minutes. Dilute with methanol to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.

**Application volume:** 20  $\mu$ L.

**Developing solvent system:** a mixture of ethyl acetate, methanol, and ammonium hydroxide (80:20:15).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution <711>—

**TEST 1—**

**Medium:** water, 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  dissolved by employing the following method.

**Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed in the *Assay* under *Benazepril Hydrochloride*.

**Procedure**—Inject about 60  $\mu$ L, or an amount of a filtered portion of the solution under test, equivalent to about 1.2  $\mu$ g of benazepril, into the chromatograph. The amount of benazepril injected should not exceed 1.5  $\mu$ g. Record the chromatogram, and measure the responses for the major peaks. Determine the quantity, in mg, of  $C_{24}H_{28}N_2O_5 \cdot HCl$  dissolved in comparison with a *Standard solution* having a known concentration of USP Benazepril Hydrochloride RS in the same *Medium* and similarly chromatographed.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  is dissolved in 30 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium, Apparatus, and Procedure**—Proceed as directed for *Test 1*.

**Time:** 45 minutes.

**Tolerances**—Not less than 70% ( $Q$ ) of the labeled amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay*.

**Test preparation**—Transfer 1 Tablet to a suitable volumetric flask, add a volume of *Mobile phase* equivalent to about 50% of the volume of the flask, sonicate for 5 minutes, and then shake by mechanical means for not less than 10 minutes. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a final concentration of about 0.2 mg per mL, mix, and pass a portion of the solution through a suitable filter, discarding the first 6 mL of the filtrate.

**Procedure**—Proceed as directed in the *Assay*, except to inject the *Test preparation* instead of the *Assay preparation*. Calculate the quantity, in mg, of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) in the Tablet taken by the formula:

$$VDC(r_U / r_S)$$

in which  $V$  is the volume, in mL, of the initial flask used to prepare the *Test preparation*;  $D$  is the dilution factor in subsequent dilutions of  $V$ , if necessary, to prepare the *Test preparation*;  $C$  is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the benazepril hydrochloride peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

**Related compounds—**

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system—*Proceed as directed in the Assay.

**Standard solution—**Dissolve an accurately weighed quantity of USP Benazepril Related Compound C RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.006 mg per mL.

**Test solution—**Use the Assay preparation.

**Procedure—**Separately inject equal volumes (about 80  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of the peaks for benazepril related compound C. Calculate the percentage of benazepril related compound C in the portion of Tablets taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Benazepril Related Compound C RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of benazepril hydrochloride in the *Test solution*; and  $r_U$  and  $r_S$  are the peak responses for benazepril related compound C obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% of benazepril related compound C is found. Calculate the percentage of each impurity (other than benazepril related compound C) in the portion of Tablets taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the sum of the responses of all the peaks (including benazepril related compound C): not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found, the results for all impurities (excluding benazepril related compound C) being added.

**Assay—**

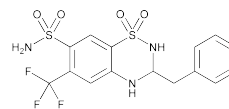
*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system—*Proceed as directed in the Assay under Benazepril Hydrochloride.

**Assay preparation—**Finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of benazepril hydrochloride, to a 250-mL volumetric flask. Add about 150 mL of *Mobile phase*, and shake by mechanical means for 30 minutes. Dilute with *Mobile phase* to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.

**Procedure—**Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the quantity, in mg, of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the benazepril hydrochloride peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Bendroflumethiazide**

$C_{15}H_{14}F_3N_3O_4S_2$  421.41  
2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 3,4-dihydro-3-(phenylmethyl)-6-(trifluoromethyl)-, 1,1-dioxide, ( $\pm$ ); ( $\pm$ )-3-Benzyl-3,4-dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [73-48-3].

**DEFINITION**

Bendroflumethiazide contains NLT 98.0% and NMT 102.0% of  $C_{15}H_{14}F_3N_3O_4S_2$ , calculated on the anhydrous basis.

**IDENTIFICATION****A. INFRARED ABSORPTION** (197K)

**Sample:** Previously dried over silica gel for 4 h

**Acceptance criteria:** Meets the requirements

**B. ULTRAVIOLET ABSORPTION** (197U)

**Analytical wavelength:** 271 nm

**Sample solution:** 10  $\mu$ g/mL in methanol

**Acceptance criteria:** Absorptivities, calculated on the anhydrous basis, do not differ by more than 4.0%.

**C.**

**Sample solution:** Mix 5 mL of dilute hydrochloric acid (50% v/v) with 20 mg of Bendroflumethiazide, boil gently for 1 min, and cool in an ice bath.

**Analysis:** To the *Sample solution* add, in succession, 0.5 mL of sodium nitrite solution (1 mg/mL), 0.5 mL of ammonium sulfamate solution (5 mg/mL), and 0.5 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 mg/mL).

**Acceptance criteria:** A deep red color is produced.

**ASSAY****PROCEDURE**

**Sample:** 190 mg of Bendroflumethiazide

**Analysis:** Dissolve the *Sample* in 80 mL of pyridine in a tall-form, 250-mL beaker in a well-ventilated hood. Add 3 drops of a saturated solution of azo violet in methanol, cover the beaker, and gently bubble nitrogen through the solution for 5 min, being careful to avoid any contact between the solution and the cover. Raise the nitrogen delivery tube above the solution surface and, maintaining a gentle flushing with nitrogen and stirring with a magnetic or mechanical stirring device, add 0.1 N sodium methoxide VS from a 10-mL buret inserted through an opening in the cover. Titrate to a blue endpoint, approaching the endpoint at a rate of 1 or 2 drop/s. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium methoxide is equivalent to 21.07 mg of  $C_{15}H_{14}F_3N_3O_4S_2$ .

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****RESIDUE ON IGNITION** (281): NMT 0.2%**HEAVY METALS, Method II** (231): 20 ppm**SELENIUM** (291)

**Sample:** 100 mg of Bendroflumethiazide and 100 mg of magnesium oxide

**Acceptance criteria:** The absorbance from the *Test solution* is NMT one-half that from the *Standard solution* (NMT 30 ppm).

**LIMIT OF 2,4-DISULFAMYL-5-TRIFLUOROMETHYLANILINE**

[NOTE—Use low-actinic glassware for the *Standard solution* and the *Sample solution*.]

**Mobile phase:** Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of

water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

**Standard solution:** 0.75 µg/mL of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in methanol

**Sample solution:** 50 µg/mL of Bendroflumethiazide in methanol

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 30-cm; packing L11

**Column temperature:** 35° ± 5°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.4 between the methanol and 2,4-disulfamyl-5-trifluoromethylaniline peaks

**Relative standard deviation:** NMT 3.0% for five replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2,4-disulfamyl-5-trifluoromethylaniline in the portion of Bendroflumethiazide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Bendroflumethiazide in the *Sample solution* (µg/mL)

**Acceptance criteria:** NMT 1.5%

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* <921>: NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>
  - USP Bendroflumethiazide RS
  - USP 2,4-Disulfamyl-5-trifluoromethylaniline RS
  - C<sub>7</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 319.29

## Bendroflumethiazide Tablets

#### DEFINITION

Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bendroflumethiazide (C<sub>15</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>).

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

[NOTE—Use low-actinic glassware for the *Sample solution* and the *Standard solution*.]

**Mobile phase:** Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial

acetic acid and 800 mL of methanol, and dilute with water to volume.

**Standard solution:** 50 µg/mL of USP Bendroflumethiazide RS in methanol

**Sample solution:** Nominally equivalent to 50 µg/mL from finely powdered Tablets (NLT 20). Initially add methanol (70% of the volume of the flask) and sonicate for 15 min with occasional shaking. Dilute further with methanol to the required concentration, and centrifuge for 15 min.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 30-cm; packing L11

**Temperature:** 35 ± 5°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0% for five replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>15</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bendroflumethiazide RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of bendroflumethiazide in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION <711>

[NOTE—Protect solutions from light throughout this test.]

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Detector:** UV 271 nm

**Sample solution:** Sample per *Dissolution* <711>.

**Standard solution:** Prepare a stock solution of USP Bendroflumethiazide RS in an appropriate organic solvent, and dilute this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.

**Analysis:** Determine the amount of C<sub>15</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> dissolved by using UV absorption on filtered portions of the *Sample solution*, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Bendroflumethiazide RS.

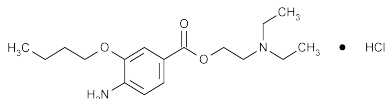
**Tolerances:** NLT 75% (Q) of the labeled amount of C<sub>15</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>
  - USP Bendroflumethiazide RS

## Benoxinate Hydrochloride



$C_{17}H_{28}N_2O_3 \cdot HCl$  344.88

Benzoic acid, 4-amino-3-butoxy-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate monohydrochloride [5987-82-6].

» Benoxinate Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of  $C_{17}H_{28}N_2O_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Benoxinate Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197K): previously dried.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 15 µg per mL.

*Medium:* water.

**C:** A solution (1 in 100) responds to the tests for *Chloride* (191).

**pH** (791): between 5.0 and 6.0, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Eluant:* a mixture of chloroform, cyclohexane, and diethylamine (5:4:1).

*Visualization:* 12.

**Assay**—Dissolve about 250 mg of Benoxinate Hydrochloride, accurately weighed, in a mixture of 20 mL of glacial acetic acid and 20 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.49 mg of  $C_{17}H_{28}N_2O_3 \cdot HCl$ .

## Benoxinate Hydrochloride Ophthalmic Solution

» Benoxinate Hydrochloride Ophthalmic Solution is a sterile solution of Benoxinate Hydrochloride in water. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{17}H_{28}N_2O_3 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Benoxinate Hydrochloride RS

**Identification**—Dilute a volume of Solution, equivalent to about 50 mg of benoxinate hydrochloride, with 0.01 N hy-

drochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator": the solution meets the requirements of the test.

**Sterility** (71): meets the requirements.

**pH** (791): between 3.0 and 6.0.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Benoxinate Hydrochloride RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 400 µg per mL.

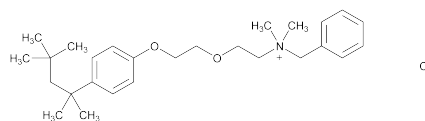
**Assay preparation**—Transfer a volume of Ophthalmic Solution, equivalent to about 20 mg of benoxinate hydrochloride, to a separator containing 15 mL of water, add 1 mL of ammonium hydroxide, and extract with five 20-mL portions of ether. Wash the combined ether extracts with 10 mL of water, extract the water washing with 10 mL of ether, and add this ether extract to the main extract. Extract the ether solution with three 5-mL portions of 0.1 N hydrochloric acid, collect the acid extracts in a 50-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and 0.1 N hydrochloric acid to provide a blank, to separate 200-mL volumetric flasks. Dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 308 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_{17}H_{28}N_2O_3 \cdot HCl$  in each mL of the Ophthalmic Solution taken by the formula:

$$(0.05C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Benoxinate Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Benzethonium Chloride



$C_{27}H_{42}ClNO_2$

448.08

Benzenemethanaminium, *N,N*-dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride; Benzyldimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride [121-54-0].

**DEFINITION**

Benzethonium Chloride contains NLT 97.0% and NMT 103.0% of  $C_{27}H_{42}ClNO_2$ , calculated on the dried basis.

**IDENTIFICATION**

• **A. PROCEDURE**

**Sample solution:** 10 mg/mL

**Analysis:** To 1 mL of the *Sample solution* add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

**Acceptance criteria:** A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

• **B. INFRARED ABSORPTION** (197K)

**ASSAY**

• **PROCEDURE**

**Sample:** 0.3 g of Benzethonium Chloride

**Analysis:** Dissolve the *Sample* in 75 mL of water contained in a glass-stoppered, 250-mL flask. Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of  $C_{27}H_{42}ClNO_2$ .

**Acceptance criteria:** 97.0%–103.0% on the dried basis

**IMPURITIES**

**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Organic Impurities**

- **PROCEDURE: LIMIT OF AMMONIUM COMPOUNDS:** To 5 mL of a solution (1 in 50) add 3 mL of 1 N sodium hydroxide, and heat to boiling: the odor of ammonia is not perceptible.

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 158°–163°, the specimen having been dried previously
- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 5.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Benzethonium Chloride RS

## Benzethonium Chloride Concentrate

» Benzethonium Chloride Concentrate contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**Labeling**—The label states that this article is not intended for direct administration to humans or animals.

**Change to read:**

**Identification—**

• **A.**  
Evaporate a volume of Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath. To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS. A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

• **B.**  
Evaporate a volume of Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath. The residue so obtained forms precipitate with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

• **C.**  
Evaporate a volume of Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath. To the residue

add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide. The solution turns orange-red, and a brown precipitate may be formed. • (ERR 1-May-2012)

**Oxidizing substances**—To 5 mL of Concentrate add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid: the solution does not acquire a yellow color.

**Limit of nitrites**—To 1 drop of Concentrate on a spot plate add 1 drop each of glacial acetic acid, sulfanilic acid in acetic acid solution (1 in 100), and 1-naphthylamine-acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid): no red color develops in the resulting solution within 10 minutes.

**Assay**—Transfer a volume of Concentrate, equivalent to about 200 mg of benzethonium chloride, to a glass-stoppered flask, and proceed as directed in the Assay under *Benzethonium Chloride*, beginning with "Add 0.4 mL of bromophenol blue solution (1 in 2000)."

## Benzethonium Chloride Topical Solution

» Benzethonium Chloride Topical Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Change to read:**

**Identification—**

• **A.**  
Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath. To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS. A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

• **B.**  
Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath. The residue so obtained forms precipitate with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

• **C.**  
Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath. To the residue add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide. The solution turns orange-red, and a brown precipitate may be formed. • (ERR 1-May-2012)

**Oxidizing substances**—To 5 mL add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid: the solution does not acquire a yellow color.

**Limit of nitrites**—To 1 drop of Topical Solution on a spot plate add 1 drop each of glacial acetic acid, sulfanilic acid in

acetic acid (1 in 100), and 1-naphthylamine–acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid): no red color develops in the resulting solution within 10 minutes.

**Assay**—Transfer a volume of Topical Solution, equivalent to about 200 mg of benzethonium chloride, to a glass-stoppered flask, and proceed as directed in the Assay under *Benzethonium Chloride*, beginning with “Add 0.4 mL of bromophenol blue solution (1 in 2000)”.

## Benzethonium Chloride Tincture

### DEFINITION

Benzethonium Chloride Tincture contains, in each 100 mL, NLT 190 mg and NMT 210 mg of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

Prepare Benzethonium Chloride Tincture 2 mg/mL as follows.

Benzethonium Chloride	2 g
Alcohol	685 mL
Acetone	100 mL
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Benzethonium Chloride* in a mixture of *Alcohol* and *Acetone*. Add sufficient *Purified Water* to make 1000 mL. [NOTE—Benzethonium Chloride Tincture may be colored by the addition of any suitable color or combination of colors certified by the FDA for use in drugs.]

### IDENTIFICATION

#### A. PROCEDURE

**Sample:** 50 mL

**Analysis:** To the residue obtained by evaporating the *Sample* on a steam bath, add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

**Acceptance criteria:** A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

#### B. PROCEDURE

**Sample:** 50 mL

**Analysis:** Evaporate the *Sample* on a steam bath.

**Acceptance criteria:** The residue obtained forms precipitates with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

### ASSAY

#### PROCEDURE

**Sample:** 50 mL

**Analysis:** Transfer the *Sample* to a 150-mL beaker, and add, with continuous stirring, 10 mL of 25 mg/mL of sodium tetraphenylboron solution. Cover, and allow to stand for 16 h. Decant the supernatant into a tared sintered-glass crucible, applying vacuum filtration. Suspend the precipitate in 20 mL of water. Transfer the precipitate to the crucible, washing well with water. Dry the precipitate and the crucible at 105° for 1 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.6122, represents its equivalent of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Acceptance criteria:** 190–210 mg

### OTHER COMPONENTS

#### ALCOHOL AND ACETONE CONTENT

**Standard solution A (alcohol low standard solution):** Add 5.0 mL of methanol and 11.0 mL of dehy-

drated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

#### Standard solution B (alcohol high standard solution):

Add 5.0 mL of methanol and 14.0 mL of dehydrated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

#### Standard solution C (acetone low standard solution):

Add 5.0 mL of methanol and 1.7 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

#### Standard solution D (acetone high standard solution):

Add 5.0 mL of methanol and 2.2 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 20 mL of Tincture to a 100-mL volumetric flask, add 5.0 mL of methanol as the internal standard, and dilute with water to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 120-cm × 4-mm packed with a suitable type of support, such as 80- to 100-mesh S3

**Carrier gas:** Dry helium

**Temperature**

**Injector port:** 240°

**Detector block:** 240°

**Column:** 120°

**Flow rate:** 90 mL/min

**Injection size:** 0.8 µL

### Analysis

**Samples:** *Standard solutions A, B, C, and D, and Sample solution*

From the respective chromatograms obtained as described previously, calculate the ratios of peak areas for alcohol to internal standard and for acetone to internal standard.

Calculate the percentage of alcohol and of acetone in the portion of Tincture taken:

$$\text{Result} = [A(Y - Z) + B(Z - X)] / (Y - X)$$

*A* = percentage of alcohol, or of acetone, in *Standard solution A* and *Standard solution C*, respectively

*Y* = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution B* and *Standard solution D*, respectively

*Z* = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for the *Sample solution*

*B* = percentage of alcohol, or of acetone, in *Standard solution B* and *Standard solution D*, respectively

*X* = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution A* and *Standard solution C*, respectively

### Acceptance criteria

**Alcohol ( $C_2H_5OH$ ):** 62.0%–68.0%

**Acetone ( $C_3H_6O$ ):** 9.0%–11.0%

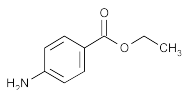
### SPECIFIC TESTS

• **SPECIFIC GRAVITY** <841>: 0.868–0.876

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

## Benzocaine



$C_9H_{11}NO_2$  165.19

Benzoic acid, 4-amino-, ethyl ester.

Ethyl *p*-aminobenzoate [94-09-7].

» Benzocaine, dried over phosphorus pentoxide for 3 hours, contains not less than 98.0 percent and not more than 102.0 percent of  $C_9H_{11}NO_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Benzocaine RS

**Identification**—

**A:** *Infrared Absorption* (197K): previously dried over phosphorus pentoxide for 3 hours.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 5 µg per mL.

*Medium:* chloroform.

Absorptivities at 278 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Dissolve about 20 mg in 10 mL of water with the aid of a few drops of 3 N hydrochloric acid, and add 5 drops of a solution of sodium nitrite (1 in 10), followed by 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**Melting range**, *Class I* (741): between 88° and 92°, but the range between beginning and end of melting does not exceed 2°.

**Reaction**—Dissolve 1.0 g in 10 mL of neutralized alcohol: a clear solution results. Dilute this solution with 10 mL of water, and add 2 drops of phenolphthalein TS and 1 drop of 0.10 N sodium hydroxide: a red color is produced.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.

**Readily carbonizable substances** (271)—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

**Residue on ignition** (281): not more than 0.1%.

**Chloride**—To a solution of 200 mg in 5 mL of alcohol, previously acidified with a few drops of diluted nitric acid, add a few drops of silver nitrate TS: no turbidity is produced immediately.

**Heavy metals**, *Method II* (231): 0.001%.

**Ordinary impurities** (466)—

*Test solution:* dehydrated alcohol.

*Standard solution:* dehydrated alcohol.

*Application volume:* 10 µL.

*Eluant:* chloroform containing about 0.75% of dehydrated alcohol as a preservative, in a nonequilibrated chamber.

*Visualization:* 1.

*Limit*—The total of any ordinary impurities observed does not exceed 1%.

**Assay**—

*Aqueous solution*—To 980 mL of water, add 20 mL of acetic acid and 1 mL of triethylamine, and mix well. The pH should be between 2.95 and 3.0; adjust as needed.

*Mobile phase*—Prepare a filtered and degassed mixture of *Aqueous solution* and methanol (60:40).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Benzocaine RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.024 mg per mL.

*Assay preparation*—Transfer about 24 mg of Benzocaine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Dilute 10 mL of this solution with *Mobile phase* to 100 mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 285-nm detector and a 2.0-mm × 15-cm column that contains 5-µm packing L11. The flow rate is about 0.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor of the benzocaine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the benzocaine peaks. Calculate the quantity, in mg, of  $C_9H_{11}NO_2$  in the portion of Benzocaine taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Benzocaine RS in the *Standard preparation*;  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and *Standard preparation*, respectively; and 1000 is the dilution factor for the *Assay preparation*.

## Benzocaine Topical Aerosol

### DEFINITION

Benzocaine Topical Aerosol is a solution of Benzocaine in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ).

### IDENTIFICATION

#### • A.

**Sample:** An amount of Topical Aerosol, equivalent to 5 mg of benzocaine

**Analysis:** Spray the *Sample* into a beaker, and heat on a steam bath for a few min to expel residual propellant. Add 20 mL of 0.5 N hydrochloric acid, warm gently to disperse, cool, and filter. To 10 mL of the filtrate add 5 drops of sodium nitrite solution (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide.

**Acceptance criteria:** An orange-red precipitate is formed.

### ASSAY

#### • PROCEDURE

**Sample solution:** Spray a portion of the Topical Aerosol into a beaker, and heat on a steam bath for a few min to expel residual propellant. Cool, and transfer a portion of the solution, equivalent to 200 mg of benzocaine, to a 250-mL beaker. Add 50 mL of water and 5 mL of hydrochloric acid, and stir. Cool the solution in an ice bath to 10°.



**Analysis:** Titrate slowly with 0.1 M sodium nitrite VS potentiometrically, using a calomel–platinum electrode system. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 M sodium nitrite is equivalent to 16.52 mg of benzocaine ( $C_9H_{11}NO_2$ ).

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **OTHER REQUIREMENTS:** It meets the requirements in *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* <601>, *Pressure Test*, *Minimum Fill*, and *Leakage Test*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.

### Benzocaine Cream

» Benzocaine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_{11}NO_2$  in a suitable cream base.

**Packaging and storage—**Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

**Identification—**Place an amount of Cream, equivalent to about 50 mg of benzocaine, in a small beaker, add 20 mL of 0.5 N hydrochloric acid, warm gently to disperse the Cream, cool, and filter. To 10 mL of the filtrate add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**Microbial enumeration tests <61> and Tests for specified microorganisms <62>—**It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill <755>:** meets the requirements.

**Assay—**Weigh accurately an amount of Cream, equivalent to about 200 mg of benzocaine, in a tared 250-mL beaker. Add 50 mL of water and 5 mL of hydrochloric acid, and stir by mechanical means, with gentle warming, until solution is effected. Cool the solution in an ice bath to about 10°, and titrate slowly with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically using a calomel–platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 16.52 mg of  $C_9H_{11}NO_2$ .

### Benzocaine Gel

» Benzocaine Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_{11}NO_2$ .

**Packaging and storage—**Preserve in well-closed containers.

**USP Reference standards <11>—**  
USP Benzocaine RS

#### Identification—

**A:** Transfer an amount of Gel, equivalent to about 5 mg of benzocaine, to a beaker, add 20 mL of 0.5 N hydrochloric acid, warm gently to disperse the Gel, cool, and filter, if

necessary, to obtain a clear solution. To 10 mL of the clear solution add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**B:** The retention time of the major peak of benzocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial enumeration tests <61> and Tests for specified microorganisms <62>—**It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill <755>:** meets the requirements.

#### Assay—

**Diluent—**Prepare a mixture of methanol and water (1:1).

**Mobile phase—**Prepare a mixture of methanol, water, and glacial acetic acid (56:40:4). Make adjustments if necessary see *System Suitability* under *Chromatography* <621>).

**Standard preparation—**Prepare a solution of USP Benzocaine RS in *Diluent* having a known concentration of about 0.02 mg per mL.

**Assay preparation—**Transfer an accurately weighed portion of Gel, equivalent to about 200 mg of benzocaine, to a 100-mL volumetric flask, add 80 mL of *Diluent*, and shake by mechanical means for 15 minutes. Dilute with *Diluent* to volume, mix, and filter, if necessary, to obtain a clear solution, discarding the first 5 mL of the filtrate. Transfer 1.0 mL of the clear solution to a second 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 294-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure—**[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 50 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_9H_{11}NO_2$  in the portion of Gel taken by the formula:

$$10,000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Benzocaine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the benzocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Benzocaine Lozenges

» Benzocaine Lozenges contain not less than 85.0 percent and not more than 120.0 percent of the labeled amount of  $C_9H_{11}NO_2$ .

**Packaging and storage—**Preserve in well-closed containers.

**USP Reference standards <11>—**  
USP Benzocaine RS

#### Identification—

**A:** Dissolve a quantity of powdered Lozenges, equivalent to about 20 mg of benzocaine, in 10 mL of water with the aid of a few drops of 3 N hydrochloric acid, and filter, if necessary, to obtain a clear solution. Add 5 drops of a solu-

tion of sodium nitrite (1 in 10), followed by 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**B:** The retention time of the major peak for benzocaine in the chromatograms of the *Assay preparations* corresponds to that in the chromatograms of the respective *Standard preparations*, as obtained in the *Assay*.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and 1.0 M monobasic potassium phosphate solution previously adjusted with phosphoric acid to a pH of 3.0 (700:250:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation 1**—Prepare a solution of USP Benzocaine RS in 0.1 N hydrochloric acid having a known concentration of about 0.01 mg per mL.

**Standard preparation 2**—Prepare a solution of USP Benzocaine RS in a mixture of acetonitrile and water (1:1) having a known concentration of about 0.01 mg per mL.

**Assay preparations**—Weigh and finely powder not fewer than 20 Lozenges. Transfer accurately weighed portions of the powder, each equivalent to about 40 mg of benzocaine, to two separate 200-mL volumetric flasks. To one flask add about 150 mL of 0.1 N hydrochloric acid, and stir for not less than 2 hours. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix (*Assay preparation 1*). To the second flask add about 150 mL of a mixture of acetonitrile and water (1:1), and stir for not less than 30 minutes. Dilute with the mixture of acetonitrile and water (1:1) to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with the mixture of acetonitrile and water (1:1) to volume, and mix (*Assay preparation 2*).

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparations*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of *Assay preparation 1*, *Assay preparation 2*, *Standard preparation 1*, and *Standard preparation 2* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of total C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> in the portion of Lozenges taken to prepare *Assay preparation 1* by the formula:

$$200C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Benzocaine RS in *Standard preparation 1*, and  $r_u$  and  $r_s$  are the benzocaine peak areas obtained from *Assay preparation 1* and *Standard preparation 1*, respectively. Calculate the quantity, in mg, of free C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> in the portion of Lozenges taken to prepare *Assay preparation 2* by the formula:

$$200C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Benzocaine RS in *Standard preparation 2*, and  $r_u$  and  $r_s$  are the benzocaine peak responses obtained from *Assay preparation 2* and *Standard preparation 2*, respectively.

## Benzocaine Ointment

» Benzocaine Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> in a suitable ointment base.

**Packaging and storage**—Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

#### Identification—

**Ointments having water-soluble bases**—Transfer an amount of Ointment, equivalent to about 5 mg of benzocaine, to a small beaker, add 20 mL of 0.5 N hydrochloric acid, warm gently to disperse the Ointment, cool, and filter, if necessary. To 10 mL of the filtrate add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of sodium hydroxide TS: an orange-red precipitate is formed.

**Ointments having water-insoluble bases**—Transfer an amount of Ointment, equivalent to about 2.5 mg of benzocaine, to a small separator, and dissolve in 20 mL of ether. Extract with 10 mL of 0.5 N hydrochloric acid, and filter the aqueous phase through paper into a small beaker. Add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill (755):** meets the requirements.

#### Assay—

**Ointments having water-soluble bases**—Accurately weigh an amount of Ointment, equivalent to about 200 mg of benzocaine, in a tared 250-mL beaker. Add 50 mL of water and 5 mL of hydrochloric acid, and stir by mechanical means until solution is effected. Cool the solution in an ice bath to about 10°, and titrate slowly with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically using a calomel-platinum electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 16.52 mg of C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>.

**Ointments having water-insoluble bases**—Accurately weigh an amount of Ointment, equivalent to about 200 mg of benzocaine, and transfer to a 125-mL separator. Suspend the Ointment in 50 mL of ether by shaking, and extract with three successive 25-mL portions of 1 N hydrochloric acid, filtering each portion into a 250-mL beaker, and proceed as directed under *Ointments having water-soluble bases*, beginning with "Cool the solution."

## Benzocaine Otic Solution

» Benzocaine Otic Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—Transfer a volume of Otic Solution, equivalent to about 2.5 mg of benzocaine, to a small beaker, add

10 mL of water, and dissolve with the aid of a few drops of 3 N hydrochloric acid. Add 5 drops of sodium nitrite solution (1 in 10), then add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli* and for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*; the total aerobic microbial count is less than 100 cfu per mL.

**Assay**—Transfer an accurately measured volume of Otic Solution, equivalent to about 400 mg of benzocaine, to a beaker, add 150 mL of cold 1.6 N hydrochloric acid, and stir until a fine dispersion is obtained. Cool the solution in an ice bath to about 10°, and titrate slowly with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 16.52 mg of  $C_9H_{11}NO_2$ .

## Benzocaine Topical Solution

» Benzocaine Topical Solution is a solution of Benzocaine in a suitable solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_{11}NO_2$ . It contains a suitable antimicrobial agent.

**Packaging and storage**—Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

**Identification**—Transfer an amount of Topical Solution, equivalent to about 5 mg of benzocaine, to a small beaker, add 20 mL of 0.5 N hydrochloric acid, warm gently to disperse, cool, and filter. To 10 mL of the filtrate add 5 drops of sodium nitrite solution (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide: an orange-red precipitate is formed.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Assay**—Transfer an accurately weighed portion of Topical Solution, equivalent to about 200 mg of benzocaine, to a 250-mL beaker. Add 50 mL of water and 5 mL of hydrochloric acid, and stir. Cool the solution in an ice bath to about 10°, and titrate slowly with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 16.52 mg of  $C_9H_{11}NO_2$ .

## Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol

### DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol is Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution packaged in a pressurized container with a suitable inert propellant. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of ben-

zocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** Methanol and water (60:40)

**Mobile phase:** Methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20)

**Standard solution:** Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask. Swirl with the aid of 25 mL of methanol, and transfer 140/ mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water, *f* being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Topical Aerosol. Transfer 140/*f* mg of USP Tetracaine Hydrochloride RS into the same volumetric flask with the aid of 25 mL of water, *f'* being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Topical Aerosol. Sonicate for 1 min, and dilute with *Diluent* to volume.

**Sample stock solution:** Fit the valve of the Topical Aerosol container with a cannula, and expel the contents of the container into a 100-mL, glass-stoppered, round-bottom flask. Attach the flask to a rotary evaporator, and evaporate under a vacuum of 600 mm of mercury for 2.5 h. Transfer a portion of the material in the round-bottom flask, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Sonicate for 1 min, dilute with methanol to volume, and mix.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 75 mL of *Diluent*. Shake, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 313 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are 0.3, 0.8, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between the benzocaine and butamben peaks; NLT 2 between the butamben and tetracaine peaks

**Relative standard deviation:** NMT 2.0% for each of the three analyte peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the individual percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding analyte from the *Sample solution*

$r_S$  = peak response of the corresponding analyte from the *Standard solution*

$C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of benzocaine, butamben, and tetracaine hydrochloride

#### SPECIFIC TESTS

- **OTHER REQUIREMENTS:** It meets the requirements in *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* (601), *Pressure Test, Minimum Fill, and Leakage Test*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)
  - USP Benzocaine RS
  - USP Butamben RS
  - USP Tetracaine Hydrochloride RS

### Benzocaine, Butamben, and Tetracaine Hydrochloride Gel

» Benzocaine, Butamben, and Tetracaine Hydrochloride Gel is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable gel base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

#### USP Reference standards (11)—

USP Benzocaine RS  
USP Butamben RS  
USP Tetracaine Hydrochloride RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

#### Assay—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution*.

*Assay preparation*—Transfer an accurately weighed portion of Gel, equivalent to about 1400 mg of benzocaine, to a 100-mL volumetric flask. Add about 75 mL of methanol, and mix. Sonicate for about 1 minute, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add about 75 mL of *Diluent*, shake, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution*. Calculate the individual quantities, in mg, of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Gel taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment

» Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

#### USP Reference standards (11)—

USP Benzocaine RS  
USP Butamben RS  
USP Tetracaine Hydrochloride RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

#### Assay—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution*.

*Assay preparation*—Transfer an accurately weighed portion of Ointment, equivalent to about 1400 mg of benzocaine, to a 100-mL volumetric flask. Add about 75 mL of methanol, and mix. Sonicate for about 1 minute, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add about 75 mL of *Diluent*, shake, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution*. Calculate the individual quantities, in mg, of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Ointment taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution

» Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**USP Reference standards** (11)—

USP Benzocaine RS

USP Butamben RS

USP Tetracaine Hydrochloride RS

**Identification**—The retention times of the major peaks in the chromatograms of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Diluent*—Prepare a mixture of methanol and water (60:40).

*Mobile phase*—Prepare a mixture of methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 140 mg of USP Benzocaine RS, accurately weighed, to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer about 140 *J* mg of USP Butamben RS, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J* being the ratio of the labeled amount, in percent, of butamben to the labeled amount, in percent, of benzocaine in the Topical Solution. Transfer about 140 *J'* mg of USP Tetracaine Hydrochloride RS, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J'* being the ratio of the labeled amount, in percent, of tetracaine hydrochloride to the labeled amount, in percent, of benzocaine in the Topical Solution. Sonicate for about 1 minute, dilute with *Diluent* to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Topical Solution, equivalent to about 1400 mg of benzocaine, to a 100-mL volumetric flask. Add about 75 mL of methanol, and mix. Sonicate for about 1 minute, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add about 75 mL of *Diluent*, shake, dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 313-nm detector, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for benzocaine, 0.8 for butamben, and 1.0 for tetracaine; the resolution, *R*, between the benzocaine peak and the butamben peak and between the butamben peak and the tetracaine peak is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0% for each of the three analyte peaks.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the major peaks. Calculate the individual quantities, in mg, of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>), butamben (C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub>), and tetracaine hydrochloride (C<sub>15</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> · HCl) in the portion of Topical Solution taken by the formula:

$$1000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas in the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Benzocaine and Menthol Topical Aerosol

**DEFINITION**

Benzocaine and Menthol Topical Aerosol is a solution of Benzocaine and Menthol with suitable propellants in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>) and menthol (C<sub>10</sub>H<sub>20</sub>O).

**IDENTIFICATION**• **A.**

**Sample:** An amount of Topical Aerosol equivalent to 5 mg of benzocaine

**Analysis:** Transfer the *Sample* to a beaker. Add 20 mL of 0.5 N hydrochloric acid, and warm gently to disperse the solution. Cool, and filter if necessary, to obtain a clear solution. To 10 mL of the clear solution add 5 drops of a solution of sodium nitrite (1 in 10) and 2 drops of methyl red TS, and neutralize with 1 N sodium hydroxide. Add 2 mL of a solution of 100 mg of 2-naphthol in 5 mL of 1 N sodium hydroxide.

**Acceptance criteria:** An orange-red precipitate is formed.

- **B.** The retention time of the major peak for benzocaine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the major peak for menthol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **BENZOCAINE**

**Mobile phase:** Methanol, water, and glacial acetic acid (56:40:4)

**Diluent:** Methanol and water (1:1)

**Standard solution:** 0.02 mg/mL of USP Benzocaine RS in *Diluent*

**Sample solution:** Nominally 0.02 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol containing 200 mg of benzocaine to a suitable volumetric flask, and dilute with *Diluent*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 294 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

• **MENTHOL**

**Internal standard solution:** 1 mg/mL of decanol in *n*-hexane

**Standard stock solution:** 1 mg/mL of USP Menthol RS in *n*-hexane

**Standard solution:** 0.05 mg/mL each of USP Menthol RS and decanol in ether from *Standard stock solution* and *Internal standard solution*

**Sample stock solution:** Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol equivalent to 50 mg of menthol to a separator, and extract with three 15-mL portions of ether, collecting the ether extracts in a 50-mL volumetric flask. Dilute with ether to volume.

**Sample solution:** Nominally 0.05 mg/mL of menthol prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 5.0 mL of *n*-hexane and 5.0 mL of *Internal standard solution*, and dilute with ether to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm × 1.8-m; packed with 10% phase G16 on support S1AB

**Carrier gas:** Helium

**Temperatures**

**Column:** 170°

**Injection port:** 260°

**Detector:** 240°

**Flow rate:** 50 mL/min

**Injection volume:** 2 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for menthol and decanol are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between the menthol and decanol peaks

**Relative standard deviation:** NMT 2% of the ratio of the peak response of menthol to that of decanol

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of menthol (C<sub>10</sub>H<sub>20</sub>O) in the portion of Topical Aerosol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of menthol to decanol from the *Sample solution*

$R_S$  = peak response ratio of menthol to decanol from the *Standard solution*

$C_S$  = concentration of USP Menthol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of menthol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** <755>: Meets the requirements

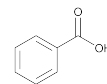
#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **OTHER REQUIREMENTS:** It meets the requirements in *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* <601>, *Pressure Test*, and *Leakage Test*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** <11>  
USP Benzocaine RS  
USP Menthol RS

## Benzoic Acid



C<sub>7</sub>H<sub>6</sub>O<sub>2</sub>

122.12

Benzoic acid [65-85-0].

#### DEFINITION

Benzoic Acid contains NLT 99.5% and NMT 100.5% of benzoic acid (C<sub>7</sub>H<sub>6</sub>O<sub>2</sub>), calculated on the anhydrous basis.

#### IDENTIFICATION

##### • A.

**Sample solution:** Prepare a saturated solution of Benzoic Acid in water, and filter twice.

**Analysis 1:** To one portion of the filtrate add ferric chloride TS.

**Acceptance criteria 1:** A salmon-colored precipitate is formed.

**Analysis 2:** To a separate 10-mL portion of the filtrate add 1 mL of 7 N sulfuric acid, and cool the mixture.

**Acceptance criteria 2:** A white precipitate forms in 10 min. This precipitate is soluble in ether.

#### ASSAY

##### • PROCEDURE

**Sample:** 500 mg of Benzoic Acid

**Analysis:** Dissolve the *Sample* in 25 mL of diluted alcohol that previously has been neutralized with 0.1 N sodium hydroxide. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a pink color. Each mL of 0.1 N sodium hydroxide is equivalent to 12.21 mg of benzoic acid (C<sub>7</sub>H<sub>6</sub>O<sub>2</sub>).

**Acceptance criteria:** 99.5%–100.5% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.05%

- **HEAVY METALS** <231>

**Test preparation:** Dissolve 2.0 g in 25 mL of acetone, and add 2 mL of water.

**Analysis:** Add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of pH 3.5 *Acetate Buffer*, and allow to stand for 5 min.

**Acceptance criteria:** NMT 10 µg/g; any color produced is not darker than that of a control made with 25 mL of acetone and 2.0 mL of *Standard Lead Solution* and treated in the same manner.

#### SPECIFIC TESTS

- **CONGEALING TEMPERATURE** <651>: 121°–123°

- **WATER DETERMINATION, Method I** <921>

**Sample solution:** A 1-in-2 solution of methanol in pyridine is used as the solvent.

**Acceptance criteria:** NMT 0.7%

- **READILY CARBONIZABLE SUBSTANCES TEST** <271>

**Sample solution:** 500 mg in 5 mL of sulfuric acid

**Acceptance criteria:** The solution has no more color than *Matching Fluid Q*.

- **READILY OXIDIZABLE SUBSTANCES**

**Sample solution:** Add 1.5 mL of sulfuric acid to 100 mL of water. Heat to boiling, and add 0.1 N potassium permanganate, dropwise, until the pink color persists for 30 s. Dissolve 1.00 g of Benzoic Acid in the hot solution.

**Analysis:** Titrate with 0.1 N potassium permanganate VS to a pink color that persists for 15 s.

**Acceptance criteria:** NMT 0.50 mL of 0.10 N potassium permanganate is consumed.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Benzoic and Salicylic Acids Ointment****DEFINITION**

Benzoic and Salicylic Acids Ointment is Benzoic Acid and Salicylic Acid, present in a ratio of 2:1, in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzoic acid ( $C_7H_6O_2$ ) and salicylic acid ( $C_7H_6O_3$ ).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHY**

**Diluent:** Mixture of chloroform and methanol (1:1)

**Standard solution A:** 2.4 mg/mL of USP Benzoic Acid RS in *Diluent*

**Standard solution B:** 1.2 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Equivalent to 60 mg of benzoic acid and 30 mg of salicylic acid from Ointment, in 25 mL of *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L of each solution at separate points 2.5 cm from the bottom edge of a 20-  $\times$  20-cm thin-layer chromatographic plate

**Developing solvent system:** Chloroform, acetone, isopropyl alcohol, methanol, and ammonium hydroxide (30:30:15:15:10)

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. View the chromatogram under short-wavelength (254 nm) UV radiation.

**Acceptance criteria:** The two major fluorescent spots from the *Sample solution* correspond in color and in  $R_f$  value to those from *Standard solution A* and *Standard solution B*.

**ASSAY**

- **PROCEDURE**

**Ferric chloride-urea reagent:** On the day of use, dissolve, without heating, 18 g of urea in a mixture of 2.5 mL of ferric chloride solution (6 in 10) and 12.5 mL of 0.05 N hydrochloric acid.

**Column A:** Insert a small pledget of glass wool above the stem constriction of a 20-  $\times$  2.5-cm chromatographic tube. Mix 1 g of chromatographic siliceous earth with 0.5 mL of dilute phosphoric acid (3 in 10) to form a uniform, fluffy mixture; transfer to the chromatographic tube; and pack evenly over the glass wool, exerting gentle pressure. Similarly, mix 4 g of chromatographic siliceous earth with 3 mL of *Ferric chloride-urea reagent*, and pack uniformly over the first layer. Cover the column with a pad of glass wool.

**Column B:** Insert a small pledget of glass wool above the stem constriction of a second 20-  $\times$  2.5-cm chromatographic tube. Mix 4 g of chromatographic siliceous earth with 2 mL of sodium bicarbonate solution (1 in 12), prepared just before use, to a uniform, fluffy mixture; and pack evenly over the glass wool. Cover the column with a pad of glass wool.

**Diluent:** Glacial acetic acid in chloroform (3 in 100)

**Standard solution A:** 20  $\mu$ g/mL of USP Salicylic Acid RS in *Diluent*

**Standard solution B:** 40  $\mu$ g/mL of USP Benzoic Acid RS in *Diluent*

**Sample solution:** Transfer an amount of the Ointment, equivalent to 100 mg of benzoic acid and 50 mg of salicylic acid, to a 250-mL volumetric flask, and dissolve in 150 mL of chloroform by warming on a steam bath. Cool. Dilute with chloroform to volume to obtain a solution having a nominal concentration of 200  $\mu$ g/mL of salicylic acid and 400  $\mu$ g/mL of benzoic acid.

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Mount *Column A* directly over *Column B*, then pipet 10 mL of *Sample solution* onto *Column A*, and allow it to pass into the column. Wash the columns with two 40-mL portions of chloroform, allowing the first portion to recede to the top of each column before adding the second portion. Discard the eluates, and separate the columns.

**Salicylic acid content:** Elute *Column A* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume, and mix. Concomitantly determine the absorbances of the eluate and *Standard solution A* in 1-cm cells at the wavelength of maximum absorbance at 311 nm, with a suitable spectrophotometer, using *Diluent* as the blank.

Calculate the percentage of the labeled amount of salicylic acid ( $C_7H_6O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

$A_U$  = absorbance of the diluted eluate from *Column A*

$A_S$  = absorbance of *Standard solution A*

$C_S$  = concentration of USP Salicylic Acid RS in *Standard solution A* ( $\mu$ g/mL)

$C_U$  = nominal concentration of the salicylic acid in the *Sample solution* ( $\mu$ g/mL)

$F$  = sample dilution factor, 10

**Acceptance criteria:** 90.0%–110.0%

**Benzoic acid content:** Elute *Column B* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume, and mix. Concomitantly determine the absorbances of eluate and *Standard solution B* in 1-cm cells at the wavelength of maximum absorbance at 275 nm, with a suitable spectrophotometer, using *Diluent* as the blank.

Calculate the percentage of the labeled amount of benzoic acid ( $C_7H_6O_2$ ) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

$A_U$  = absorbance of the diluted eluate from *Column B*

$A_S$  = absorbance of *Standard solution B*

$C_S$  = concentration of USP Benzoic Acid RS in *Standard solution B* ( $\mu$ g/mL)

$C_U$  = nominal concentration of benzoic acid in the *Sample solution* ( $\mu$ g/mL)

$F$  = sample dilution factor, 10

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL <755>:** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and avoid exposure to temperatures exceeding 30°.
- **LABELING:** Label Ointment to indicate the concentrations of Benzoic Acid and Salicylic Acid and to indicate

whether the ointment base is water-soluble or water-insoluble.

- **USP REFERENCE STANDARDS** <11>  
USP Benzoic Acid RS  
USP Salicylic Acid RS

## Benzoïn

### DEFINITION

Benzoïn is the balsamic resin obtained from *Styrax benzoïn* Dryander or *Styrax paralleloneurus* Perkins, known in commerce as Sumatra Benzoïn, or from *Styrax tonkinensis* (Pi  re) Craib ex Hartwich, or other species of the Section *Anthostyrax* of the genus *Styrax*, known in commerce as Siam Benzoïn (Fam. *Styraceae*).

Sumatra Benzoïn yields NLT 75.0% of alcohol-soluble extractive, and Siam Benzoïn yields NLT 90.0% of alcohol-soluble extractive.

### IDENTIFICATION

- **A.** A solution in alcohol becomes milky upon the addition of water, and the mixture is acid to litmus paper.
- **B. IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN** <563>  
**Analysis:** Heat a few fragments in a test tube.  
**Acceptance criteria:** Sumatra Benzoïn evolves a sublimate consisting of plates and small, rod-like crystals of cinnamic acid and its esters that strongly polarize light. Siam Benzoïn evolves a sublimate directly above the melted mass, consisting of numerous long, rod-shaped crystals of benzoic acid that do not strongly polarize light.

### ASSAY

#### • PROCEDURE

**Sample:** Place 2 g of Benzoïn in a tared extraction thimble, and insert the thimble in a continuous-extraction apparatus. Place 100 mg of sodium hydroxide in the receiving flask of the apparatus, and extract the Benzoïn with alcohol for 5 h, or until completely extracted. Dry the extraction thimble containing the insoluble residue at 105   for 2 h.

**Analysis:** On a separate portion of Benzoïn, determine the water content as directed for *Water Determination* <921>, *Method II*. Calculate the weight of water in the quantity of the Benzoïn taken for the Assay, and subtract it from the original weight of the Benzoïn taken. The difference between this result and the weight of the residue in the extraction thimble represents the alcohol-soluble extractive.

**Acceptance criteria:** The alcohol-soluble extractive is NLT 75.0% for Sumatra Benzoïn and NLT 90.0% for Siam Benzoïn.

### OTHER COMPONENTS

#### • CONTENT OF BENZOIC ACID

**Analysis:** Treat 1 g of powdered Benzoïn with 15 mL of warm carbon disulfide. Filter through a small pledget of cotton, wash the cotton with an additional 5 mL of carbon disulfide, and allow the filtrate to evaporate spontaneously.

**Acceptance criteria:** The weight of the residue is NLT 6.0% of the weight of Benzoïn taken for Sumatra Benzoïn and NLT 12.0% for Siam Benzoïn. This residue meets the requirements for *Identification Tests—General* <191>, *Benzoate*.

### IMPURITIES

#### Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN**, *Acid-Insoluble Ash* <561>: NMT 1.0% in Sumatra Benzoïn; NMT 0.5% in Siam Benzoïn

### Organic Impurities

- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN**, *Foreign Organic Matter* <561>: NMT 1.0% in Siam Benzoïn

### SPECIFIC TESTS

#### • BOTANIC CHARACTERISTICS

**Sumatra Benzoïn:** Blocks or lumps of varying size, made up of tears, compacted together, with a reddish brown, reddish gray, or grayish brown resinous mass; the tears are externally yellowish or rusty brown, milky white on fresh fracture; hard and brittle at ordinary temperatures, but softened by heat.

**Siam Benzoïn:** Pebble-like tears of variable size and shape, compressed, yellowish brown to rusty brown externally, milky white on fracture, separate or very slightly agglutinated; hard and brittle at ordinary temperatures, but softened by heat.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is Sumatra Benzoïn or Siam Benzoïn.

## Compound Benzoïn Tincture

» Prepare Compound Benzoïn Tincture as follows.

Benzoïn, in moderately coarse powder . . . . .	100 g
Aloe, in moderately coarse powder . . . . .	20 g
Storax . . . . .	80 g
Tolu Balsam . . . . .	40 g
To make . . . . .	1000 mL

Prepare a *Tincture by Process M* (see *Pharmaceutical Dosage Forms* <1151>), using alcohol as the menstruum.

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

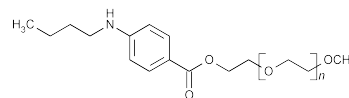
**Labeling**—Label it to indicate that it is flammable.

**Specific gravity** <841>: between 0.870 and 0.885.

**Limit of nonvolatile residue**—Evaporate 3 mL of Tincture in a suitable tared dish on a steam bath, and dry the residue at 100   for 2 hours: the weight of the residue is between 525 mg and 675 mg.

**Alcohol content**, *Method II* <611>: between 74.0% and 80.0% of C<sub>2</sub>H<sub>5</sub>OH, the dilution to approximately 2% alcohol being made with methanol instead of with water.

## Benzonatate



C<sub>30</sub>H<sub>53</sub>NO<sub>11</sub> (av.) 603.74 (average)  
Benzoic acid, 4-(butylamino)-, 2,5,8,11,14,17,20,23,26-nonaoxaoctacos-28-yl ester.



2,5,8,11,14,17,20,23,26-Nonaaoctacosan-28-yl  
*p*-(butylamino)benzoate [104-31-4].

» Benzonatate contains not less than 95.0 per-  
 cent and not more than 105.0 percent of  
 $C_{30}H_{53}NO_{11}$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Benzonatate RS

**Identification**—

**A:** *Infrared Absorption* (197F).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 15 µg per mL.

*Medium:* water.

**Refractive index** (831): between 1.509 and 1.511 at 20°.

**Water, Method I** (921): not more than 0.3%.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Mix 20 mL of a solution (1 in 10) with 20 mL of water and 1 mL of nitric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of silver nitrate TS. Dilute with water to 50 mL, mix, and allow to stand protected from light for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N hydrochloric acid (0.0035%).

**Sulfate** (221)—Mix 5 mL of a solution (1 in 20) with 5 mL of water and 1 mL of 3 N hydrochloric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of barium chloride TS. Mix, and allow to stand for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (0.04%).

**Heavy metals, Method II** (231): 0.001%.

**Assay**—Weigh accurately about 5 g of Benzonatate, and reflux with 25.0 mL of 0.5 N sodium hydroxide VS for 1 hour. Cool, add 25 mL of water and 10 drops of bromothymol blue TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations under Titrimetry* (541)). Each mL of 0.5 N sodium hydroxide is equivalent to 301.5 mg of  $C_{30}H_{53}NO_{11}$ .

## Benzonatate Capsules

» Benzonatate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of benzonatate ( $C_{30}H_{53}NO_{11}$  av.).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Benzonatate RS

**Identification**—

**A:** The contents of the Capsules meet the requirements of *Identification* test A under *Benzonatate*. If a difference is observed, or if excipients are present, use an amount of the contents of Capsules equivalent to about 100 mg of benzonatate, mixed with 25 mL of 0.01 N hydrochloric acid, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator."

**B:** The contents of the Capsules respond to *Identification* test B under *Benzonatate*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

Determine the amount of benzonatate dissolved by employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.04 M monobasic potassium phosphate (3:1). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard solution**—Transfer 50 mg, accurately weighed, of USP Benzonatate RS to a 100-mL volumetric flask, and add about 50 mL of water. Sonicate for 10 minutes, cool, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

**Test solution**—Pass a portion of the solution under test through a 0.45-µm filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 310-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of benzonatate dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of USP Benzonatate RS in the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and LC is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of benzonatate is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Standard preparation**—Transfer about 50 mg of USP Benzonatate RS, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Mix a number of Capsules, equivalent to about 500 mg of benzonatate, with 40 mL of chloroform in a suitable high-speed blender, and dilute with chloroform to 100.0 mL. Transfer 10.0 mL of this solution, equivalent to about 50 mg of benzonatate, to a 100-mL volumetric flask, and evaporate the chloroform on a steam bath with the aid of a current of air. Dissolve the residue in water, dilute with water to volume, and mix.

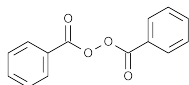
**Procedure**—Transfer 4.0 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide the blank, to separate test tubes. To each tube add in succession 1.0 mL of 1 M hydroxylamine hydrochloride and 1.0 mL of 3.5 N sodium hydroxide, mixing after each addition. Allow to stand for 10 minutes, accurately timed, then add 1.0 mL of 3.5 N hydrochloric acid, mix, add 1.0 mL of ferric chloride solution (8 in 100), and mix. Allow to stand for 30 minutes, accurately timed. Gently swirl the tubes for 1 minute to remove any gas bubbles present, then concomitantly determine the absorbances of the solutions in 1-cm cells, at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of benzonatate.

tate ( $C_{30}H_{53}NO_{11}$  av.) in the number of Capsules taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Benzonatate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrous Benzoyl Peroxide



$C_{14}H_{10}O_4$  (anhydrous) 242.23  
Peroxide, dibenzoyl;  
Benzoyl peroxide [94-36-0].

### DEFINITION

Hydrous Benzoyl Peroxide contains NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{14}H_{10}O_4$ . It contains a minimum of 20% of water for the purpose of reducing flammability and shock sensitivity.

**[CAUTION]**—Hydrous Benzoyl Peroxide may explode at temperatures higher than  $60^\circ$  or cause fires in the presence of reducing substances. Store it in the original container, treated to reduce static charges.]

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 10 mg/mL of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in methanol

**Sample solution:** 10 mg/mL of benzoyl peroxide in methanol

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu\text{L}$

**Developing solvent system:** Toluene, dichloromethane, and glacial acetic acid (50:2:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Place the plate in a developing chamber containing and equilibrated with the *Developing solvent system*. Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### • B. The *Sample solution* in the test for *Organic Impurities* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard solution*.

### ASSAY

#### • PROCEDURE

**Sample:** 300 mg of previously mixed Hydrous Benzoyl Peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the *Sample*.

**Analysis:** Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is

approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of  $C_{14}H_{10}O_4$ .

**Acceptance criteria:** 90.0%–110.0% of the labeled amount

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**Solution A:** Prepare a mixture of acetonitrile and glacial acetic acid (1000:1).

**Solution B:** Prepare a mixture of water and glacial acetic acid (1000:1).

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

**System suitability solution:** 100  $\mu\text{g/mL}$  of benzoic acid and 60  $\mu\text{g/mL}$  of methylparaben in acetonitrile

**Standard solution:** Dissolve a quantity of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in acetonitrile to obtain a solution containing 0.32 mg/mL.

**Sample solution:** 0.32 mg/mL of benzoyl peroxide in acetonitrile

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10  $\mu\text{L}$

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between benzoic acid and methylparaben

**Tailing factors:** NMT 2.0 for the benzoic acid and methylparaben peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the area, as a percentage, of each peak in the chromatogram of the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for any individual peak other than the principal peak in the *Sample solution*

$r_T$  = sum of the peak responses of all the individual peaks including the principal peak in the *Sample solution*

**Acceptance criteria:** The area of any individual peak other than the principal peak is NMT 1.5% of the total area. The sum of the areas of all peaks other than the principal peak is NMT 2.0% of the total area.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in the original container, at room temperature. [NOTE—Do not transfer Hydrous Benzoyl Peroxide to metal or glass containers fitted with friction tops. Do not return unused material to its original container, but destroy it by treatment with sodium hydroxide solution (1 in 10) until addition of a crystal of potassium iodide results in no release of free iodine.]

## Benzoyl Peroxide Gel

### DEFINITION

Benzoyl Peroxide Gel is benzoyl peroxide in a suitable gel base. It contains NLT 90.0% and NMT 125.0% of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ).

### IDENTIFICATION

**A.** The retention time of the major peak for benzoyl peroxide of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile in water (5 in 10)

**Internal standard solution:** 3.6 mg/mL of ethyl benzoate in acetonitrile

**Standard stock solution:** 0.8 mg/mL of benzoyl peroxide prepared as follows. Transfer a suitable quantity of benzoyl peroxide, recently subjected to the *Assay* in Hydrous Benzoyl Peroxide, into a weighed conical flask fitted with a glass stopper. Weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile.

**Standard solution:** 0.32 mg/mL of benzoyl peroxide prepared as follows. Mix 10 mL of *Standard stock solution* and 5 mL of *Internal standard solution*, and dilute with acetonitrile to 25 mL.

**Sample stock solution:** Transfer an equivalent to 40 mg of benzoyl peroxide from Gel into a 50-mL volumetric flask, add 40 mL of acetonitrile, and shake until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.

**Sample solution:** 10 mL of *Sample stock solution* and 5 mL of *Internal standard solution*; dilute with acetonitrile to 25 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution* (three replicate injections)

[NOTE—The retention times for ethyl benzoate and benzoyl peroxide are 7 and 14 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between ethyl benzoate and benzoyl peroxide

**Tailing factors:** NMT 2.0 for the ethyl benzoate and benzoyl peroxide peaks

**Peak response ratios:** The lowest and highest peak response ratios ( $R_s$ ) agree within 2.0%.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Sample solution*

$R_S$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Standard solution*

$C_S$  = concentration of benzoyl peroxide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzoyl peroxide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–125.0%

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A:** Acetonitrile and glacial acetic acid (1000:1)

**Solution B:** Water and glacial acetic acid (1000:1)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

**System suitability solution:** 100  $\mu$ g/mL of benzoic acid and 60  $\mu$ g/mL of methylparaben in acetonitrile

**Standard solution A:** 500  $\mu$ g/mL of benzoic acid in acetonitrile

**Standard solution B:** 20  $\mu$ g/mL of ethyl benzoate in acetonitrile

**Standard solution C:** 20  $\mu$ g/mL of benzaldehyde in acetonitrile

**Standard solution D:** Equivalent to 40  $\mu$ g/mL of anhydrous benzoyl peroxide in acetonitrile, prepared from hydrous benzoyl peroxide, which has been analyzed as follows. Place 300 mg of previously mixed hydrous benzoyl peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the sample. Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of hydrous benzoyl peroxide ( $C_{14}H_{10}O_4$ ).

**Sample solution:** Transfer an amount of Gel equivalent to 100 mg of benzoyl peroxide to a 50-mL volumetric flask, and add 25 mL of acetonitrile. Shake vigorously to disperse the specimen, sonicate for 5 min, dilute with acetonitrile to volume, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between benzoic acid and methylparaben

**Tailing factor:** NMT 2.0 for the benzoic acid and methylparaben peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The responses of any peaks from the *Sample solution* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are NMT those of the main peaks from *Standard solution A* (25%), *Standard solution B* (1%), and *Standard solution C* (1%), respectively. The response of any other impurity peak from the *Sample solution*—other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzalde-

hyde, methylparaben, or propylparaben peak, and any solvent peak—is NMT that from *Standard solution D* (2%); and the sum of the responses of all the impurity peaks—other than those of benzoic acid, ethyl benzoate, and benzaldehyde—is NMT that from *Standard solution D* (2%).

**SPECIFIC TESTS**

- **pH** (791): 2.8–6.6

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

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**Benzoyl Peroxide Lotion**


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» Benzoyl Peroxide Lotion is benzoyl peroxide in a suitable lotion base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{14}H_{10}O_4$ .

**Packaging and storage**—Preserve in tight containers.

**Identification—**

**A:** Dilute a quantity of Lotion with acetone to obtain a solution having a concentration of benzoyl peroxide equivalent to 10 mg per mL, and proceed with the solution so obtained as directed in the *Identification* test A under *Hydrous Benzoyl Peroxide*, beginning with “Apply 5  $\mu$ L of this solution.” The solution responds to the test.

**B:** It responds to the *Identification* test under *Benzoyl Peroxide Gel*.

**pH** (791): between 2.8 and 6.6.

**Related compounds—**

*Solution A, Solution B, Mobile phase, Standard preparation A, Standard preparation B, Standard preparation C, Standard preparation D, System suitability solution, and Chromatographic system*—Proceed as directed in the test for *Related compounds* under *Benzoyl Peroxide Gel*.

**Test preparation**—Transfer an accurately weighed quantity of Lotion, equivalent to about 100 mg of benzoyl peroxide, to a 50-mL volumetric flask, add 25 mL of acetonitrile, shake vigorously to disperse the specimen, sonicate for 5 minutes, dilute with acetonitrile to volume, mix, and filter.

**Procedure**—Proceed with Lotion as directed for *Procedure* in the test for *Related compounds* under *Benzoyl Peroxide Gel*; it meets the limits stated.

**Assay—**

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Benzoyl Peroxide Gel*.

**Assay preparation**—Prepare as directed for *Assay preparation* in the *Assay* under *Benzoyl Peroxide Gel*, using Lotion.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Benzoyl Peroxide Gel*. Calculate the quantity, in mg, of  $C_{14}H_{10}O_4$  in the portion of Lotion taken by the formula:

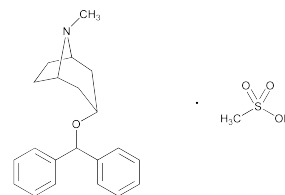
$$125C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of benzoyl peroxide in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of benzoyl peroxide peak response to ethyl benzoate peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Benztropine Mesylate**


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$C_{21}H_{25}NO \cdot CH_4O_3S$  403.53

8-Azabicyclo[3.2.1]octane, 3-(diphenylmethoxy)-, *endo*-, methanesulfonate;  
3 $\alpha$ -(Diphenylmethoxy)-1 $\alpha$ H,5 $\alpha$ H-tropane methanesulfonate [132-17-2].

**DEFINITION**

Benztropine Mesylate contains NLT 98.0% and NMT 100.5% of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**ASSAY**

- **PROCEDURE**

**Sample:** 60 mg of Benztropine Mesylate

**Analysis:** Dissolve the *Sample* in 25 mL of water, add 5 mL of sodium carbonate TS, and extract with four 10-mL portions of chloroform. Wash the combined chloroform extracts with about 10 mL of water, and extract the wash solution with 5 mL of chloroform. Filter the combined chloroform extracts through a tightly packed pledget of cotton, and wash the cotton with about 5 mL of chloroform. Add methyl red TS, and titrate the chloroform solution with 0.01 N perchloric acid in dioxane VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.01 N perchloric acid is equivalent to 4.035 mg of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ).  
**Acceptance criteria:** 98.0%–100.5% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 141°–148°
- **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Benztropine Mesylate RS

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**Benztropine Mesylate Injection**


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**DEFINITION**

Benztropine Mesylate Injection is a sterile solution of Benztropine Mesylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ).

**IDENTIFICATION**

- **A.**  
**Standard stock solution:** 0.2 mg/mL of USP Benztropine Mesylate RS  
**Standard solution:** In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide.

Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Sample stock solution:** Dilute a volume of Injection, equivalent to 10 mg of bzotropine mesylate, in a separator to 50 mL with water (0.2 mg/mL).

**Sample solution:** In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

#### Chromatographic system

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 1  $\mu$ L

**Developing solvent system:** Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Buffer:** Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** 1 mg/mL of USP Bzotropine Mesylate RS

**Sample solution:** Nominally 1 mg/mL of bzotropine mesylate from the volume of Injection

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 1.3 mL/min adjusted, as needed, to obtain a retention time of 7 min for bzotropine mesylate

**Injection volume:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of bzotropine mesylate ( $C_{21}H_{25}NO \cdot CH_3O_3S$ ) in each mL of the Injection:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bzotropine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bzotropine mesylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 55.6 USP Endotoxin Units/mg of bzotropine mesylate
- **pH (791):** 5.0–8.0
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* <1>

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**  
USP Bzotropine Mesylate RS  
USP Endotoxin RS

## Bzotropine Mesylate Tablets

#### DEFINITION

Bzotropine Mesylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bzotropine mesylate ( $C_{21}H_{25}NO \cdot CH_3O_3S$ ).

#### IDENTIFICATION

##### • A.

**Standard stock solution:** 0.2 mg/mL of USP Bzotropine Mesylate RS

**Standard solution:** In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Sample stock solution:** Dissolve a portion of finely powdered Tablets, equivalent to 10 mg of bzotropine mesylate, in 50 mL of water, shake by mechanical means for 30 min, and filter into a separator (0.2 mg/mL).

**Sample solution:** In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

#### Chromatographic system

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 1  $\mu$ L

**Developing solvent system:** Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY****• PROCEDURE**

**Buffer:** Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute to volume, and adjust with phosphoric acid to a pH of 3.0.

**Diluent:** Isopropyl alcohol, water, and phosphoric acid (40: 60: 0.1)

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Standard solution:** 0.04 mg/mL of USP Benztropine Mesylate RS in *Diluent*

**Sample solution:** Nominally 0.04 mg/mL of benztropine mesylate from a suitable amount of powdered Tablets in *Diluent* prepared as follows. Add a suitable amount of fine powder from NLT 20 Tablets to a portion of *Diluent* corresponding to 60% of the final volume. Mix by mechanical means for NLT 60 min, and dilute with *Diluent* to volume. Centrifuge a portion of this mixture, and filter the supernatant layer.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 0.7 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 4.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Benztropine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benztropine mesylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) dissolved by using the following method.

**Buffer:** Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute to volume, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** USP Benztropine Mesylate RS in *Medium*. Dilute to obtain a solution having a known concentration similar to that of the *Sample solution*.

**Sample solution:** Use a filtered portion of the solution under test from the dissolution vessel.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection volume:** 300 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Benztropine Mesylate RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Acceptance criteria:** NLT 80% (Q) of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

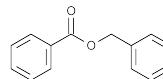
- **USP REFERENCE STANDARDS <11>**

USP Benztropine Mesylate RS

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**Benzyl Benzoate**

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$C_{14}H_{12}O_2$

212.24

Benzoic acid, phenylmethyl ester;  
Benzyl benzoate [120-51-4].

**DEFINITION**

Benzyl Benzoate contains NLT 99.0% and NMT 100.5% of  $C_{14}H_{12}O_2$ .

**IDENTIFICATION**

- **A. INFRARED ABSORPTION <197F>**

**ASSAY****• PROCEDURE**

**Sample:** 2 g of Benzyl Benzoate

**Analysis:** Transfer the *Sample* to a conical flask fitted with a reflux condenser. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, and boil gently for 1 h. Cool, add phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Titrimetry* <541>). Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of Benzyl Benzoate ( $C_{14}H_{12}O_2$ ).

**Acceptance criteria:** 99.0%–100.5%

**IMPURITIES****• LIMIT OF ALDEHYDES**

**Sample solution:** Transfer 10.0 g to a 125-mL conical flask containing 50 mL of alcohol and 5 mL of hydroxylamine hydrochloride solution (3.5 in 100), mix, and allow to stand for 10 min.

**Analysis:** Add 1 mL of bromophenol blue TS, and titrate with 0.1 N sodium hydroxide VS to a light green endpoint. Perform a blank determination, and match the color of the endpoint with that of the titrated *Sample solution*.

**Acceptance criteria:** The net volume of 0.1 N sodium hydroxide consumed does not exceed 0.50 mL (0.05% as benzaldehyde).

#### SPECIFIC TESTS

- **SPECIFIC GRAVITY** (841): 1.116–1.120
- **CONGEALING TEMPERATURE** (651): Congelation may be brought about by the addition of a fragment of previously congealed Benzyl Benzoate when the temperature has reached the expected congealing temperature.  
**Acceptance criteria:** NLT 18.0°
- **REFRACTIVE INDEX** (831): 1.568–1.570 at 20°
- **ACIDITY:** Add 2 drops of phenolphthalein TS to 25 mL of alcohol, and add 0.020 N sodium hydroxide until a pink color is produced. Add 5.0 g of Benzyl Benzoate, and titrate with 0.020 N sodium hydroxide.  
**Acceptance criteria:** NMT 1.5 mL of 0.020 N sodium hydroxide is required to restore the pink color.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, well-filled, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)  
USP Benzyl Benzoate RS

### Benzyl Benzoate Lotion

» Benzyl Benzoate Lotion contains not less than 26.0 percent and not more than 30.0 percent (w/w) of  $C_{14}H_{12}O_2$ .

Benzyl Benzoate . . . . .	250 mL
Triethanolamine . . . . .	5 g
Oleic Acid . . . . .	20 g
Purified Water . . . . .	750 mL
To make about . . . . .	1000 mL

Mix the Triethanolamine with the Oleic Acid, add the Benzyl Benzoate, and mix. Transfer the mixture to a suitable container of about 2000-mL capacity, add 250 mL of Purified Water, and shake the mixture thoroughly. Finally add the remaining Purified Water, and again shake thoroughly.

**Packaging and storage**—Preserve in tight containers.

**pH** (791): between 8.5 and 9.2.

**Assay**—Place about 5 g of Lotion, accurately weighed, in a conical flask. Add 25 mL of alcohol and 2 drops of phenolphthalein TS. Cool the solution to about 15°, and titrate quickly with 0.1 N sodium hydroxide to a slight pink color. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, connect the flask to a reflux condenser, and boil gently for 1 hour. Cool, promptly add phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of  $C_{14}H_{12}O_2$ .

### Benzylpenicilloyl Polylysine Concentrate

» Benzylpenicilloyl Polylysine Concentrate has a molar concentration of benzylpenicilloyl moiety ( $C_{16}H_{19}N_2O_5S$ ) of not less than 0.0125 M and not more than 0.020 M. It contains one or more suitable buffers.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label states that this article is not intended for direct administration to humans or animals.

**USP Reference standards** (11)—

USP L-Lysine Hydrochloride RS

**pH** (791): between 6.5 and 8.5, the undiluted Concentrate being used.

**Limit of penicillenate and penamaldate**—Transfer 1 mL of Concentrate to a 50-mL volumetric flask, dilute with *Saline phosphate buffer*, prepared as directed in the *Assay*, to volume, and mix. Using a suitable spectrophotometer and using *Saline phosphate buffer* as a blank, determine the absorbances at the wavelengths of maximum absorption at about 322 nm and 282 nm. Calculate the molar concentration of penicillenate taken by the formula:

$$50A_{322} / 26,600b$$

in which  $A_{322}$  is the absorbance at 322 nm, 26,600 is the molar absorptivity of the penicillenate moiety at pH 7.6, and  $b$  is the length of the cell, in cm: not more than 0.00020 M is found. Calculate the molar concentration of penamaldate taken by the formula:

$$50A_{282} / 22,325b$$

in which  $A_{282}$  is the absorbance at 282 nm, 22,325 is the molar absorptivity of the penamaldate moiety at pH 7.6, and  $b$  is the length of the cell, in cm: not more than 0.00060 M is found.

#### Benzylpenicilloyl substitution—

**Citrate buffer**—Dissolve 19.69 g of sodium citrate dihydrate, 0.1 mL of pentachlorophenol, and 5 mL of 2,2'-thiodiethanol in 900 mL of 0.2 N hydrochloric acid, adjust with hydrochloric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

**Ninhydrin reagent**—Dissolve 18 g of ninhydrin and 0.7 g of hydrindantin in 675 mL of dimethyl sulfoxide, add 225 mL of 4 M lithium acetate solution previously adjusted with glacial acetic acid to a pH of 5.2, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP L-Lysine Hydrochloride RS in *Citrate buffer* to obtain a solution having a known concentration of about 91 µg per mL ( $5 \times 10^{-4}$  M).

**Test preparation**—Transfer 1.0 mL of Concentrate to a 10-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to an ampul, add 1.5 mL of 6 N hydrochloric acid, and seal the ampul under nitrogen. Heat the ampul at 110° for 22 hours. Transfer the contents of the ampul to a round-bottom, 50-mL flask, and dry by vacuum rotary evaporation. Dissolve the residue three times, using 5-mL portions of water, evaporating to dryness after each dissolution. Dissolve the residue in 10 mL of *Citrate buffer*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 1.75-mm × 50-cm column that contains a packing of 8-µm 8% cross-linked sulfonated divinylbenzene polystyrene cation-exchange resin. The column effluent is mixed continuously with flowing *Ninhydrin reagent*, and the flowing mixture is heated at 130° for 1.5 minutes in a reaction coil. The absorbance of

the reaction mixture is measured continuously by a 570-nm detector. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 57 minutes for L-lysine. Calculate the molar concentration of lysine in the Concentrate taken by the formula:

$$(0.1C/182.65)(r_U / r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP L-Lysine Hydrochloride RS in the *Standard preparation*; 182.65 is the molecular weight of anhydrous lysine hydrochloride; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Calculate the percentage of benzylpenicilloyl substitution taken by the formula:

$$100(B/L)$$

in which *B* is the molar concentration of benzylpenicilloyl moiety in the Concentrate, as determined in the *Assay*; and *L* is the molar concentration of lysine in the Concentrate: not less than 50% and not more than 70% is found.

#### Assay—

**Saline phosphate buffer**—Dissolve 9 g of sodium chloride and 1.38 g of monobasic sodium phosphate in 900 mL of water, adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 7.6, dilute with water to 1000 mL, and mix.

**Mercuric chloride solution**—Dissolve 35 mg of mercuric chloride in 500 mL of water, and mix.

**Assay preparation**—Transfer 1.0 mL of Concentrate to a 500-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

**Procedure**—Transfer 3.0 mL of *Assay preparation* to a spectrophotometric cell. Using a suitable spectrophotometer and using *Saline phosphate buffer* as the blank, determine the initial absorbance at the wavelength of maximum absorbance at about 282 nm. Add 0.02 mL of *Mercuric chloride solution* to the *Assay preparation* in the spectrophotometric cell, mix, and determine the absorbance at the same wavelength after 1 and 3 minutes. Repeat the addition of 0.02-mL portions of *Mercuric chloride solution* until a maximum absorbance reading is obtained. Calculate the molar concentration of benzylpenicilloyl moiety in the Concentrate taken by the formula:

$$500\{[A_m(3 + 0.02n)/3] - A_i\}/22,325b$$

in which  $A_m$  is the highest absorbance observed;  $A_i$  is the initial absorbance, *n* is the number of 0.02-mL portions of *Mercuric chloride solution* added to the *Assay preparation* to obtain the maximum absorbance; 22,325 is the molar absorptivity of the penamaldate formed by the reaction of benzylpenicilloyl with mercuric chloride at pH 7.6; and *b* is the length of the cell, in cm: between 0.0125 M and 0.020 M is found.

## Benzylpenicilloyl Polylysine Injection

» Benzylpenicilloyl Polylysine Injection has a molar concentration of benzylpenicilloyl moiety

( $C_{16}N_2H_{19}O_5S$ ) of not less than  $5.4 \times 10^{-5}$  M and not more than  $7.0 \times 10^{-5}$  M. It contains one or more suitable buffers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a refrigerator.

#### USP Reference standards (11)—

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 5833.0 USP Endotoxin Units per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.5 and 8.5.

#### Assay—

**Saline phosphate buffer** and **Mercuric chloride solution**—Prepare as directed in the *Assay* under *Benzylpenicilloyl Polylysine Concentrate*.

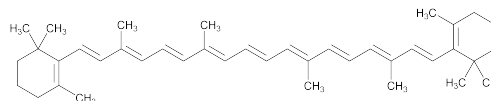
**Assay preparation**—Combine the contents of a sufficient number of containers to obtain not less than 3 mL of Injection. Transfer 3.0 mL of Injection to a 10-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Benzylpenicilloyl Polylysine Concentrate*. Calculate the molar concentration of benzylpenicilloyl moiety in the Injection taken by the formula:

$$(10 / 3)\{[A_m(3 + 0.02n) / 3] - A_i\} / 22,325b$$

in which the terms are as defined therein.

## Beta Carotene



$C_{40}H_{56}$

536.87

$\beta,\beta$ -Carotene;

all-*trans*- $\beta$ -Carotene;

(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene] [7235-40-7].

#### DEFINITION

Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene ( $C_{40}H_{56}$ ). It contains NLT 95.0% of all-*trans*-beta carotene in the total carotenoids content.

#### IDENTIFICATION

##### • A.

**Sample solution:** Prepare as directed for the *Sample solution* in the test for *Content of Total Carotenoids*.

**Analysis:** Record the UV-Vis spectrum from 300–600 nm.

**Acceptance criteria:** The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio  $A_{455}/A_{483}$  is 1.14–1.18.

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Content of Beta Carotene*.



**ASSAY****COMPOSITION****• CONTENT OF TOTAL CAROTENOIDS**

[NOTE—Use low-actinic glassware.]

**Sample stock solution:** 0.1 mg/mL of Beta Carotene in tetrahydrofuran**Sample solution:** Transfer 3.0 mL of *Sample stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume.**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* (851).)**Analytical wavelength:** 457 nm**Cell path:** 1 cm**Blank:** Cyclohexane**Analysis****Sample:** *Sample solution*Calculate the percentage of total carotenoids (*T*) as beta carotene (C<sub>40</sub>H<sub>56</sub>):

$$T = A/(F \times C)$$

*A* = absorbance of the *Sample solution**F* = 2505, coefficient of extinction (E<sup>1%</sup>) of pure all-*trans*-beta carotene in cyclohexane (100 mL · g<sup>-1</sup> · cm<sup>-1</sup>)*C* = concentration of *Sample solution* (g/mL)**Acceptance criteria:** 96.0%–101.0% of total carotenoids as beta carotene (C<sub>40</sub>H<sub>56</sub>)**• CONTENT OF BETA CAROTENE**

[NOTE—Use low-actinic glassware.]

**Mobile phase:** Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyl-diisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.**Diluent:** 50 µg/mL of butylated hydroxytoluene in alcohol**System suitability solution:** Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water, 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a membrane filter of 0.45-µm pore size, and use the clear filtrate.**Standard solution:** 10 µg/mL of USP Beta Carotene RS in tetrahydrofuran and *Diluent* (1:9). Dissolve an appropriate amount of USP Beta Carotene RS in a volumetric flask first with tetrahydrofuran, using 10% of the volume of the flask, then complete with *Diluent* to volume.**Sample solution:** Dilute the freshly prepared *Sample stock solution* as prepared in the test for *Content of Total Carotenoids* (1 in 10) with *Diluent*.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 448 nm**Column:** 4.6-mm × 25-cm; 5-µm packing L68**Column temperature:** 30°**Flow rate:** 0.6 mL/min**Injection size:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1*.**Table 1**

Analyte	Relative Retention Time	Relative Response Factor
all- <i>trans</i> -Alpha carotene	0.93	1.1
all- <i>trans</i> -Beta carotene	1.00	1
9- <i>cis</i> -Beta carotene	1.07	1
13- <i>cis</i> -Beta carotene	1.17	1.2
15- <i>cis</i> -Beta carotene	1.21	1.4

**Suitability requirements****Chromatogram similarity:** The chromatogram from the *System suitability solution* is similar to the Reference Chromatogram provided with the USP Beta Carotene System Suitability RS being used.**Resolution:** NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene and between all-*trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution***Tailing factor:** NMT 2.0 for the all-*trans*-beta carotene peak, *Standard solution***Relative standard deviation:** NMT 2.0% for the all-*trans*-beta carotene peak from replicate injections, *Standard solution***Analysis****Sample:** *Sample solution*Record the chromatograms, and identify the peaks of the relevant analytes in the chromatogram of the *Sample solution* by comparing with those in the chromatogram of the *System suitability solution*. Measure the peak area responses.Calculate the percentage of all-*trans*-beta carotene relative to total carotenoids in the sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

*r<sub>U</sub>* = peak area response of all-*trans*-beta carotene from the *Sample solution**r<sub>T</sub>* = [(peak area of all-*trans*-alpha carotene × 1.1) + (peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) + (sum of peak area of other *cis*-isomers of beta carotene)] from the *Sample solution***Acceptance criteria:** NLT 95.0% of all-*trans*-beta carotene in the total carotenoids content**• ALPHA CAROTENE AND OTHER RELATED COMPOUNDS****Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the test for *Content of Beta Carotene*.**Analysis****Sample:** *Sample solution***Injection size:** 20 µL

Calculate the percentage of alpha carotene and other individual related compounds relative to total carotenoids in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

*r<sub>U</sub>* = (peak area response of all-*trans*-alpha carotene × 1.1) or (peak area response of other individual related compounds × appropriate relative response factor, *Table 1*) in the *Sample solution**r<sub>T</sub>* = [(peak area of all-*trans*-alpha carotene × 1.1) + (peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) + (sum of peak area of other *cis*-isomers of beta carotene)] in the *Sample solution*

**Acceptance criteria****Alpha carotene:** NMT 1.0%**Total related compounds** (including alpha carotene): NMT 5.0%**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%, 2 g of specimen being used
- **HEAVY METALS, Method II** (231): NMT 10 ppm

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 176°–182°, with decomposition
- **LOSS ON DRYING** (731): Dry a sample in vacuum over phosphorus pentoxide at 40° for 4 h: it loses NMT 0.2% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Beta Carotene RS
  - (all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene].
  - USP Beta Carotene System Suitability RS

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**Beta Carotene Capsules**


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**DEFINITION**

Beta Carotene Capsules contain NLT 90% and NMT 125.0% of the labeled amount of total beta carotene ( $C_{40}H_{56}$ ), of which NLT 95.0% is the all-*trans*-beta carotene isomer.

**IDENTIFICATION**

- **A.**

**Sample solution:** Dilute the *Sample stock solution* of the test for *Content of Total Beta Carotene* with cyclohexane to a final concentration of between 1 and 5 µg/mL of beta carotene. Pass through a membrane filter of 0.45-µm pore size.

**Analysis:** Record the UV-Vis spectrum from 300 to 600 nm.

**Acceptance criteria:** The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio  $A_{455}/A_{483}$  is between 1.14 and 1.18.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Total Beta Carotene*.

**ASSAY**• **CONTENT OF TOTAL BETA CAROTENE**

[NOTE—Use low-actinic glassware.]

**Mobile phase:** Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyl-diisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.

**Diluent:** 50 mg/L of butylated hydroxytoluene in alcohol

**System suitability solution:** Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass through a membrane filter of 0.45-µm pore size, and use the clear filtrate.

**Standard stock solution:** 60 µg/mL of USP Beta Carotene RS in tetrahydrofuran. [NOTE—The USP Beta Carotene RS is subjected to the spectrophotometric purity

test at the time of analysis; see the determination of the concentration of *Standard solution A*, below.]

**Standard solution A:** Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of tetrahydrofuran, and dilute with *Diluent* to volume. Determine the concentration of *Standard solution A* according to the *Analysis of Standard solution B*. [NOTE—The concentration of *Standard solution B* equals the concentration of *Standard solution A*.]

**Standard solution B:** Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.

**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* (851).)**Analytical wavelength:** 457 nm**Cell:** 1 cm**Blank:** Cyclohexane**Analysis****Sample:** *Standard solution B*

Calculate the concentration of total beta carotene (µg/mL) as all-*trans*-beta carotene ( $C_{40}H_{56}$ ) in *Standard solution B*:

$$\text{Result} = (A_U/a) \times F$$

$A_U$  = average absorbance of the three preparations of *Standard solution B*

$a$  = absorptivity of pure all-*trans*-beta carotene in cyclohexane at 457 nm ( $\text{mL} \cdot \text{mg}^{-1} \cdot \text{cm}^{-1}$ ), 250

$F$  = conversion factor from mg to µg (µg/mg), 1000

**Sample stock solution:** Randomly select a number of Capsules, equivalent to 10–50 mg of beta carotene, with a total weight not exceeding 5 g. For powder-containing Capsules, empty the shell, and transfer shell and contents into a 250-mL volumetric flask. For Capsules containing liquid formulations, place the Capsules directly into a 250-mL volumetric flask. Add 250 mg of butylated hydroxytoluene, 0.5 mL of alkaline protease R, and 15 mL of water. Swirl the flask gently to wet the entire contents. Sonicate the flask at 50° for 30 min, and swirl the flask every 10 min. Add 100 mL of alcohol to the warm suspension, and shake vigorously. Add 110 mL of methylene chloride, and shake vigorously again. Disperse any clumps with homogenizer, and rinse the homogenizer probe with 15 mL of methylene chloride into the flask. Allow the solution to stand in the dark until it reaches room temperature (about 2 h), dilute with methylene chloride to volume, shake vigorously, and allow the solids to settle.

**Sample solution:** Dilute a volume of the *Sample stock solution* with a *Diluent*–methylene chloride mixture (1:1) so that the final concentration of beta carotene is between 1 and 5 µg/mL. Pass through a membrane filter of 0.45-µm pore size.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 448 nm**Column:** 4.6-mm × 25-cm; 5-µm packing L68**Column temperature:** 30°**Flow rate:** 0.6 mL/min**Injection size:** 20 µL**System suitability**

**Samples:** *System suitability solution* and *Standard solution A*

[NOTE—The approximate relative retention times of the components in the *System suitability solution* are listed in Table 1.]

Table 1

Name	Relative Retention Time	Relative Response Factor
all- <i>trans</i> -Alpha carotene	0.93	1.1
all- <i>trans</i> -Beta carotene	1.00	1
9- <i>cis</i> -Beta carotene	1.07	1
13- <i>cis</i> -Beta carotene	1.17	1.2
15- <i>cis</i> -Beta carotene	1.21	1.4

**Suitability requirements**

**Chromatogram similarity:** The chromatogram from the *System suitability solution* is similar to the Reference Chromatogram provided with the USP Beta Carotene System Suitability RS being used.

**Resolution:** NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene and between all-*trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

**Tailing factor:** NMT 2.0 for the all-*trans*-beta carotene peak, *Standard solution A*

**Relative standard deviation:** NMT 2.0% for the all-*trans*-beta carotene peak from replicate injections, *Standard solution A*

**Analysis**

**Samples:** *Standard solution A* and *Sample solution*  
Identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution*. Measure the peak area responses. Calculate the percentage of the labeled amount of total beta carotene in the Capsules taken:

$$\text{Result} = (\Sigma r_U / r_S) \times (C_S / C_U) \times 100$$

$\Sigma r_U$  = [(peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene  $\times$  1.2) + (peak area of 15-*cis*-beta carotene  $\times$  1.4) from the *Sample solution*]

$r_S$  = peak area of all-*trans*-beta carotene from *Standard solution A*

$C_S$  = concentration of all-*trans*-beta carotene in *Standard solution A* as determined above ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of total beta carotene in the *Sample solution* ( $\mu\text{g/mL}$ )

Calculate the percentage of all-*trans*-beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_{\text{all-trans}} / \Sigma r_U) \times 100$$

$r_{\text{all-trans}}$  = peak area of all-*trans*-beta carotene from the *Sample solution*

$\Sigma r_U$  = [(peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene  $\times$  1.2) + (peak area of 15-*cis*-beta carotene  $\times$  1.4) from the *Sample solution*]

**Acceptance criteria:** 90%–125.0% of the labeled amount of total beta carotene ( $\text{C}_{40}\text{H}_{56}$ ), of which NLT 95.0% is the all-*trans*-beta carotene isomer

**SPECIFIC TESTS**• **ALPHA CAROTENE AND OTHER RELATED COMPOUNDS**

**Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the test for *Content of Total Beta Carotene*.

**Analysis**

**Sample:** *Sample solution*

**Injection size:** 20  $\mu\text{L}$

Calculate the percentage of alpha carotene and other individual related compounds relative to total beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak area of alpha carotene or other individual related compounds

$r_T$  = sum of the areas of all the peaks

**Acceptance criteria**

**Alpha carotene:** NMT 1.0%

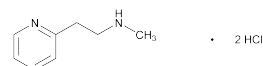
**Total related compounds** (including alpha carotene): NMT 5.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** The label states the name and content of any carriers and antioxidants added to the formulation and the content of total carotenoids as beta carotene.
- **USP REFERENCE STANDARDS** (11)  
USP Beta Carotene RS  
(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene].  
 $\text{C}_{40}\text{H}_{56}$  536.87  
USP Beta Carotene System Suitability RS

**Betahistine Hydrochloride**

$\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{HCl}$  209.12

2-Pyridineethanamine, *N*-methyl-, dihydrochloride.

2-[2-(Methylamino)ethyl]pyridine dihydrochloride [5579-84-0].

» Betahistine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{HCl}$ , calculated on the dried basis.

**USP Reference standards** (11)—

USP Betahistine Hydrochloride RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** (791): between 2.0 and 3.0, in a solution (1 in 10).

**Loss on drying** (731)—Dry it between 100° and 105° to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Related compounds—**

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay*.

**Test solution**—Transfer about 38 mg of Betahistine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Procedure**—Inject about 10  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Betahistine Hydrochloride taken by the formula:

$$100F(r_i/r_s)$$

in which *F* is the response factor of the respective impurity (see *Table 1*) and 1.0 for all other peaks; *r<sub>i</sub>* is the peak response for each impurity; and *r<sub>s</sub>* is the sum of the responses of all of the peaks, adjusted for the relative response factor.

Table 1

Impurity Name	Relative Retention Time	Response Factor ( <i>F</i> )	Limit (%)
2-(2-Hydroxyethyl)pyridine	0.3	0.5	0.2
2-Vinylpyridine	0.4	0.4	0.2
<i>N</i> -Methyl- <i>N,N</i> -bis(2-pyridin-2-yl-ethyl)-amine	2.4	1.4	0.2

In addition to not exceeding the limits for impurities in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 0.5 % of total impurities is found.

#### Assay—

**Ammonium acetate buffer**—Dissolve about 0.69 g of ammonium acetate in 1000 mL of water. Adjust with glacial acetic acid to a pH of 4.7.

**Mobile phase**—Prepare a filtered and degassed mixture of 350 mL of acetonitrile and 650 mL of *Ammonium acetate buffer*, containing 2.88 g of sodium lauryl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betahistine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.38 mg per mL.

**Assay preparation**—Transfer about 38 mg of Betahistine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

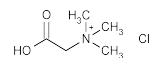
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and 3.0-mm  $\times$  15-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate is about 0.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates; the tailing factor for the betahistine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_8H_{12}N_2 \cdot 2HCl$  in the portion of Betahistine Hydrochloride taken by the formula:

$$100C(r_u/r_s)$$

in which *C* is the concentration, in mg per mL, of USP Betahistine Hydrochloride RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betaine Hydrochloride



$C_5H_{11}NO_2 \cdot HCl$  153.61

Methanaminium, 1-carboxy-*N,N,N*-trimethyl-, chloride. Betaine hydrochloride.

(Carboxymethyl)trimethylammonium chloride [590-46-5].

» Betaine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of  $C_5H_{11}NO_2 \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Betaine Hydrochloride RS

#### Identification—

**A: Infrared Absorption** <197K>.

**B:** A solution (1 in 20) responds to the tests for *Chloride* <191>.

**pH** <791>: between 0.8 and 1.2, in a solution (1 in 4).

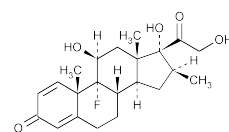
**Water, Method I** <921>: not more than 0.5%.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals** <231>: 0.001%.

**Assay**—Transfer about 400 mg of Betaine Hydrochloride, accurately weighed, to a conical flask, add 50 mL of glacial acetic acid, and heat gently with swirling until solution is complete. Add 25 mL of mercuric acetate TS, cool, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.36 mg of  $C_5H_{11}NO_2 \cdot HCl$ .

## Betamethasone



$C_{22}H_{29}FO_5$  392.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11 $\beta$ ,16 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione [378-44-9].

» Betamethasone contains not less than 97.0 percent and not more than 103.0 percent of  $C_{22}H_{29}FO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 30°.

**USP Reference standards** <11>—

USP Betamethasone RS

#### Identification—

**A: Infrared Absorption** <197M>.

**B: Thin-Layer Chromatographic Identification Test** <201>—

**Test solution**—Prepare a solution of Betamethasone in dehydrated alcohol containing 0.5 mg per mL.

**Developing solvent system**: a mixture of chloroform and diethylamine (2:1).

**Procedure**—Proceed as directed in the chapter, except to locate the spots by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear.

**Specific rotation** (781S): between +118° and +126°, calculated on the dried basis.

**Test solution:** 5 mg per mL, in methanol.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.

**Ordinary impurities** (466)—

**Test solution:** methanol.

**Standard solution:** methanol.

**Application volume:** 10  $\mu$ L.

**Eluant:** a mixture of toluene, acetone, methyl ethyl ketone, and formic acid (55:20:20:5), in a nonequilibrated chamber.

**Visualization:** 5.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (63:37). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of propylparaben in alcohol having a known concentration of about 0.25 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone RS in alcohol to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 10.0 mL of this solution to a suitable vial, and add 10.0 mL of *Internal standard solution*, to obtain a *Standard preparation* having known concentrations of about 0.1 mg of betamethasone and about 0.125 mg of propylparaben per mL.

**Assay preparation**—Using about 80 mg of Betamethasone, accurately weighed, prepare as directed for *Standard preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for betamethasone and 1.4 for propylparaben; the resolution, *R*, between betamethasone and propylparaben is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Betamethasone taken by the formula:

$$800C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak height ratios of the betamethasone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Cream

» Betamethasone Cream contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$  in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or in tight containers.

**USP Reference standards** (11)—

USP Betamethasone RS

**Thin-layer chromatographic identification test** (201)—

**Test solution:** 1 mg per mL, prepared by concentrating 10 mL of the *Assay preparation* on a steam bath to 1 mL.

**Standard solution**—Prepare a solution of USP Betamethasone RS in dehydrated alcohol having a concentration of 1 mg per mL.

**Developing solvent system:** a mixture of chloroform and diethylamine (2:1).

**Spray reagent:** a mixture of sulfuric acid, methanol, and nitric acid (10:10:1).

**Procedure**—Proceed as directed in the chapter, except to spray the plate with the *Spray reagent*, and heat at 105° for 10 minutes.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Mobile phase**, **Internal standard solution**, **Standard preparation**, and **Chromatographic system**—Prepare as directed in the *Assay* under *Betamethasone*.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 2 mg of betamethasone, into a capped 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution* and 10.0 mL of alcohol. Mix by rotation for about 20 minutes. Centrifuge at 2500 rpm for about 10 minutes. Transfer a portion of the supernatant to a suitable vial.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Cream taken by the formula:

$$20C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak height ratios of the betamethasone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Oral Solution

» Betamethasone Oral Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Store at controlled room temperature, protected from light. Preserve in a tight container.

**USP Reference standards** (11)—

USP Betamethasone RS

**Identification**—**A:**

**Test solution**—Place a volume of Oral Solution equivalent to about 1 mg of betamethasone in a centrifuge tube. Add 15 mL of 0.1 N hydrochloric acid and 20 mL of ethyl acetate. Shake the tube for about 1 minute. Centrifuge to separate the phases. Transfer the upper phase (ethyl acetate) to a suitable container. Evaporate it to dryness on a steam bath under a gentle stream of nitrogen.

Allow to cool to room temperature. Dissolve the residue in about 0.5 mL of chloroform and methanol (1:1), using a vortex mixer. Transfer the solution to a 2-mL volumetric flask with small portions of a mixture of chloroform and methanol (1:1), dilute with the mixture of chloroform and methanol (1:1) to volume, and mix.

Evaporate 1 mL of the resulting solution on a steam bath just to dryness, and dissolve the residue in 0.5 mL of alcohol.

**Standard solution**—Prepare a solution of USP Betamethasone RS in alcohol having a concentration of about 1 mg betamethasone per mL.

**Developing solvent system:** a mixture of chloroform and diethylamine (2:1).

**Procedure**—Proceed as directed in the chapter. Locate the spots by lightly spraying with dilute sulfuric acid (1 in 2); heat on a hot plate or under a lamp until spots appear.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial limits** (61)—The total aerobic microbial count does not exceed 100 cfu per mL, and the total combined molds and yeasts count does not exceed 10 cfu per mL. It meets the requirements of the test for absence of *Escherichia coli*.

**pH** (791): between 2.8 and 3.6.

**Deliverable volume** (698)—For Oral Solution packaged in multiple-unit containers: meets the requirements.

**Related compounds**—[NOTE—Protect all standard and test solutions from light.]

*Diluent, Buffer, Solution A, Solution B, Mobile phase, Standard stock preparation, System suitability preparation, and Assay preparation*—Prepare as directed in the *Assay*.

**Standard solution**—Quantitatively dilute with *Diluent* an aliquot of the *Standard stock preparation*, as prepared in the *Assay*, to obtain a solution having a known concentration of about 0.48 µg per mL.

**Quantitative limit solution**—Quantitatively dilute with *Diluent* an aliquot of the *Standard solution* to obtain a solution having a known concentration of about 0.024 µg per mL.

**Test solution**—Prepare as directed for the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—Proceed as directed in the *Assay*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for betamethasone and 1.2 for beclomethasone; the resolution, *R*, between betamethasone and beclomethasone is not less than 4.0; and the relative standard deviation of the betamethasone peak for replicate injections of the *Quantitative limit solution* is not more than 10%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution*, the *Quantitative limit solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for the betamethasone related compounds listed in *Table 1* and for betamethasone. Calculate the percentage of each related

compound in the portion of Oral solution taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which *C<sub>S</sub>* is the concentration, in mg per mL, of betamethasone in the *Standard solution*; *C<sub>T</sub>* is the concentration, in mg per mL, of betamethasone in the *Test solution*, based on the label claim; *r<sub>i</sub>* is the peak area response for each related compound obtained from the *Test solution*; and *r<sub>S</sub>* is the peak area response of the betamethasone peak obtained from the *Standard solution*. The limits are as specified in *Table 1*.

**Table 1**

Related Compound	Relative Retention Time	Limit (%)
Betamethasone	1.0	—
9,11-Expoxy-17α,21-dihydroxy-16β-methylpregna-1,4 diene-3,20-dione	1.25	1.3
17α,21-Dihydroxy-16β-methylpregna-1,4,11-triene-3,20-dione	1.33	0.7

**Assay**—[NOTE—Protect all standard and assay preparations from light.]

**Diluent**—Prepare a mixture of water and dehydrated alcohol (3:2).

**Buffer**—Accurately weigh 6.8 g of monobasic potassium phosphate, and transfer to a 1-L flask. Add 1 L of water, and mix until completely dissolved. Adjust with phosphoric acid to a pH of 2.9, and mix.

**Solution A**—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (75:25).

**Solution B**—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (55:45).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock preparation**—Dissolve an accurately weighed quantity of USP Betamethasone RS in dehydrated alcohol, and sonicate until completely dissolved to obtain a solution having a known concentration of about 0.3 mg per mL. Quantitatively dilute with water an aliquot of the solution to obtain a solution having a known concentration of about 0.12 mg per mL.

**System suitability preparation**—Dissolve an accurately weighed quantity of beclomethasone in dehydrated alcohol, and sonicate until completely dissolved to obtain a solution having a concentration of about 0.3 mg per mL. Quantitatively dilute with water an aliquot to obtain a solution having a known concentration of about 0.12 mg per mL. Pipet 10.0 mL of this solution and 10.0 mL of the *Standard stock preparation* into a 25-mL volumetric flask, and dilute with *Diluent* to volume.

**Standard preparation**—Quantitatively dilute with *Diluent* an aliquot of the *Standard stock preparation* to obtain a solution having a known concentration of about 0.048 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 1.2 mg of betamethasone, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–25.0	100→0	0→100	linear gradient
25.0–25.1	0→100	100→0	linear gradient
25.1–35.0	100	0	re-equilibration

Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for betamethasone and 1.2 for beclomethasone; the resolution,  $R$ , between betamethasone and beclomethasone is not less than 4.0; the tailing factor of the betamethasone peak is not more than 1.5; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses for the betamethasone peaks. Calculate the content of betamethasone ( $C_{22}H_{29}FO_5$ ) as a percentage of the labeled content of betamethasone in the Oral Solution taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of betamethasone in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of betamethasone in the *Assay preparation*, based on the label claim; and  $r_U$  and  $r_S$  are the peak area responses of the betamethasone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Tablets

» Betamethasone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight containers. Store between 2° and 25°, excursions permitted between 15° and 30°. [NOTE—Protect the 21-tablet pack from excessive moisture.]

**USP Reference standards** (11)—  
USP Betamethasone RS

**Identification**—Evaporate 50 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Proceed as directed for *Identification test B* under *Betamethasone*, beginning with “Apply 10  $\mu$ L of this solution.”

**Dissolution**, *Procedure for a Pooled Sample* (711)—

**Medium**: water; 900 mL. Add 1.0 mL of *Internal standard solution* to each vessel.

**Apparatus 2**: 50 rpm.

**Time**: 45 minutes.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution in methanol of testosterone having a final concentration of about 0.5 mg per mL.

**Standard solution**—Prepare a solution of USP Betamethasone RS, in methanol, having an accurately known concentration of about 0.5 mg per mL. Pipet 1 mL of this solution and 1 mL of the *Internal standard solution* into a container, and quantitatively dilute with water to 900 mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between betamethasone and testosterone is not less than 1.5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 200  $\mu$ L) of the *Standard solution* and filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for betamethasone and 1.0 for testosterone. Calculate the quantity of  $C_{22}H_{29}FO_5$  dissolved in comparison with the *Standard solution*, similarly chromatographed.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{22}H_{29}FO_5$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—

**Standard preparation**—Prepare as directed under *Assay for Steroids* (351), using USP Betamethasone RS, to obtain a solution having a known concentration of about 12  $\mu$ g per mL instead of 10  $\mu$ g per mL.

**Test preparation**—Weigh and finely powder 1 Tablet. Transfer to a 125-mL separator, add 20 mL of water, and shake. Extract the betamethasone completely, using three 15-mL portions of chloroform, filtering each extract through chloroform-washed cotton into a 50-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol.

**Procedure**—Proceed as directed under *Assay for Steroids* (351), except to keep the flasks in a constant-temperature bath at  $45 \pm 1^\circ$  for 90 minutes, then add 1.0 mL of glacial acetic acid, and cool. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the Tablet by the formula:

$$(TC/D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of betamethasone in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Betamethasone RS in the *Standard preparation*;  $D$  is the concentration, in  $\mu$ g per mL, of betamethasone in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 25 mg of beclomethasone to a 200-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone RS in methanol, and dilute quantitatively and stepwise, if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Mix equal volumes, accurately measured, of this solution and the *Internal standard solution* to obtain a *Standard preparation* having a final known concentration of about 0.05 mg of USP Betamethasone RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.5 mg of betamethasone, to a 125-mL separator. Add 25 mL of water, and shake by mechanical means for about 15 minutes. Add 5.0 mL of *Internal standard solution*. Extract with four 25-mL portions of

chloroform. Filter the chloroform extracts through about 4 g of chloroform-washed anhydrous sodium sulfate, collecting the extracts in a 150-mL beaker. Evaporate the extracts on a steam bath with the aid of a stream of nitrogen to dryness, taking care to avoid overheating. Dissolve the residue in 2 mL of methanol, and transfer to a 10-mL volumetric flask. Rinse the beaker with small portions of methanol, transferring the rinses to the same flask. Dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak heights as directed for *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the heights of the major peaks. The relative retention times are about 1.4 for beclomethasone and 1.0 for betamethasone. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Tablets taken by the formula:

$$10C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Acetate

$C_{24}H_{31}FO_6$  434.50

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(acetyloxy)-, (11 $\beta$ ,16 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 21-acetate [987-24-6].

» Betamethasone Acetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{24}H_{31}FO_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 30°.

**USP Reference standards** <11>—

USP Betamethasone Acetate RS

**Identification**—

**A:** Infrared Absorption <197M>.

**B:** Thin-Layer Chromatographic Identification Test <201>—

*Test solution:* 0.5 mg per mL in dehydrated alcohol.

*Developing solvent system:* a mixture of chloroform and diethylamine (2:1).

**Procedure**—Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with 10% sulfuric acid in alcohol and heating on a hot plate or under a lamp until spots appear.

**Specific rotation** <781S>: between +120° and +128°.

*Test solution:* 10 mg per mL, in dioxane.

**Water, Method I** <921>: not more than 4.0%.

**Residue on ignition** <281>: not more than 0.2%, a platinum crucible being used.

**Ordinary impurities** <466>—

*Test solution:* methanol.

*Standard solution:* methanol.

*Application volume:* 10  $\mu$ L.

*Eluant:* a mixture of toluene and isopropyl alcohol (90:10), in a nonequilibrated chamber.

*Visualization:* 5.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (800:700:1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Transfer about 35 mg of progesterone to a 50-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.5 mg per mL. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Betamethasone Acetate RS per mL.

**Assay preparation**—Transfer about 50 mg of Betamethasone Acetate, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 3 for progesterone and 1.0 for betamethasone acetate; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{31}FO_6$  in the portion of Betamethasone Acetate taken by the formula:

$$500C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Acetate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Benzoate

$C_{29}H_{33}FO_6$  496.57

Pregna-1,4-diene-3,20-dione, 17-(benzoyloxy)-9-fluoro-11,21-dihydroxy-16-methyl-, (11 $\beta$ ,16 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17-benzoate [22298-29-9].

» Betamethasone Benzoate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{29}H_{33}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 30°.

**USP Reference standards** <11>—

USP Betamethasone Benzoate RS



**Identification, Infrared Absorption** (197M).

**Specific rotation** (781S): between +60° and +66°.

*Test solution:* 40 mg per mL, in dioxane.

**Loss on drying** (731)—Dry about 200 mg, accurately weighed, at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Related steroids—**

*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture.

*Test solution—*Dissolve 100.0 mg in 5.0 mL of methanol.

*Standard solution 1—*Dissolve a suitable quantity of USP Betamethasone Benzoate RS in methanol to obtain a solution having a known concentration of about 5 mg per mL.

*Standard solution 2—*Dilute a portion of *Standard solution 1*, quantitatively and stepwise, with methanol to obtain a solution having a known concentration of about 100 µg per mL.

*Application volume:* 10 µL.

*Developing solvent system:* a mixture of toluene, acetone, and methanol (75:25:4).

*Procedure—*Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate under short-wavelength UV light: the principal spot from the *Test solution* corresponds in  $R_f$  value to that of *Standard solution 1*; and the *Test solution* shows not more than 3 additional spots, the intensity and size of which do not exceed those of the spot from *Standard solution 2*.

**Assay—**

*Mobile phase—*Prepare a suitable filtered solution of acetonitrile and water (60:40).

*Internal standard solution—*Prepare a solution of betamethasone dipropionate in methanol containing 0.6 mg per mL.

*Standard preparation—*Using an accurately weighed quantity of USP Betamethasone Benzoate RS, prepare a solution in methanol having a known concentration of about 0.6 mg per mL. Mix 5.0 mL of this solution and 10.0 mL of the *Internal standard solution* to obtain a *Standard preparation* having a known concentration of about 0.2 mg of betamethasone benzoate per mL.

*Assay preparation—*Transfer about 60 mg of Betamethasone Benzoate, accurately weighed, to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. Mix 5.0 mL of this solution and 10.0 mL of the *Internal standard solution*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%; and the resolution factor between betamethasone benzoate and the internal standard is not less than 3.

*Procedure—*Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention times are about 7 and 5 minutes for betamethasone dipropionate and betamethasone benzoate, respectively. Calculate the quantity, in mg, of  $C_{29}H_{33}FO_6$  in the portion of Betamethasone Benzoate taken by the formula:

$$300C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Benzoate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the betamethasone benzoate peak and the internal standard

peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Benzoate Gel

» Betamethasone Benzoate Gel contains an amount of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ).

**Packaging and storage—**Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.

**USP Reference standards** (11)—

USP Betamethasone Benzoate RS

USP Methyltestosterone RS

**Identification—**The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay—**

*Mobile phase—*Prepare a filtered and degassed mixture of methanol, water, and acetonitrile (23:18:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution—*Dissolve suitable quantities of USP Methyltestosterone RS in methanol to obtain a solution containing about 250 µg per mL.

*Standard preparation—*Dissolve an accurately weighed quantity of USP Betamethasone Benzoate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

*Assay preparation—*Transfer an accurately weighed portion of Gel, equivalent to about 0.5 mg of betamethasone benzoate, to a 125-mL separatory funnel, add 20 mL of water and 2 mL of saturated sodium acetate solution, shake to disperse the Gel, add 2.0 mL of *Internal standard solution*, and mix. Extract this solution with one 50-mL portion of chloroform followed by three 40-mL portions of chloroform. Discard the aqueous layer. Wash the chloroform extract with 10 mL of water, allow to stand for 10 minutes, then pass through chloroform-wetted glass fiber filter paper and anhydrous sodium sulfate into a suitable container. Evaporate to dryness under vacuum at 30°. Dissolve the residue in 10 mL of methanol.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 236-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.33 for betamethasone benzoate and 1.0 for methyltestosterone; the resolution,  $R$ , between methyltestosterone and betamethasone benzoate is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.0%.

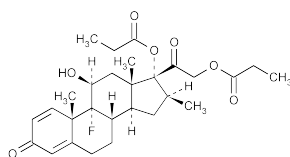
*Procedure—*Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ) in the portion of Gel taken by the formula:

$$0.01C(R_U / R_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Betamethasone Benzoate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained for betamethasone benzoate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate



$C_{28}H_{37}FO_7$  504.59

Pregna-1,4-diene-3,20-dione, 9-fluoro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11 $\beta$ ,16 $\beta$ ).  
9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4].

» Betamethasone Dipropionate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{28}H_{37}FO_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

USP Betamethasone Valerate RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution:* 1 mg per mL, in chloroform.

*Developing solvent system:* a mixture of chloroform and acetone (7:1).

**Specific rotation** (781S): between +63° and +70°.

*Test solution:* 10 mg per mL, in dioxane.

**Loss on drying** (731): Dry it at 105° for 3 hours; it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.

### Chromatographic purity—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve accurately weighed quantities of USP Betamethasone Dipropionate RS and USP Betamethasone Valerate RS in *Mobile phase* to obtain a solution having final concentrations of about 0.05 mg of each per mL.

*Test solution*—Transfer about 3 mg of Betamethasone Dipropionate, accurately weighed, to a suitable flask. Add 10 mL of *Mobile phase*, and shake until dissolved.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the

*System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between betamethasone valerate and betamethasone dipropionate is not less than 4.0; and the column efficiency is not less than 8000 theoretical plates.

*Procedure*—Inject a volume (about 10  $\mu\text{L}$ ) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Betamethasone Dipropionate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses for all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

### Assay—

*Mobile phase*—Prepare a suitable acetonitrile solution (about 1 in 2), degassed by ultrasonic vibration for 5 to 10 minutes, such that the retention time of betamethasone dipropionate is approximately 14 minutes and that of beclomethasone dipropionate is approximately 18 minutes. [NOTE—Do not leave the mobile phase in the column overnight, but flush the system after use with water for 15 minutes, followed by methanol for 15 minutes.]

*Internal standard solution*—Prepare a solution of USP Beclomethasone Dipropionate RS in a solution of acetic acid in methanol (1 in 1000) having a known concentration of about 0.9 mg per mL.

*Standard preparation*—Prepare a solution of USP Betamethasone Dipropionate RS in a solution of acetic acid in methanol (1 in 1000) having a known concentration of about 0.6 mg per mL. Transfer 5.0 mL of this solution to a suitable vial, and add 5.0 mL of *Internal standard solution* to obtain a solution having known concentrations of about 0.3 mg of betamethasone dipropionate and about 0.45 mg of beclomethasone dipropionate per mL.

*Assay preparation*—Accurately weigh about 60 mg of Betamethasone Dipropionate. Dilute quantitatively and stepwise with a solution of acetic acid in methanol (1 in 1000) to obtain a solution containing about 0.6 mg per mL. Transfer 5.0 mL of this solution to a suitable vial, and add 5.0 mL of *Internal standard solution*.

*Procedure*—Separately inject equal volumes (between 5  $\mu\text{L}$  and 25  $\mu\text{L}$ ) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the internal standard in the *Standard preparation* is about 0.6 full-scale. Typically, the apparatus is fitted with a 4-mm  $\times$  30-cm column that contains packing L1, and is equipped with a UV detector capable of monitoring absorption at 254 nm or 240 nm and a suitable recorder, and is capable of operating at a column pressure of up to 3500 psi. In a suitable chromatogram, the lowest and highest peak area ratios ( $R_s$ ) of three successive injections of the *Standard preparation* agree within 2.0%. Determine the ratio of the peak heights, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of  $C_{28}H_{37}FO_7$  in the portion of Betamethasone Dipropionate taken by the formula:

$$200C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of betamethasone dipropionate to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate Topical Aerosol

» Betamethasone Dipropionate Topical Aerosol is a solution, in suitable propellants in a pressurized container, of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight, pressurized containers, and avoid exposure to excessive heat. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

### Thin-layer chromatographic identification test (201)—

**Test solution**—Place the container in a dry ice-methanol bath for about 5 minutes. Open the can by means of a tube-cutter, and allow the propellant to evaporate under a gentle stream of nitrogen for about 1 hour. Transfer about 3 mL of the residue to a 50-mL centrifuge tube. Add 10 mL of a mixture of methanol and water (4:1), and shake vigorously. Centrifuge to clarify.

**Standard solution**: USP Betamethasone Dipropionate RS in methanol containing 3.2 mg per mL.

**Application volume**: 25  $\mu$ L.

**Developing solvent system**: a mixture of toluene and ethyl acetate (1:1).

**Procedure**—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 minutes.

**Other requirements**—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

### Assay—

**Mobile phase**—Prepare as directed for *Mobile phase* in the *Assay* under *Betamethasone Dipropionate*.

**Internal standard solution**—Prepare a solution of USP Beclomethasone Dipropionate RS, having a known concentration of about 0.90 mg per mL, in isopropyl alcohol containing glacial acetic acid (1 in 1000).

**Standard preparation**—Prepare a solution of USP Betamethasone Dipropionate RS, having a known concentration of about 0.642 mg per mL, in isopropyl alcohol containing acetic acid (1 in 1000). Transfer 10.0 mL of this solution and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask, add isopropyl alcohol containing acetic acid (1 in 1000) to volume, and mix, to obtain a solution having known concentrations of about 0.09 mg of beclomethasone dipropionate and about 0.0642 mg of betamethasone dipropionate per mL.

**Assay preparation**—Discharge the entire contents of the container of Topical Aerosol into a 100-mL volumetric flask. Allow the solution to warm to room temperature slowly to prevent it from boiling out of the flask, then evaporate the propellant by swirling the flask in a water bath at about 25° until the solution stops bubbling. Add 10.0 mL of *Internal standard solution*, and dilute with glacial acetic acid in isopropyl alcohol (1 in 1000) to volume. Pass the solution through a 0.45- $\mu$ m filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Dipropionate*. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) equivalent to the quan-

tity of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) in the container of the Topical Aerosol taken by the formula:

$$(392.46/504.60)(100C)(R_U / R_S)$$

in which 392.46 and 504.60 are the molecular weights of betamethasone and betamethasone dipropionate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of the betamethasone dipropionate and beclomethasone dipropionate peaks in the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate Cream

» Betamethasone Dipropionate Cream contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.

### USP Reference standards (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

### Thin-layer chromatographic identification test (201)—

**Test solution**—Transfer about 1.5 g of Cream to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of a methanol-hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 mL of water, and mix. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150  $\mu$ g of betamethasone dipropionate per mL.

**Standard solution**: USP Betamethasone Dipropionate RS in chloroform containing 150  $\mu$ g per mL.

**Application volume**: 40  $\mu$ L.

**Developing solvent system**: a mixture of chloroform and acetone (7:1).

**Procedure**—Proceed as directed in the chapter.

**Minimum fill** (755): meets the requirements.

### Assay—

**Mobile phase**—Prepare as directed in the *Assay* under *Betamethasone Dipropionate*.

**Internal standard solution**—Prepare a solution of USP Beclomethasone Dipropionate RS in methanol containing acetic acid (1 in 1000) having a known concentration of about 0.45 mg per mL.

**Standard preparation**—Prepare a solution of USP Betamethasone Dipropionate RS in methanol containing acetic acid (1 in 1000) having a known concentration of about 0.2 mg per mL. Transfer 10.0 mL of this solution to a suitable vial, and add 5.0 mL of *Internal standard solution*, to obtain a *Standard preparation* having known concentrations

of about 0.133 mg of betamethasone dipropionate and about 0.15 mg of beclomethasone dipropionate per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 2 mg of betamethasone dipropionate, into a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of methanol containing acetic acid (1 in 1000). Heat in a water bath at 60°, shaking intermittently, until the assay specimen melts. Remove from the bath, and shake vigorously until the specimen has resolidified. Repeat the heating and shaking. Freeze in an ice-methanol bath for about 15 minutes, and centrifuge at 2500 rpm for about 5 minutes. Transfer a portion of the supernatant to a suitable vial.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Dipropionate*. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Cream taken by the formula:

$$(392.46/504.60)(15C)(R_U / R_S)$$

in which 392.46 and 504.60 are the molecular weights of betamethasone and betamethasone dipropionate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of the betamethasone dipropionate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate Lotion

» Betamethasone Dipropionate Lotion contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable lotion base.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from light and freezing.

**USP Reference standards** (11)—  
USP Beclomethasone Dipropionate RS  
USP Betamethasone Dipropionate RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Transfer a quantity of Lotion, equivalent to about 0.6 mg of betamethasone dipropionate, to a 50-mL vial. Add 10 mL of 0.1 N hydrochloric acid, then add 4 mL of chloroform. Disperse on a vortex mixer for about 1 minute, then shake vigorously for 10 minutes, and centrifuge at 2000 rpm for about 5 minutes. Transfer the chloroform layer to a suitable vial.

**Standard solution**: USP Betamethasone Dipropionate RS in chloroform containing 150 µg per mL.

**Application volume**: 40 µL.

**Developing solvent system**: a mixture of chloroform and acetone (7:1).

**Procedure**—Proceed as directed in the chapter.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay* under *Betamethasone Dipropionate*.

**Internal standard solution**—Prepare as directed in the *Assay* under *Betamethasone Dipropionate*, except to use chloroform as the solvent.

**Standard preparation**—Prepare as directed in the *Assay* under *Betamethasone Dipropionate*, except to use chloroform as the solvent. To 10.0 mL of 0.1 N hydrochloric acid in a capped 5-mL centrifuge tube add 4.0 mL of the prepared solution, and treat this solution as follows. Cap, and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 mL of methanol, and swirl to dissolve the residue.

**Assay preparation**—Transfer an accurately weighed quantity of Lotion, equivalent to about 1.2 mg of betamethasone dipropionate, to a capped 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, shake to disperse, then add 2.0 mL of *Internal standard solution* and 2.0 mL of chloroform. Proceed as directed for *Standard preparation* beginning with "Cap, and shake."

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Dipropionate*. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Lotion taken by the formula:

$$(392.46/504.60)(4C)(R_U / R_S)$$

in which 392.46 and 504.60 are the molecular weights of betamethasone and betamethasone dipropionate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of the betamethasone dipropionate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate Ointment

» Betamethasone Dipropionate Ointment contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.

**USP Reference standards** (11)—  
USP Beclomethasone Dipropionate RS  
USP Betamethasone Dipropionate RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Transfer about 1.5 g of Ointment to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of methanol-hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 mL of water, and mix. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150 µg of betamethasone dipropionate per mL.

**Standard solution**: USP Betamethasone Dipropionate RS in chloroform containing 150 µg per mL.

*Application volume:* 40  $\mu$ L.

*Developing solvent system:* a mixture of chloroform and acetone (7:1).

*Procedure*—Proceed as directed in the chapter.

**Minimum fill** (755): meets the requirements.

#### Assay—

*Mobile phase*—Prepare as directed in the Assay under *Betamethasone Dipropionate*.

*Internal standard solution and Standard preparation*—Prepare as directed in the Assay under *Betamethasone Dipropionate Cream*, except to use alcohol containing acetic acid (1 in 1000) as the solvent.

*Assay preparation*—Transfer an accurately weighed quantity of Ointment, equivalent to about 2 mg of betamethasone dipropionate, to a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of alcohol containing acetic acid (1 in 1000). Heat in a water bath at 70°, shaking intermittently until the assay specimen melts. Remove from the bath, and shake vigorously until the ointment has solidified. Repeat the heating and shaking operation. Proceed as directed for *Assay preparation* in the Assay under *Betamethasone Dipropionate Cream*, beginning with "Freeze in an ice-methanol bath."

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Betamethasone Dipropionate Cream*.

### Betamethasone Sodium Phosphate

$C_{22}H_{28}FNa_2O_8P$  516.40

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonoxy)-, disodium salt, (11 $\beta$ ,16 $\beta$ )-9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 21-(disodium phosphate) [151-73-5].

» Betamethasone Sodium Phosphate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{22}H_{28}FNa_2O_8P$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Betamethasone Sodium Phosphate RS

#### Identification—

**A:** *Infrared Absorption* (197M).

**B:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution:* 1 mg per mL.

*Standard solution*—Prepare a solution of USP Betamethasone Sodium Phosphate RS in methanol having a concentration of 1 mg per mL.

*Developing solvent system*—Place 500 mL of butyl alcohol and 200 mL of dilute hydrochloric acid (1 in 12) in a separatory funnel, and mix. Use the organic layer as the developing solvent.

*Spray reagent:* a mixture of sulfuric acid, methanol, and nitric acid (10:10:1).

*Procedure*—Proceed as directed in the chapter except to spray the plate with *Spray reagent*, and heat at 105° for 10 minutes.

**C:** Ignite it at 800° (see *Residue on Ignition* (281)): the residue responds to the tests for *Sodium* (191) and for *Phosphate* (191).

**Specific rotation** (781S): between +99° and +105°.

*Test solution:* 10 mg per mL, in water.

**Water, Method I** (921): not more than 10.0%.

#### Limit of phosphate ions—

*Standard Phosphate Solution and Phosphate reagent A*—Prepare as directed under *Phosphate in reagents* (see *General Tests for Reagents under Reagents, Indicators, and Solutions*).

*Phosphate reagent B*—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium metabisulfite, mix to dissolve, and dilute with water to 100 mL.

*Procedure*—Dissolve about 50 mg of Betamethasone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25.0 mL, mix, and allow to stand at room temperature for 30 minutes. Similarly and concomitantly prepare a Standard solution, using 5.0 mL of *Standard Phosphate Solution* instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the Standard solution. The limit is 1.0% of phosphate ( $PO_4$ ).

#### Limit of free betamethasone—

*Test solution*—Dissolve 25.0 mg of Betamethasone Sodium Phosphate in water to make 25.0 mL. Transfer 5.0 mL of the solution to a separator, and extract with three 25-mL portions of chloroform. Filter each extract through a chloroform-saturated cotton pledget, combining the filtrates in a conical flask. Evaporate the chloroform on a steam bath with the aid of a current of air to dryness, and dissolve the residue in methanol to make 25.0 mL.

*Blank solution*—Transfer 5.0 mL of water to a separator, and proceed as directed under *Test solution*. The methanolic solution so obtained is the *Blank solution*.

*Procedure*—Determine the absorbance (*A*) of the *Test solution* in a 1-cm cell at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using *Blank solution* as the blank. Calculate the quantity, in mg, of free betamethasone in the portion of Betamethasone Sodium Phosphate taken by the formula:

$$3.125A.$$

The limit is 250  $\mu$ g (1.0%).

#### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and 0.07 M anhydrous monobasic potassium phosphate (3:2). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Betamethasone Sodium Phosphate RS in a mixture of methanol and water (3:2), and dilute quantitatively, and stepwise if necessary, with the same mixture of methanol and water to obtain a solution having a known concentration of about 0.17 mg per mL.

*Assay preparation*—Transfer about 34 mg of Betamethasone Sodium Phosphate, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with a mixture of methanol and water (3:2) to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{28}FNa_2O_8P$  in the portion of Betamethasone Sodium Phosphate taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Sodium Phosphate Injection

» Betamethasone Sodium Phosphate Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injection. It contains an amount of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Betamethasone Sodium Phosphate RS  
USP Endotoxin RS

**Identification**—Dilute the Injection with methanol, if necessary, to obtain a solution containing about 2 mg of betamethasone sodium phosphate per mL. Separately apply 10  $\mu$ L of this test solution and 10  $\mu$ L of a solution of USP Betamethasone Sodium Phosphate RS in methanol containing 2 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with chromatographic silica gel mixture. Develop the chromatogram in an equilibrated chamber containing *n*-butyl alcohol previously shaken with 1 N hydrochloric acid, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, then spray with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1). Heat the plate at 105° for 10 minutes: the  $R_f$  value of the principal spot from the test solution corresponds to that obtained from the Standard solution.

**Bacterial endotoxins** (85)—It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.

**pH** (791): between 8.0 and 9.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 0.05 M monobasic potassium phosphate (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 100 mg of butylparaben to a 100-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Using an accurately weighed quantity of USP Betamethasone Sodium Phosphate RS, prepare a solution in water containing 4 mg per mL. Transfer 3.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of

**Internal standard solution**, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Betamethasone Sodium Phosphate RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 9 mg of betamethasone, to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.4 for butylparaben and 1.0 for betamethasone sodium phosphate. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Injection taken by the formula:

$$(392.47 / 516.41)(25C / V)(r_U / r_S)$$

in which 392.47 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension

» Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension is a sterile preparation of Betamethasone Sodium Phosphate in solution and Betamethasone Acetate in suspension in Water for Injection. It contains an amount of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone acetate ( $C_{24}H_{31}FO_6$ ).

**Packaging and storage**—Preserve in multiple-dose containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Betamethasone Acetate RS  
USP Betamethasone Sodium Phosphate RS  
USP Endotoxin RS

### Identification—

**A: Thin-layer chromatographic identification test** (201)—

**Test solution**—Dilute 2 mL with 2 mL of methanol.

**Standard solution**—Prepare a solution of USP Betamethasone Sodium Phosphate RS in a mixture of methanol and water (1:1) having a concentration of 2 mg per mL.

*Developing solvent system, Spray reagent, and Procedure*—Proceed as directed for *Identification test B* under *Betamethasone sodium phosphate*.

**B:** *Test solution*—Use the *Test solution* prepared for *Identification test A*.

*Standard solution*—Prepare a solution of USP Betamethasone Acetate RS in a mixture of methanol and water (1:1) having a concentration of 1.5 mg per mL.

*Developing solvent system and Procedure*—Proceed as directed for *Identification test B* under *Betamethasone*.

**Bacterial endotoxins** (85)—It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.

**pH** (791): between 6.8 and 7.2.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and 0.075 M monobasic potassium phosphate (7:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Transfer about 50 mg of methyltestosterone to a 50-mL volumetric flask, add methanol to volume, and mix.

*Standard preparation*—Transfer about 63 mg of USP Betamethasone Sodium Phosphate RS, accurately weighed, to a 25-mL volumetric flask, add *Mobile phase* to volume, and mix (*Solution 1*). Transfer about 45 mg of USP Betamethasone Acetate RS, accurately weighed, to a 25-mL volumetric flask, add methanol to volume, and mix (*Solution 2*). Pipet 5 mL each of *Solution 1* and *Solution 2* into a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 126 µg of USP Betamethasone Sodium Phosphate RS per mL and 90 µg of USP Betamethasone Acetate RS per mL.

*Assay preparation*—Using a “To contain” pipet transfer an accurately measured volume of the well-mixed Injectable Suspension, equivalent to about 9 mg of betamethasone acetate, to a 100-mL volumetric flask. Rinse the pipet with about 10 mL of *Mobile phase*, collecting the rinse in the volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R_s$ , between the betamethasone phosphate and betamethasone acetate peaks is not less than 5.0, and the resolution,  $R_s$ , between the betamethasone acetate and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for betamethasone phosphate, 1.7 for methyltestosterone, and 1.0 for betamethasone acetate. Calculate the quantity, in mg, of betamethasone acetate ( $C_{24}H_{31}FO_6$ ) in each mL of the Injectable Suspension taken by the formula:

$$0.1 C / V(R_U / R_S)$$

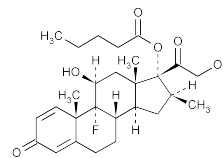
in which  $C$  is the concentration, in µg per mL, of USP Betamethasone Acetate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injectable Suspension taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for betamethasone acetate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) equivalent to the quantity of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ), in each mL of the Injectable Suspension taken by the formula:

$$(392.46/516.41)(0.1 C/V)(R_U / R_S)$$

in which 392.46 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively;  $C$  is the concentration, in µg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injectable Suspension taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for betamethasone phosphate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate



$C_{27}H_{37}FO_6$  476.58

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11β,16β)-

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-valerate [2152-44-5].

» Betamethasone Valerate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{37}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Valerate RS

#### Identification—

**A:** *Infrared Absorption* (197M).

**B:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution*: 1 mg per mL, in alcohol.

*Developing solvent system*: a mixture of toluene and ethyl acetate (1:1).

*Procedure*—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 minutes.

**Specific rotation** (781S): between +75° and +82°.

*Test solution*: 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.

#### Chromatographic purity—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (550:450:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Test solution*—Transfer about 4 mg of Betamethasone Valerate, accurately weighed, to a suitable flask. Add 10 mL of *Mobile phase*, and shake until dissolved.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the

*Test solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between betamethasone valerate and any impurity is not less than 1.5; and the column efficiency is not less than 9000 theoretical plates.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Betamethasone Valerate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of all the peak responses: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

#### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Transfer about 40 mg of beclomethasone dipropionate to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix.

*Standard preparation*—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

*Assay preparation*—Transfer about 60 mg of Betamethasone Valerate, accurately weighed, to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution,  $R$ , between betamethasone valerate and beclomethasone dipropionate is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{27}H_{37}FO_6$  in the portion of Betamethasone Valerate taken by the formula:

$$300C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate Cream

» Betamethasone Valerate Cream contains an amount of betamethasone valerate ( $C_{27}H_{37}FO_6$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount

of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or in tight containers.

#### USP Reference standards (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Valerate RS

**Identification**—Transfer an amount of Cream, equivalent to about 2 mg of betamethasone, to a separator, add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120), and mix. Extract with four 50-mL portions of chloroform, and combine the extracts. Filter through a cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing about 1 mg per mL. Proceed as directed in *Identification test B* under *Betamethasone Valerate*, beginning with "Apply 10  $\mu$ L of this solution."

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill (755):** meets the requirements.

#### Assay—

*Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Betamethasone Valerate*.

*Assay preparation*—Transfer an accurately weighed portion of Cream, equivalent to about 2.5 mg of betamethasone, to a 50-mL centrifuge tube. Add 10.0 mL of the *Internal standard solution* and 5.0 mL of a 1 in 1000 solution of glacial acetic acid in methanol. Insert the stopper into the tube, and place in a water bath held at 60° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 minutes, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Valerate*. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Cream taken by the formula:

$$(392.46 / 476.59)(15C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively;  $C$  is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate Lotion

» Betamethasone Valerate Lotion contains an amount of Betamethasone Valerate ( $C_{27}H_{37}FO_6$ ) equivalent to not less than 95.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Betamethasone Valerate RS

**Identification**—Mix an amount of Lotion, equivalent to about 5 mg of betamethasone, with a mixture of methanol and chloroform (2:1) to make 10 mL. Apply 20  $\mu$ L of this



solution and 20  $\mu\text{L}$  of a Standard solution of USP Betamethasone Valerate RS in a mixture of methanol and chloroform (2:1) containing 0.6 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and ethyl acetate (1:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. View the spots under UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

**pH** <791>: between 4.0 and 6.0.

#### Assay—

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay under *Betamethasone Valerate*.

*Internal standard solution*—Transfer about 50 mg of beclomethasone dipropionate to a 25-mL volumetric flask, add chloroform to volume, and mix.

*Standard preparation*—Transfer about 40 mg of USP Betamethasone Valerate RS, accurately weighed, to a 25-mL volumetric flask, add chloroform to volume, and mix. Pipet 2 mL of this solution into a 50-mL centrifuge tube, add 10 mL of 0.1 N hydrochloric acid, then add 2.0 mL of *Internal standard solution*. Insert the stopper into the tube, shake vigorously for about 2 minutes, and centrifuge to separate the phases. Using a syringe, transfer the lower, chloroform phase to a small stoppered vial. Evaporate the chloroform on a steam bath, at low heat, with the aid of a stream of nitrogen. Add 4.0 mL of a 1 in 1000 solution of glacial acetic acid in methanol, and swirl to dissolve the residue.

*Assay preparation*—Transfer an accurately weighed portion of Lotion, equivalent to about 2.5 mg of betamethasone, to a stoppered, 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake to disperse the specimen. Add 2.0 mL of chloroform and 2.0 mL of *Internal standard solution*, insert the stopper, and proceed as directed for *Standard preparation*, beginning with “shake vigorously for about 2 minutes.”

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Betamethasone Valerate*. Calculate the quantity, in mg, of betamethasone ( $\text{C}_{22}\text{H}_{29}\text{FO}_5$ ) in the portion of Lotion taken by the formula:

$$(392.46 / 476.59)(4C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate Ointment

» Betamethasone Valerate Ointment contains an amount of betamethasone valerate ( $\text{C}_{27}\text{H}_{37}\text{FO}_6$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $\text{C}_{22}\text{H}_{29}\text{FO}_5$ ), in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or in tight containers, and avoid exposure to excessive heat.

#### USP Reference standards <11>—

USP Betamethasone Valerate RS

**Identification**—It responds to the *Identification* test under *Betamethasone Valerate Cream*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

#### Assay—

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay under *Betamethasone Valerate*.

*Internal standard solution*—Transfer about 20 mg of beclomethasone dipropionate to a 50-mL volumetric flask, add a 1 in 1000 solution of glacial acetic acid in alcohol to volume, and mix.

*Standard preparation*—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a 1 in 1000 solution of glacial acetic acid in alcohol to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

*Assay preparation*—Transfer an accurately weighed portion of Ointment, equivalent to about 2.5 mg of betamethasone, to a 50-mL centrifuge tube. Add 10.0 mL of the *Internal standard solution* and 5.0 mL of a 1-in-1000 solution of glacial acetic acid in alcohol. Insert the stopper into the tube, and place in a water bath held at 70° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 minutes, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.

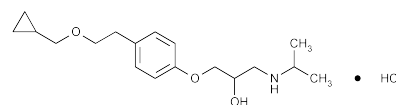
*Procedure*—Proceed as directed for *Procedure* in the Assay under *Betamethasone Valerate*. Calculate the quantity, in mg, of  $\text{C}_{22}\text{H}_{29}\text{FO}_5$  in the portion of Ointment taken by the formula:

$$(392.46 / 476.59)(15C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betaxolol Hydrochloride

#### Change to read:



$\text{C}_{18}\text{H}_{29}\text{NO}_3 \cdot \text{HCl}$  343.89  
 ▲2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride, ( $\pm$ ); ▲*USP36*  
 ( $\pm$ )-1-[p-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride [63659-19-8].

**DEFINITION****Change to read:**

Betaxolol Hydrochloride contains  $\Delta$ NLT 98.0% and NMT 102.0% $\Delta$ <sup>USP36</sup> of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** <191>  
**Analysis:** Proceed as directed for alkaloidal hydrochlorides.  
**Acceptance criteria:** Meets the requirements

**ASSAY****Change to read:**• **PROCEDURE**

$\Delta$ **Buffer:** 0.025 M monobasic potassium phosphate containing 0.1% (w/v) of tetrabutyl ammonium bromide. Adjust with 0.025 M phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and Buffer (15:85)

**System suitability solution:** 2.0 mg/mL of USP Betaxolol Hydrochloride RS and 1.0 mg/mL of alprenolol hydrochloride in *Mobile phase*

**Standard solution:** 2.0 mg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*

**Sample solution:** 2.0 mg/mL of Betaxolol Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for alprenolol and betaxolol are 0.9 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between alprenolol and betaxolol

**Tailing factor:** NMT 2.0 for the alprenolol and betaxolol peaks

**Relative standard deviation:** NMT 1.0% for the betaxolol peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Betaxolol Hydrochloride in the *Sample solution* (mg/mL) $\Delta$ <sup>USP36</sup>

**Acceptance criteria:**  $\Delta$ 98.0%–102.0% $\Delta$ <sup>USP36</sup> on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS, Method II** <231>: NMT 20 ppm

**Change to read:**• **ORGANIC IMPURITIES**

$\Delta$ **Buffer, Mobile phase, System suitability solution, Sample solution, and System suitability:** Proceed as directed in the *Assay*. $\Delta$ <sup>USP36</sup>

**Chromatographic system:**  $\Delta$ Proceed as directed in the *Assay*, except to use a run time of NLT 5 times the retention time of betaxolol. $\Delta$ <sup>USP36</sup>

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual peak other than the main betaxolol peak

$r_T$  = sum of all the peak responses

**Acceptance criteria:** NMT 1.0% for the sum of all impurities

**SPECIFIC TESTS****Delete the following:**

- $\Delta$ • **MELTING RANGE OR TEMPERATURE, Class I** <741>:

113°–117° $\Delta$ <sup>USP36</sup>

- **pH** <791>

**Sample solution:** 20 mg/mL

**Acceptance criteria:** 4.5–6.5

- **LOSS ON DRYING** <731>

**Analysis:** Dry under vacuum at 65° for 2 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS****Change to read:**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

$\Delta$ Store at room temperature. $\Delta$ <sup>USP36</sup>

- **USP REFERENCE STANDARDS** <11>

USP Betaxolol Hydrochloride RS

**Betaxolol Ophthalmic Solution**

» Betaxolol Ophthalmic Solution is a sterile, aqueous, isotonic solution of Betaxolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betaxolol ( $C_{18}H_{29}NO_3$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Betaxolol Hydrochloride RS

**Identification**—Prepare a test solution by diluting a suitable volume of it with water to obtain a solution containing about 2.5 mg of betaxolol per mL. Separately apply 5  $\mu$ L of the test solution and 5  $\mu$ L of a Standard solution of USP Betaxolol Hydrochloride RS in water containing about 2.75 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm

layer of silica gel. Allow the spots to dry, and develop the chromatogram in a chromatographic chamber, using a solvent system consisting of a mixture of chloroform, isopropyl alcohol, and ammonium hydroxide (70:30:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Spray the plate with a 1 in 1000 solution of ninhydrin in isopropyl alcohol, and heat the plate at 105° for 10 minutes. Locate the spots on the plate: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 8.0.

#### Assay—

**pH 3.0 Buffer**—Dissolve 7.1 g of anhydrous dibasic sodium phosphate in about 800 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to make 1000 mL of solution.

**Mobile phase**—Prepare a suitable filtered and degassed mixture of pH 3.0 Buffer and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betaxolol Hydrochloride RS in pH 3.0 Buffer to obtain a solution having a known concentration of about 0.11 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of betaxolol, to a 100-mL volumetric flask. Dilute with pH 3.0 Buffer to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the main betaxolol peak is between 1 and 3; the tailing factor is not less than 0.8 and not more than 2.0; the column efficiency is not less than 750 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{29}NO_3$  in each mL of the Ophthalmic Solution taken by the formula:

$$(307.43 / 343.89)(100C / V)(r_U / r_S)$$

in which 307.43 and 343.89 are the molecular weights of betaxolol and betaxolol hydrochloride, respectively;  $C$  is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the betaxolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betaxolol Tablets

» Betaxolol Tablets contain an amount of Betaxolol Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Tablets to state both the content of the betaxolol active moiety and the content of betaxolol hydrochloride used in formulating them.

#### USP Reference standards (11)—

USP Betaxolol Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

**Medium**: 0.01 N hydrochloric acid; 500 mL.

**Apparatus 2**: 50 rpm.

**Time**: 30 minutes.

**Procedure**—Determine the amount of  $C_{18}H_{29}NO_3 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test in comparison with a Standard solution having a known concentration of USP Betaxolol Hydrochloride RS in the same *Medium*. A 5-cm pathlength cell may be used for lower dosage levels.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{18}H_{29}NO_3 \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a volumetric flask of appropriate size to obtain a concentration, based on the labeled amount of betaxolol hydrochloride per Tablet, of about 0.1 mg per mL when diluted. Add an amount of 0.1 N hydrochloric acid equal to about 70% of the volume of the flask, shake by mechanical means until dissolved, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter the mixture, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of the clear filtrate and of a Standard solution of USP Betaxolol Hydrochloride RS in 0.1 N hydrochloric acid having a known concentration of about 0.1 mg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 274 nm, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in the Tablet taken by the formula:

$$(CV)(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the Standard solution;  $V$  is the volume, in mL, of 0.1 N hydrochloric acid used to dissolve the Tablet; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

#### Assay—

**Mobile phase**—Prepare a filtered mixture of 0.025 M pH 6.0 ammonium phosphate buffer, acetonitrile, and methanol (35:35:30). Mix, and degas under vacuum while stirring. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of acetonitrile and water (1:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betaxolol Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 2 mg per mL.

**Assay preparation**—Dissolve not fewer than 20 Tablets in an appropriate accurately measured volume of *Diluent* so that the final concentration, based on the labeled amount per Tablet, is about 2 mg of betaxolol hydrochloride per mL. Sonicate until the Tablets are disintegrated. Cool to room temperature, dilute with *Diluent* to volume, mix, and filter. Use the clear filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 15-cm column that contains packing L1.

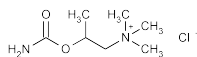
The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, record the chromatogram, and measure the peak response as directed for *Procedure*: the tailing factor is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation*, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in each Tablet taken by the formula:

$$(CV/N)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the *Standard preparation*; V is the volume of *Diluent* used to dissolve the Tablets; N is the number of Tablets taken; and  $r_U$  and  $r_S$  are the betaxolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bethanechol Chloride



$C_7H_{17}ClN_2O_2$  196.68

1-Propanaminium, 2-(aminocarbonyloxy)-*N,N,N*-trimethyl-, chloride, ( $\pm$ )-.

( $\pm$ )-(2-Hydroxypropyl)trimethylammonium chloride carbamate [590-63-6].

» Bethanechol Chloride contains not less than 98.0 percent and not more than 101.5 percent of  $C_7H_{17}ClN_2O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Bethanechol Chloride RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** Dissolve about 50 mg in 2 mL of water, add 0.1 mL of cobaltous chloride solution (1 in 100), then add 0.1 mL of potassium ferrocyanide TS: an emerald-green color is produced, and almost entirely fades in 5 to 10 minutes (*distinction from choline chloride, which gives the same reaction but the color does not fade*).

**C:** To 1 mL of a solution (1 in 100) add 0.1 mL of iodine TS: a brown precipitate is formed, and it rapidly changes to a dark olive-green color.

**D:** A solution of it responds to the tests for *Chloride* (191).

**pH** (791): between 5.5 and 6.5, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231)—Dissolve 667 mg in 10 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is 0.003%.

**Chloride content**—Dissolve about 400 mg, previously dried and accurately weighed, in 30 mL of water. Add 40.0 mL of 0.1 N silver nitrate VS, then add 3 mL of nitric acid and 5 mL of nitrobenzene, shake for a few minutes, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl is between 17.7% and 18.3%.

## Related compounds—

**Buffer solution**—Transfer about 0.48 g of methanesulfonic acid to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1  $\mu$ g of USP Bethanechol Chloride RS per mL.

**Test solution**—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix.

**System suitability solution**—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatography system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductivity detector and a 3.9-  $\times$  150-mm column containing packing L55. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35° and 30°, respectively. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention time is about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for bethanechol; the resolution,  $R$ , between 2-hydroxypropyltrimethyl ammonium chloride and bethanechol is not less than 0.8. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10.0% for bethanechol chloride.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Mobile phase*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for all the peaks. Calculate the percentage of each impurity in the portion of Bethanechol Chloride taken by the formula:

$$25,000C(F/W)(r_i / r_S)$$

in which C is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard solution*; F is the relative response factor and is equal to 0.79 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for any other impurity; W is the weight, in mg, of Bethanechol Chloride taken to prepare the *Test solution*;  $r_i$  is the peak response for any impurity in the *Test solution*; and  $r_S$  is the peak response of USP Bethanechol Chloride RS in the *Standard solution*. Not more than 1.0% of 2-hydroxypropyltrimethyl ammonium is found; not more than 0.1% of any other impurity is found; and the sum of all the impurities is not more than 1.5%.

**Assay**—

**Buffer solution**—Transfer about 29 mg of edetic acid to a 1000-mL volumetric flask, and dissolve in 500 mL of water. Add 300  $\mu$ L of nitric acid to the volumetric flask, and dilute with water to volume. Pass through a 0.45- $\mu$ m nylon membrane filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N

hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg of USP Bethanechol Chloride RS per mL.

**Assay preparation**—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a conductivity detector and a 3.9- × 150-mm column containing packing L55. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35° and 30°, respectively. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for bethanechol; and the resolution,  $R$ , between 2-hydroxypropyltrimethyl ammonium chloride and bethanechol is not less than 0.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 3.5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_7H_{17}ClN_2O_2$  in the portion of Bethanechol Chloride taken by the formula:

$$250C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the bethanechol chloride peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bethanechol Chloride Injection

» Bethanechol Chloride Injection is a sterile solution of Bethanechol Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_7H_{17}ClN_2O_2$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** <11>—

USP Bethanechol Chloride RS

USP Endotoxin RS

**Identification**—It responds to *Identification tests B, C, and D* under *Bethanechol Chloride*.

**Bacterial endotoxins** <85>—It contains not more than 25.0 USP Endotoxin Units per mg of bethanechol chloride.

**pH** <791>: between 5.5 and 7.5.

**Limit of 2-hydroxypropyltrimethyl ammonium chloride**—

*Diluent, Mobile phase, System suitability solution, and Chromatographic system*—Prepare as directed in the *Assay*.

*2-Hydroxypropyltrimethyl ammonium chloride solution*—Transfer 50.0 mg of bethanechol chloride into a 50-mL volu-

metric flask. Add about 40 mL of 0.1 N sodium hydroxide, and sonicate until fully dissolved. Dilute with 0.1 N sodium hydroxide to volume, and allow to stand for five days to allow adequate time for conversion from bethanechol to 2-hydroxypropyltrimethyl ammonium chloride. Chromatograph as directed for *Procedure* to verify the presence and location of the peak for 2-hydroxypropyltrimethyl ammonium chloride.

**Standard solution**—Use the *Standard preparation*, prepared as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *2-Hydroxypropyltrimethyl ammonium chloride solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the bethanechol and 2-hydroxypropyltrimethyl ammonium chloride peaks. Calculate the percentage of 2-hydroxypropyltrimethyl ammonium chloride in each mL of the Injection taken by the formula:

$$100(C_s / C_i)(r_i / r_s)$$

in which  $C_s$  is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard solution*;  $C_i$  is the concentration, in mg per mL, of bethanechol chloride in the *Test solution*;  $r_i$  is the peak response for 2-hydroxypropyltrimethyl ammonium chloride obtained from the *Test solution*; and  $r_s$  is the peak response for bethanechol obtained from the *Standard solution*. Not more than 4.0% is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

**Diluent**—Transfer 10 mg of calcium chloride and 10 mg of magnesium chloride to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed solution of 20 mM methanesulfonic acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Transfer 25 mg of USP Bethanechol Chloride RS, accurately weighed, to a 25-mL volumetric flask, and add 15 mL of water, 2.0 mL of the *Diluent*, and 0.5 mL of 0.1 N sodium hydroxide. Dilute with water to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 1.0 mg per mL.

**Assay preparation**—Dilute an accurately measured volume of Injection, if necessary, with water to obtain a solution having a concentration of about 1.0 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a conductivity detector and a 4-mm × 25-cm column that contains packing L53. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for sodium, 1.4 for magnesium, 1.6 for calcium, 2.0 for 2-hydroxypropyltrimethyl ammonium chloride, and 2.8 for bethanechol; the resolution,  $R$ , between the calcium ion and 2-hydroxypropyltrimethyl ammonium chloride is not less than 2.0; the column efficiency determined from the bethanechol peak is not less than 350 theoretical plates; the tailing factor is not more than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in each mL of the Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bethanechol Chloride Oral Solution

### DEFINITION

Bethanechol Chloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

Prepare Bethanechol Chloride Oral Solution 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Bethanechol Chloride	500 mg
Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , a sufficient quantity to make	100 mL

Add *Bethanechol Chloride* powder and about 20 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (33:67)

**Standard solution:** 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Solution with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L11

**Flow rate:** 0.7 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of bethanechol chloride is about 3 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 3.1% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of bethanechol chloride in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

• **PH (791):** 3.9–4.9

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature or in a cold place.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Bethanechol Chloride RS

## Bethanechol Chloride Oral Suspension

### DEFINITION

Bethanechol Chloride Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

Prepare Bethanechol Chloride Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Bethanechol Chloride	500 mg
Vehicle: a mixture of Vehicle for Oral Solution, (regular or sugar-free), <i>NF</i> and Vehicle for Oral Suspension, <i>NF</i> (1:1), a sufficient quantity to make	100 mL

If using *Bethanechol Chloride* tablets, add to a suitable mortar and comminute to a fine powder, or add the *Bethanechol Chloride* powder to the mortar. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (33:67)

**Standard solution:** 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Suspension with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L11

**Flow rate:** 0.7 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for bethanechol chloride is about 3 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 3.1% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of bethanechol chloride in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 3.9–4.9

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature, or in a cold place.
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded.
- **USP REFERENCE STANDARDS** (11)  
USP Bethanechol Chloride RS

**Bethanechol Chloride Tablets**

» Bethanechol Chloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Bethanechol Chloride RS

**Identification**—Pulverize a quantity of Tablets, equivalent to about 100 mg of bethanechol chloride, add 15 mL of ether, and allow to digest for 15 minutes. Decant the ether, again extract the residue with 10 mL of ether, and discard the ether extracts. To the residue add 30 mL of alcohol, shake for 10 minutes, and allow to stand for 1 hour with frequent agitation. Filter with suction, and evaporate the filtrate on a steam bath to dryness: the bethanechol chloride so obtained, recrystallized from alcohol and dried at 105° for 2 hours, responds to *Identification test A* under *Bethanechol Chloride*.

**Dissolution** (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

Determine the amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) dissolved using the following method.

*Buffer solution, Mobile phase, and Chromatographic system*—Proceed as directed in *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of USP Bethanechol Chloride RS approximately corresponding to the concentration of the solution under test.

*Procedure*—Separately inject equal volumes (about 100  $\mu\text{L}$ ) of a filtered portion of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major

peaks. Calculate the quantity of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) dissolved based on the peak responses obtained from the solution under test and the *Standard solution*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_7H_{17}ClN_2O_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—

*Buffer solution*—Transfer about 0.48 g of methanesulfonic acid to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in *Mobile phase*, and dilute quantitatively and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1  $\mu\text{g}$  of USP Bethanechol Chloride RS per mL.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 1 Tablet, to a suitable volumetric flask so that the final solution yields a concentration of about 0.1 mg per mL of bethanechol chloride. Add an amount of *Mobile phase*, about 60% to 70% of the total volume of the flask. Sonicate for 20 minutes. Shake by mechanical means for about 15 minutes. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 minutes, and pass the solution through a 1- $\mu\text{m}$  glass filter, discarding the first 3 mL of the filtrate.

*System suitability solution*—Transfer about 25 mg of bethanechol chloride, accurately weighed, to a 250-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductivity detector and a 3.9-  $\times$  150-mm column containing packing L55. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35° and 30°, respectively. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for bethanechol; and the resolution, *R*, between 2-hydroxypropyltrimethyl ammonium chloride and bethanechol is not less than 0.8. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10.0% for bethanechol chloride.

*Procedure*—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100V(F/W)C(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard solution*; *F* is the relative response factor and is equal to 0.79 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for any other impurity;  $r_i$  is the peak response for any impurity in the *Test solution*;  $r_s$  is the peak response of USP Bethanechol Chloride RS in the *Standard solution*; and *W* is the amount, in mg, of bethanechol chloride based on the average weight, labeled dose, and amount taken to prepare the *Test solution*. Not more than 1.0% of 2-hydroxypropyltrimethyl ammonium chloride is found; not more than 0.2% of any other impurity

is found; and the sum of all the impurities is not more than 1.5%.

#### Assay—

**Buffer solution**—Transfer about 29 mg of edetic acid to a 1000-mL volumetric flask, and dissolve in 500 mL of water. Add 300  $\mu$ L of nitric acid to the volumetric flask, and dilute with water to volume. Pass through a 0.45- $\mu$ m nylon membrane filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg of USP Bethanechol Chloride RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 1 Tablet, to a suitable volumetric flask so that the final solution yields a concentration of about 0.1 mg per mL of bethanechol chloride. Add an amount of *Mobile phase*, about 60% to 70% of the total volume of the flask. Sonicate for 20 minutes. Shake by mechanical means for about 15 minutes. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 minutes, and pass the solution through a 1- $\mu$ m glass filter, discarding the first 3 mL of the filtrate.

**System suitability solution**—Transfer about 25 mg of bethanechol chloride, accurately weighed, to a 250-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume, and mix.

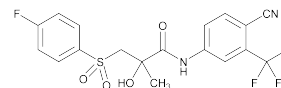
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a conductivity detector and a 3.9-  $\times$  150-mm column containing packing L55. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35° and 30°, respectively. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention time is about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for bethanechol; and the resolution, *R*, between 2-hydroxypropyltrimethyl ammonium chloride and bethanechol is not less than 0.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 3.5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of bethanechol chloride (C<sub>7</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>) in the portion of Tablets taken by the formula:

$$VC(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the *Standard preparation*; *V* is the volume, in mL, of the flask used to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the bethanechol chloride peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bicalutamide



C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>4</sub>S 430.37  
 Propanamide, N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-, (±)-; (±)-4'-Cyano- $\alpha,\alpha,\alpha$ -trifluoro-3-[(*p*-fluorophenyl)sulfonyl]-2-methyl-*m*-lactotoluidide [90357-06-5].

#### DEFINITION

Bicalutamide contains NLT 98.0% and NMT 102.0% of C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>4</sub>S, calculated on the anhydrous and solvent-free basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION** <197M>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Solution A:** 0.01% (v/v) of trifluoroacetic acid in water

**Solution B:** 0.01% (v/v) of trifluoroacetic acid in acetonitrile

**Mobile phase:** *Solution A* and *Solution B* (52:48)

**Diluent:** *Solution A* and *Solution B* (1:2)

**System suitability solution:** 5  $\mu$ g/mL of USP Bicalutamide Related Compound A RS and 50  $\mu$ g/mL of USP Bicalutamide RS in *Diluent*

**Standard solution:** 0.05 mg/mL of USP Bicalutamide RS in *Diluent*

**Sample solution:** 0.05 mg/mL of Bicalutamide in *Diluent*

**Chromatographic system** (See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.0-mm  $\times$  10-cm; 3- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

##### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for bicalutamide related compound A isomer A and bicalutamide related compound A isomer B are 0.75 and 0.78, respectively.]

##### Suitability requirements

**Resolution:** NLT 2.0 between bicalutamide related compound A isomer B and bicalutamide, *System suitability solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>4</sub>S in the portion of Bicalutamide taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis



**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, *Method II* (231): NMT 10 ppm

**Organic Impurities**• **PROCEDURE**

**Solution A, Solution B, Diluent, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	67	33
16.5	67	33
26.5	40	60
32.5	5	95
32.6	67	33
35	67	33

**Standard solution:** 1 µg/mL of USP Bicalutamide RS in *Diluent*

**Sample solution:** 1 mg/mL of Bicalutamide in *Diluent*

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution 1:** NLT 0.8 between bicalutamide related compound A isomer A and bicalutamide related compound A isomer B

**Resolution 2:** NLT 8.5 between bicalutamide related compound A isomer B and bicalutamide

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bicalutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of bicalutamide from the *Standard solution*

$C_S$  = concentration of bicalutamide in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bicalutamide in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.5%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bicalutamide aminobenzonitrile <sup>a</sup>	0.30	1.4	0.1
Bicalutamide related compound A isomer A <sup>b</sup>	0.64	1.0	0.1

<sup>a</sup> 4-Amino-2-(trifluoromethyl)benzonitrile.

<sup>b</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide.

<sup>c</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-2-hydroxy-2-methyl-3-(phenylsulfonyl)propanamide.

<sup>d</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

<sup>e</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylpropanamide.

<sup>f</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylthio)-2-hydroxy-2-methylpropanamide.

**Impurity Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bicalutamide related compound A isomer B <sup>b</sup>	0.67	1.0	0.1
Desfluoro bicalutamide <sup>c</sup>	0.83	1.1	0.2
2-Fluoro bicalutamide <sup>d</sup>	0.94	1.0	0.2
Bicalutamide	1.00	—	—
Deoxybicalutamide <sup>e</sup>	1.33	1.0	0.2
Bicalutamide sulfide <sup>f</sup>	1.56	1.0	0.1
Any unspecified impurity	—	1.0	0.1

<sup>a</sup> 4-Amino-2-(trifluoromethyl)benzonitrile.

<sup>b</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide.

<sup>c</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-2-hydroxy-2-methyl-3-(phenylsulfonyl)propanamide.

<sup>d</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

<sup>e</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylpropanamide.

<sup>f</sup> *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylthio)-2-hydroxy-2-methylpropanamide.

**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method I* (921): NMT 0.2%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Bicalutamide RS
  - USP Bicalutamide Related Compound A RS
  - [*N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide] ( $C_{18}H_{14}F_4N_2O_3S$  414.37)

**Bicalutamide Tablets**

» Bicalutamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ).

**Packaging and storage**—Preserve in tight containers. Store at controlled room temperature.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

**USP Reference standards** (11)—

USP Bicalutamide RS

USP Bicalutamide Related Compound B RS

(*RS*)-*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(3-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.  
 $C_{18}H_{14}F_4N_2O_4S$  430.37

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

TEST 1—

**Medium:** 1.0% w/v sodium lauryl sulfate in water; 1000 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

**Standard solution**—Transfer about 10 mg, accurately weighed, of USP Bicalutamide RS to a 200-mL volumetric flask, dissolve in 2 mL of tetrahydrofuran, and dilute with *Medium* to volume.

**Test solution**—Pass the solution under test through a suitable 0.45- $\mu$ m filter.

**Procedure**—Determine the amount of  $C_{18}H_{14}F_4N_2O_4S$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on portions of the *Test solution* in comparison with the *Standard solution*, using the *Medium* as the blank. Calculate the percentage of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) dissolved by the formula:

$$\frac{A_U \times C_S \times 1000 \times 100}{A_S \times L}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of bicalutamide in the *Standard solution*; 1000 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $L$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of bicalutamide is dissolved in 45 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium, Apparatus 2, Time, Standard solution, Test solution, and Procedure**—Proceed as directed for *Test 1*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of bicalutamide is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**1% Sodium lauryl sulfate solution**—Dissolve 15 g of sodium lauryl sulfate in 1.5 L of water.

**Standard solution**—Dissolve an accurately weighed quantity of USP Bicalutamide RS in a minimum amount of tetrahydrofuran, and dilute quantitatively with 1% Sodium lauryl sulfate solution to obtain a solution having a known concentration of about 0.05 mg per mL.

**Test solution**—Transfer 1 Tablet to a 100-mL volumetric flask, add about 10 mL of water, and sonicate for approximately 30 minutes. Add about 80 mL of tetrahydrofuran, and sonicate for 30 minutes to complete dissolution of the bicalutamide. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass this solution through a 0.45- $\mu$ m suitable filter unit, transfer 10.0 mL of filtrate to a 100-mL volumetric flask, and dilute with 1% Sodium lauryl sulfate solution to volume.

**Procedure**—Concomitantly determine the UV absorbances of the *Standard solution* and the *Test solution* with a suitable spectrophotometer at 270 nm, using 1% Sodium lauryl sulfate solution as the blank. Calculate the quantity, in mg, of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) in the Tablet taken by the formula:

$$1000C(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bicalutamide RS in the *Standard solution*; and  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively.

**Limit of 4-amino-2-(trifluoromethyl)benzonitrile**—

**Mobile phase and System suitability solution**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Bicalutamide RS in tetrahydrofuran to obtain a solution having a known concentration of about 0.2 mg per

mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Test solution**—Transfer an accurately weighed quantity of the powdered Tablet prepared in the *Assay preparation*, equivalent to about 50 mg of bicalutamide, to a 25-mL volumetric flask. Add about 2 mL of tetrahydrofuran, and allow to stand for 5 minutes. Add about 20 mL of *Mobile phase*, and sonicate for 10 minutes. Allow to cool to room temperature, and dilute with *Mobile phase* to volume. Pass the sample through a suitable 0.2- $\mu$ m filter.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*, except to use a liquid chromatograph equipped with a 220-nm detector.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of 4-amino-2-(trifluoromethyl)benzonitrile in the portion of Tablets taken by the formula:

$$100(1/1.4)(C_S / C_U)(r_U / r_S)$$

in which 1.4 is the relative response factor for 4-amino-2-(trifluoromethyl)benzonitrile,  $C_S$  is the concentration, in mg per mL, of USP Bicalutamide RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of bicalutamide in the *Test solution* based on the label claim;  $r_U$  is the peak area for 4-amino-2-(trifluoromethyl)benzonitrile obtained from the *Test solution*; and  $r_S$  is the peak area for bicalutamide obtained from the *Standard solution*; not more than 0.1% of 4-amino-2-(trifluoromethyl)benzonitrile is found. [NOTE—The relative retention time for 4-amino-2-(trifluoromethyl)benzonitrile is about 0.4.]

**Assay**—

**Mobile phase**—Prepare a mixture of water, tetrahydrofuran, and acetonitrile (65:20:15).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bicalutamide RS in tetrahydrofuran to obtain a solution having a known concentration of about 0.8 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Assay preparation**—Grind not fewer than 20 Tablets to a fine powder. Transfer an accurately weighed quantity of powdered Tablets, equivalent to about 50 mg of bicalutamide, to a 100-mL volumetric flask. Add about 50 mL of tetrahydrofuran, and sonicate for at least 10 minutes to complete dissolution. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass this solution through a suitable 0.45- $\mu$ m filter. Transfer 4.0 mL of the filtrate to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**System suitability solution**—Dissolve suitable quantities of USP Bicalutamide RS and USP Bicalutamide Related Compound B RS in tetrahydrofuran to obtain a solution having known concentrations of about 0.8 mg of USP Bicalutamide RS per mL and 0.4 mg of USP Bicalutamide Related Compound B RS per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 270-nm detector, and a 5-mm  $\times$  12.5-cm column that contains 3- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 50°. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the relative retention time for the bicalutamide related compound B peak is about 1.1; the resolution,  $R$ , between bicalutamide and bicalutamide related compound B is greater than 1.9; the tailing factor of the bicalutamide peak is less than 1.3; and the relative standard deviation for replicate injections calculated for the bicalutamide peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the bicalutamide peaks. Calculate the quantity, in percentage of the label claim, of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Bicalutamide RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of bicalutamide in the *Assay preparation* based on the label claim; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Biological Indicator for Dry-Heat Sterilization, Paper Carrier

» Biological Indicator for Dry-Heat Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus subtilis* subspecies *niger*, on a suitable grade of paper carrier, individually packaged in a container readily penetrable by dry heat, and characterized for predictable resistance to dry-heat sterilization. The packaged Biological Indicator for Dry-Heat Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than  $10^4$  and not more than  $10^9$  spores. When labeled for and subjected to dry-heat sterilization conditions at a particular temperature, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes) of the preparation, specified by:

*Survival time* (in minutes) = not less than (labeled *D value*)  $\times$  (log labeled spore count per carrier – 2); and

*Kill time* (in minutes) = not more than (labeled *D value*)  $\times$  (log labeled spore count per carrier + 4).

**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article used as directed in the labeling.

**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.

**Labeling**—Label it to state that it is a Biological Indicator for Dry-Heat Sterilization, Paper Carrier; to indicate its *D value* and the method used to determine such *D value*, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under the specified sterilization conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were

derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.

**Identification**—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus subtilis*, ATCC No. 9372, designated subspecies *niger*, detailed for that biological indicator organism under *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*.

#### Resistance performance tests—

*D value*—Proceed as directed for the relevant procedure for *D Value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature, and if the confidence limits of the estimate are within 10% of the determined *D value*.

*Survival time and kill time*—Proceed as directed for *Survival Time and Kill Time* in the section *Dry-Heat Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to dry-heat sterilization for the survival time show evidence of growth, while none of the specimens subjected to dry-heat sterilization for the kill time shows growth. If for either the survival time test or the kill time test not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to dry-heat sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

*Total viable spore count*—Proceed as directed for *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

#### Purity—

*Presence of contamination by other microorganisms*—By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.

**Disposal**—Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a test strip employed in any test procedures for the strips themselves.

### Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier

» Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus subtilis* subspecies *niger* on a suitable grade of paper carrier, individually packaged in a suitable container readily penetrable by ethylene oxide sterilizing gas mixture, and characterized for predictable resistance to sterilization with such gas mixture. The packaged

Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than  $10^4$  and not more than  $10^9$  spores. Where labeled for and subjected to particular ethylene oxide sterilization conditions of a stated gaseous mixture, temperature, and relative humidity, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D* value, in minutes) of the preparation, specified by:

*Survival time* (in minutes) = not less than (labeled *D* value)  $\times$  (log labeled spore count per carrier – 2), and

*Kill time* (in minutes) = not more than (labeled *D* value)  $\times$  (log labeled spore count per carrier + 4).

**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect it from light, toxic substances, excessive heat, and moisture. The packaging and container material shall be such that it does not adversely affect the performance of the article used as directed in the labeling.

**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.

**Labeling**—Label it to state that it is a Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier; to indicate its *D* value, the method used to determine such *D* value, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under specified sterilization conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D* value is reproducible only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.

**Identification**—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus subtilis*, ATCC No. 9372, designated subspecies *niger*: under microscopic examination it consists of Gram-positive rods of width 0.7 to 0.8  $\mu\text{m}$ , and length 2 to 3  $\mu\text{m}$ ; the endospores are oval and central and the cells are not swollen; when incubated aerobically in appropriate media at 30° to 35°, growth occurs within 24 hours, and similar inoculated media incubated concomitantly at 55° to 60° show no evidence of growth in the same period; agar colonies have a dull appearance and may be cream or brown-colored; when incubated in nutrient broth it develops a pellicle, and shows little or no turbidity; when examined under conventional biochemical tests for microbial characterization, it develops a black pigment with tyrosine, it liquefies gelatin, utilizes citrate but not propionate or hippurate, reduces nitrate, and hydrolyzes both starch and glucose with no gas production; it shows a positive catalase reaction and gives a positive result with the Voges-Proskauer test.

#### **Resistance performance tests—**

*D* value—Proceed as directed for the relevant procedure for *D* value under *Biological Indicators—Resistance Performance Tests* (55).

The requirements of the test are met if the determined *D* value is within 20% of the labeled *D* value for the selected sterilizing temperature and if the confidence limits of the estimate are within 10% of the determined *D* value.

*Survival time and kill time*—Proceed as directed for *Survival Time and Kill Time* in the section *Ethylene Oxide Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the ethylene oxide sterilization conditions for the survival time show evidence of growth, while none of the specimens subjected to the ethylene oxide sterilization conditions for the kill time shows evidence of growth. If for either the survival time test or the kill time test, not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to ethylene oxide sterilization meet either the survival requirement for the survival time test or the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

*Total viable spore count*—Follow the procedure for *Total Viable Spore Count* in the section *Ethylene Oxide Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

#### **Purity—**

*Presence of contamination by other microorganisms*—By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.

**Disposal**—Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a strip used in test procedures for strips themselves.

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### **Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions**

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» Liquid spore suspensions may be used to prepare biological indicators for moist heat, dry heat, and gaseous modes of sterilization. On the basis of the intended sterilization use, the suspension is prepared inoculated from a culture of viable spores derived from one of several sterilization resistant microorganisms. Cultures used for liquid spore suspensions include, among others, the following: *Clostridium sporogenes*, *Geobacillus stearothermophilus* (formerly *B. stearothermophilus*), *Bacillus atrophaeus* (formerly *B. subtilis*), *Bacillus subtilis*, or *Bacillus coagulans*. Each tube or container containing the spore suspension is individually packaged for use. The packaged biological indicator spore suspension has a particular labeled spore count of not less than  $10^3$ , and not more than  $10^9$ , spores per mL of suspension. The suspending medium or vehicle is identified according to chemical composition. It has a survival time and kill time appropriate to the labeled

spore count, and to the decimal reduction value (the *D value*, in minutes), specified by the following:

*Survival time* (in minutes) = not less than (labeled *D value*)  $\times$  (log of labeled spore count per mL from 1:100 dilution of original suspension – 2); and

*Kill time* (in minutes) = not more than (labeled *D value*)  $\times$  (log of labeled spore count per mL from 1 : 100 dilution of original suspension + 4).

**Packaging and storage**—Preserve in the original tube or container under the conditions recommended on the label, and protect the contents of the tube or container from light, toxic substances, and excessive heat. The materials of composition of the tube or container must not adversely affect the performance of the spore suspension.

**Expiration date**—The expiration date is determined on the basis of stability studies. The date of manufacture is the date on which the first determination of the total viable count was made.

**Labeling**—Label the spore suspension tube or container or package insert to state that it is a biological indicator spore suspension for use in label specified applications for moist-heat, dry-heat, and/or gaseous sterilization. State the biological indicator *D value* obtained under defined exposures to stated sterilization conditions using the Survival Curve Method of *D-value* analysis. State the *Survival time* and *kill time* for the biological indicator suspension under specified conditions on the label. The total viable spore count per mL of the suspension following heat shock treatment must also appear on the label. State in the labeling the strain and ATCC number of the microorganisms used in the spore suspension and instructions for spore recovery and for safe disposal of the suspension. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions determined by the manufacturer and that the user would not necessarily obtain the same results if different exposure conditions were used. State that the user should determine the suitability of the biological indicator spore suspension for the user's particular purpose and exposure conditions.

**Identification**—Identification for the biological indicator is of lesser importance than the more relevant concerns of population and resistance to the sterilization processes. The manufacturer should identify the species used.

**D value**—If the biological indicators are being used in moist-heat or dry-heat sterilization, proceed as directed for the relevant procedure in the section *D-Value Determination* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing conditions, and if the confidence limits of the estimate are within 10% of the determined *D value*. The *D-value* determination method used should be that identified by the biological indicator manufacturer.

**Survival time and kill time**—Follow the procedure under *Survival Time and Kill Time* in the section *D-Value Determination* under *Biological Indicators—Resistance Performance Tests* (55). The test is conducted using 1:100 dilution aliquots of the original suspension to inoculate carrier substrates that are most likely to be used by the purchaser of the spore suspensions for a given mode of sterilization. Following a total viable count analysis, the inoculated substrates are subjected to sterilization exposure conditions intended to indicate survival. The inoculated carriers must show evidence of growth among the exposed carriers. A second study is conducted to demonstrate the conditions necessary to result in total kill of the carriers. None of the carriers subjected to conditions designed to induce total kill should show growth. If for either the survival-time test or the kill-time test, not more than one carrier out of both groups fails the survival

or kill requirements, continue the corresponding test with four additional groups, each consisting of 10 carriers, according to the procedure described. For biological indicators for use with moist-heat or dry-heat sterilization, if all of the additional specimens subjected to the specific sterilization process either meet the survival requirements for the survival-time test or meet the kill requirement for the kill-time test, whichever is applicable, the requirements are met.

**Total viable spore count**—Proceed as directed for *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions* in the section *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55). The requirements for this test are met if the total viable spore count within the suspension is within  $\pm 1$  log of the value stipulated by the manufacturer.

**Purity**—There is no evidence of contamination with other microorganisms following examination of spores recovered from the metal carriers using suitable plate-culture medium.

**Shipment**—Spore suspensions must be shipped following EPA requirements for the shipment of biological and/or etiological agents.

**Disposal**—Spore suspensions that a user or manufacturer wishes to dispose of are first sterilized by moist heat by a process that achieves temperatures of approximately 121° for not less than 30 minutes. Alternative sterilization methods yielding equivalent or greater levels of lethality may be used.

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### Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers

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» Biological indicators for moist heat, dry heat, and gaseous modes of sterilization may be nonpaper carriers inoculated with a culture of viable spores derived from one of several sterilization-resistant microorganisms, based on the intended sterilization use. Cultures used for inoculation of carriers include, among others, *Clostridium sporogenes*, *Geobacillus stearothermophilus* (formerly *B. stearothermophilus*), *Bacillus atrophaeus* (formerly *B. subtilis*), or *Bacillus coagulans*. The carriers should be individually packaged for use either within the package or for use upon removal from the package as an unpackaged biological indicator. The packaged biological indicator on the carrier has a particular labeled spore count of not less than  $10^3$  and not more than  $10^9$  spores per carrier. It has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes), specified by:

*Survival time* (in minutes) = not less than (labeled *D value*)  $\times$  (log of labeled spore count per carrier – 2), and

*Kill time* (in minutes) = not more than (labeled *D value*)  $\times$  (log of labeled spore count per carrier + 4).

**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect the package from light, toxic substances, excessive heat, and high relative humidity or moisture. The packaging

or container materials do not adversely affect the performance of the article used as directed in the labeling.

**Expiration date**—The expiration date is determined on the basis of stability studies and is not more than 18 months from the date of manufacture. The date of manufacture is the date on which the first determination of the total viable count was made.

**Labeling**—Label the package or package insert to state that it is a biological indicator prepared on a carrier for use in label-specified applications for moist heat, dry heat, and/or gaseous sterilization. State the biological indicator *D value* obtained under defined exposures to stated sterilization conditions using the Survival Curve method, Spearman-Kärber method, or Stumbo-Murphy-Cochran method of *D value* analysis. State the survival time and kill time for the biological indicator carrier under specified conditions on the label. The total viable spore count per carrier following heat shock treatment must also appear on the label or package insert. State in the labeling the strain and ATCC number of the spore suspension used to inoculate the carriers and instructions for spore recovery and for safe disposal of the carriers. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions determined by the manufacturer and that the user would not necessarily obtain the same results if different exposure conditions were used. State that the user should determine the suitability of the carrier biological indicator for the user's particular purpose and exposure conditions.

**Identification**—Identification for the biological indicator is of less importance than the more relevant concerns of population and resistance to the sterilization processes. The manufacturer should identify the species used.

**D value**—Proceed as directed in the relevant procedure for *D Value Determination* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing conditions, and if the confidence limits of the estimate are within 10% of the determined *D value*. The *D-value* determination method used should be that identified by the biological indicator manufacturer.

**Survival time and kill time**—Follow the procedure in the subsection *Survival Time and Kill Time* in the section *D-Value Determination* under the chapter *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the carriers subjected to sterilization exposure conditions intended to indicate survival show evidence of growth among the exposed carriers, while none of the carriers subjected to conditions designed to induce total kill show growth. If for either the survival test or the kill time test, not more than one carrier out of both groups fails the survival or kill requirements, continue the corresponding test with four additional groups, each consisting of 10 carriers, according to the procedure described. If all of the additional specimens subjected to the specific sterilization process either meet the survival requirements for the survival test time or meet the kill requirement for the kill test, whichever is applicable, the requirements are met.

**Total viable spore count**—Proceed as directed in the subsection *Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Nonpaper Carriers* in the section *Total Viable Spore Count* under the chapter *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the average number of viable spores per carrier are within –50% and +300% of the labeled count per carrier or within a lesser range that may be stated by the manufacturer.

**Purity**—There is no evidence of contamination with other microorganisms following examination of spores recovered from the carriers using a suitable plate culture medium.

**Disposal**—Prior to destruction or discarding the carriers, sterilize by moist heat sterilization to ensure that the carrier surface is exposed to 121° for not less than 30 minutes, or by an equivalent method recommended by the manufacturer.

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## Biological Indicator for Steam Sterilization, Paper Carrier

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» Biological Indicator for Steam Sterilization, Paper Carrier, is a defined preparation of viable spores made from a culture derived from a specified strain of *Bacillus stearothermophilus*, on a suitable grade of paper carrier, individually packaged in a suitable container readily penetrable by steam, and characterized for predictable resistance to steam sterilization. The packaged Biological Indicator for Steam Sterilization, Paper Carrier, has a particular labeled spore count per carrier of not less than  $10^4$  and not more than  $10^9$  spores. When labeled for and subjected to steam sterilization conditions at a particular temperature, it has a survival time and kill time appropriate to the labeled spore count and to the decimal reduction value (*D value*, in minutes) of the preparation, specified by:

*Survival time* (in minutes) = not less than (labeled *D value*)  $\times$  (log labeled spore count per carrier – 2); and

*Kill time* (in minutes) = not more than (labeled *D value*)  $\times$  (log labeled spore count per carrier + 4).

**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect it from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article used as directed in the labeling.

**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.

**Labeling**—Label it to state that it is a Biological Indicator for Steam Sterilization, Paper Carrier; to indicate its *D value*, the method used to determine such *D value*, i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions; the survival time and kill time under specified sterilization conditions stated on the label; its particular total viable spore count with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State in the labeling the size of the paper carrier, the strain and ATCC number from which the spores were derived, and instructions for spore recovery and for safe disposal of the indicator. Indicate in the labeling that the stated *D value* is reproducible only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result and that the user should determine the suitability of the biological indicator for the particular use.

**Identification**—The biological indicator organism complies substantially with the morphological, cultural, and biochemical characteristics of the strain of *Bacillus stearothermophilus*, ATCC No. 7953 or 12980, whichever is stated in the labeling: under microscopic examination it consists of Gram-positive rods with oval endospores in subterminally swollen cells; when incubated in nutrient broth for 17 hours and used to inoculate appropriate solid media, growth occurs when the inoculated media are incubated aerobically for 24 hours at 55° to 60°, and similar inoculated media incubated concomitantly at 30° to 35° show no evidence of growth in the same period. When examined under conventional biochemical tests for microbial characterization, it shows a delayed weak positive catalase reaction, it does not utilize citrate, propionate or hippurate, it reduces nitrate, but it does not liquefy gelatin, and it gives a negative result with the Voges-Proskauer test. Organisms derived from ATCC strain No. 7953 show negative egg yolk and starch hydrolysis reactions, while those derived from ATCC strain No. 12980 show positive reactions in both tests.

**Resistance performance tests—**

*D value*—Proceed as directed for the relevant procedure for *D Value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature, and if the confidence limits of the estimate are within 10% of the determined *D value*.

*Survival time and kill time*—Follow the procedure for *Survival Time and Kill Time* in the section *Steam Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the steam sterilization for the survival time show evidence of growth, while none of the specimens subjected to the steam sterilization for the kill time shows growth. If for either the survival time test or the kill time test, not more than 1 specimen out of both groups fails the survival requirement or the kill requirement (whichever is applicable), continue the corresponding test with 4 additional groups, each consisting of 20 specimens, according to the procedure described. If all of the additional specimens subjected to steam sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

*Total viable spore count*—Proceed as directed for *Total Viable Spore Count* in the section *Steam Sterilization, Paper Carrier*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the tests are met if the log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

**Purity—**

*Presence of contamination by other microorganisms*—By examination of the spores on a suitable plate culture medium, there is no evidence of contamination with other microorganisms.

**Disposal**—Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes a test strip employed in any test procedures for the strips themselves.

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## Biological Indicator for Steam Sterilization, Self-Contained

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» Biological Indicator for Steam Sterilization, Self-Contained, is a Biological Indicator for Steam Sterilization, Paper Carrier individually packaged

in a suitable container readily penetrable by steam and designed to hold an appropriate bacteriological culture medium, so as to enable the packaged carrier, after subjection to saturated steam sterilization conditions, to be incubated in the supplied medium in a self-contained system. The supplied medium may contain a suitable indicator as a convenience for determining by a color change whether or not spores have survived. The design of the self-contained system is such that, after exposure to the specified sterilization conditions and inoculation of the medium under closed conditions as stated in the labeling, there is no loss of medium and inoculum during subsequent transport and handling, if done according to the provided instructions. The materials of which the self-contained system are made are such that there is no retention or release of any substance that may cause inhibition of growth of surviving spores under the incubation conditions stated in the labeling.

**Packaging and storage**—Preserve in the original package under the conditions recommended on the label, and protect from light, from substances that may adversely affect the contained microorganisms, from excessive heat, and from moisture.

**Expiration date**—The expiration date is determined on the basis of stability studies and is not less than 18 months from the date of manufacture, the date of manufacture being the date on which the first determination of the total viable spore count was made.

**Labeling**—Label it to state that it is a Biological Indicator for Steam Sterilization, Self-Contained; to indicate the *D value* of the self-contained system, the method used to determine such *D value* (i.e., by spore count or fraction negative procedure after graded exposures to the sterilization conditions); the survival time and kill time under the specified conditions stated on the label; its particular total viable spore count, with a statement that such count has been determined after preliminary heat treatment; and its recommended storage conditions. State on the labeling that the supplied bacteriological medium will meet requirements for growth-promoting ability, the strain and ATCC number from which the spores were derived, and the instructions for spore recovery and for safe disposal of the indicator unit. Also indicate in the labeling that the stated resistance characteristics are reproducible only under steam sterilization conditions at the stated temperature and only under the exact conditions under which it was determined, that the user would not necessarily obtain the same result, and that the user should determine the suitability of the biological indicator for the particular use.

**Identification**—It meets the requirements of the *Identification* test under *Biological Indicator for Steam Sterilization, Paper Carrier*.

**Resistance performance tests—**

*D value*—Proceed as directed for the relevant procedure for *D Value* under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if the determined *D value* is within 20% of the labeled *D value* for the selected sterilizing temperature and if the confidence limits of the estimate are within 10% of the determined *D value*.

*Survival time and kill time*—Follow the procedure for *Survival Time and Kill Time* in the section *Steam Sterilization, Self-Contained*, under *Biological Indicators—Resistance Performance Tests* (55). The requirements of the test are met if all of the specimens subjected to the steam sterilization for the

survival time show evidence of growth, while none of the specimens subjected to the steam sterilization for the kill time shows growth. If for either the survival time or the kill time requirement, not more than 1 specimen out of both groups fails the test, whichever is applicable, continue the corresponding test with 4 additional groups, each consisting of 10 specimens, according to the procedure described above. If all of the additional specimens subjected to steam sterilization either meet the survival requirement for the survival time test or meet the kill requirement for the kill time test, whichever is applicable, the requirements of the test are met.

**Total viable spore count**—Proceed as directed for *Total Viable Spore Count* under *Biological Indicators—Resistance Performance Tests* (55) using the procedure applicable to *Biological Indicator for Steam Sterilization, Paper Carrier*. The requirements of the test are met if the average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48.

#### Medium suitability—

**Sterility**—Incubate 10 self-contained biological indicator systems at 55° to 60°, or at the optimal recovery temperature specified by the manufacturer, for 48 hours, making sure that there is no contact between the individual spore strips and the supplied medium. Examine the incubated medium visually (for change in color indicator or turbidity) and microscopically (for absence of microbial growth).

**Growth promotion of medium prior to sterilization treatment**—Submerge 10 self-contained units in a water bath maintained at 95° to 100° for 15 minutes. Start timing when the temperature of the container contents reach 95°. Cool rapidly in an ice-water bath (0° to 4°). Remove the units from the ice-water bath, submerge each spore strip with the self-contained medium, incubate at 55° to 60°, or at the optimal recovery temperature specified by the manufacturer, and examine visually after 48 hours for growth (for turbidity or change in color), and microscopically (for microbial growth). All the specimens under test show growth. If one or more of the specimens do not show growth, repeat the test with 20 additional units. The additional units all show growth.

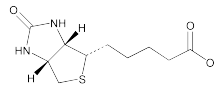
**Growth promotion of medium after exposure to sterilization conditions**—Expose the specified number of units for both the *Survival Time* and *Kill time* stated in the labeling, as described in the section *Biological Indicator for Steam Sterilization, Self-Contained* under *Biological Indicators—Resistance Performance Tests* (55). Incubate the spore strips submerged in the self-contained medium according to the instructions of the manufacturer. At the end of the incubation period confirm the existence of growth in each of the specimens that were exposed for each *Survival time* and the absence of growth in each of the specimens that were exposed for each *Kill time* by visual inspection (turbidity or color indicator change) and by separate microscopic examination of each specimen and confirm, where applicable, correspondence of the labeled color to the appearance of growth in the supplied medium.

**Ability of medium to support growth after exposure to the sterilization conditions**—Take a stated number of units (e.g., 10) after they have been exposed for each *Kill time* stated in the labeling as directed in the preceding section. Aseptically remove and pool the medium from each unit. Prepare a suspension of the indicator microorganism as directed for *Total Viable Spore Counts* under *Biological Indicator for Steam Sterilization, Paper Carrier*. Prepare a dilution of that suspension so as to contain 100 to 1000 viable microorganisms in one mL. Inoculate the pooled medium with enough suspension to contain a total of 100 to 1000 microorganisms in a 10 mL aliquot of not more than the volume from 10 units

of the pooled medium. Incubate the inoculated pooled medium as directed for *Total Viable Spore Count*. Clear evidence of growth is obtained within 7 days.

**Disposal**—Prior to destruction or discard, sterilize it by steam at 121° for not less than 30 minutes, or by not less than an equivalent method recommended by the manufacturer. This includes test strips employed in any test procedures for the strips themselves.

## Biotin



$C_{10}H_{16}N_2O_3S$  244.31  
 1*H*-Thieno[3,4-*d*]imidazole-4-pentanoic acid, hexahydro-2-oxo-, [3*aS*-(3*αα*,4*β*,6*αα*)]-;  
 (3*aS*,4*S*,6*aR*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-valeric acid [58-85-5].

### DEFINITION

Biotin contains NLT 97.5% and NMT 100.5% of biotin ( $C_{10}H_{16}N_2O_3S$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**B.** It meets the requirements in *Specific Tests for Optical Rotation, Specific Rotation* (781S).

**C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer solution:** Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer solution* (8.5: 91.5)

**Diluent:** Acetonitrile and water (1:4)

**Standard solution:** 0.1 mg/mL of USP Biotin RS in *Diluent*. Sonicate if necessary to dissolve.

**Sample solution:** 0.1 mg/mL of Biotin in *Diluent*. Sonicate if necessary to dissolve.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L7

**Flow rate:** 1.2 mL/min

**Injection size:** 50 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of biotin ( $C_{10}H_{16}N_2O_3S$ ) in the portion of Biotin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*



- $C_S$  = concentration of USP Biotin RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–100.5%

## IMPURITIES

### • RELATED COMPOUNDS

Buffer solution, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

#### Analysis

Sample: *Sample solution*

Measure the peak responses of the *Sample solution*. Calculate the percentage of each impurity in the portion of Biotin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_T$  = sum of the peak responses of all the peaks from the *Sample solution*

#### Acceptance criteria

Individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

## SPECIFIC TESTS

### • OPTICAL ROTATION, *Specific Rotation* (781S)

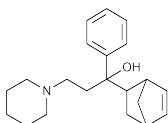
Sample solution: 20 mg/mL in 0.1 N sodium hydroxide

Acceptance criteria: +89° to +93°

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Biotin RS

## Biperiden



$C_{21}H_{29}NO$  311.46

1-Piperidinepropanol,  $\alpha$ -bicyclo[2.2.1]hept-5-en-2-yl- $\alpha$ -phenyl-

$\alpha$ -5-Norbornen-2-yl- $\alpha$ -phenyl-1-piperidinepropanol [514-65-8].

» Biperiden contains not less than 98.0 percent and not more than 101.0 percent of  $C_{21}H_{29}NO$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Biperiden RS

#### Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 900  $\mu$ g per mL. Transfer about 180 mg of it, accurately weighed, to a 200-mL volumetric flask, add 1 mL of lactic acid, dilute with water to volume, and mix. Absorptivities, at about 257 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Dissolve about 20 mg in 5 mL of phosphoric acid: a green color is produced.

D: Dissolve 200 mg in 80 mL of water with the aid of 0.5 mL of 3 N hydrochloric acid, warming, if necessary, to effect solution, and then cool. To 5 mL of the solution add 1 drop of hydrochloric acid and several drops of mercuric chloride TS: a white precipitate is formed. To a second 5-mL portion of the solution add bromine TS dropwise: a yellow precipitate forms which redissolves on shaking, and finally, upon the addition of more bromine TS, a permanent precipitate is formed.

**Melting range**, *Class I* (741): between 112° and 116°.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

#### Ordinary impurities (466)—

Test solution: methanol.

Standard solution: methanol.

Eluant: a mixture of methanol and ammonium hydroxide (100:1.5).

Visualization: 17.

**Assay**—Dissolve about 500 mg of Biperiden, accurately weighed, in 20 mL of benzene, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.15 mg of  $C_{21}H_{29}NO$ .

## Biperiden Hydrochloride

$C_{21}H_{29}NO \cdot HCl$  347.92

1-Piperidinepropanol,  $\alpha$ -bicyclo[2.2.1]hept-5-en-2-yl- $\alpha$ -phenyl-, hydrochloride.

$\alpha$ -5-Norbornen-2-yl- $\alpha$ -phenyl-1-piperidinepropanol hydrochloride [1235-82-1].

» Biperiden Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of  $C_{21}H_{29}NO \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Biperiden Hydrochloride RS

#### Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 1 mg per mL.

Medium: methanol.

Absorptivities at 257 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Dissolve about 20 mg in 5 mL of phosphoric acid: a green color is produced.

D: To 5 mL of a solution (1 in 500) add bromine TS dropwise: a yellow precipitate, which dissolves on shaking, is formed. Addition of more bromine TS produces a precipitate that does not dissolve on shaking.

E: A 5-mL portion of a solution (1 in 500) responds to the tests for *Chloride* (191).

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Eluant:* a mixture of methanol and ammonium hydroxide (100:1.5).

*Visualization:* 17.

**Assay**—Accurately weigh about 500 mg of Biperiden Hydrochloride, and dissolve in 80 mL of glacial acetic acid, warming slightly, if necessary, to effect solution. Cool, add 1 drop of crystal violet TS and 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.79 mg of  $C_{21}H_{29}NO \cdot HCl$ .

## Biperiden Hydrochloride Tablets

**DEFINITION**

Biperiden Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of  $C_{21}H_{29}NO \cdot HCl$ .

**IDENTIFICATION**

- THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** Dissolve 10 mg of USP Biperiden Hydrochloride RS in 5 mL of water, mix, and sonicate to disperse the powder. Add 5 mL of methanol to the flask, mix, and sonicate for 15 min. Filter the solution into a separator, add 2 mL of 1 N sodium hydroxide and 10 mL of chloroform, and shake for 3 min. Filter the chloroform layer into a stoppered flask, and use the chloroform filtrate.

**Sample solution:** To a quantity of finely powdered Tablets, equivalent to 10 mg of biperiden hydrochloride, add 5 mL of water, mix, and sonicate to disperse the powder. Add 5 mL of methanol to the flask, mix, and sonicate for 15 min. Filter the solution into a separator, add 2 mL of 1 N sodium hydroxide and 10 mL of chloroform, and shake for 3 min. Filter the chloroform layer into a stoppered flask, and use the chloroform filtrate.

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture. Condition by heating the plate at 105° for 1 h and allowing to cool.

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Methanol and ammonium hydroxide (100:1.5)

**Visualization:** Iodine vapor, 10 min

**Analysis:** Separately apply the *Sample solution* and the *Standard solution* to the chromatographic plate. Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by exposing the plate for 10 min to iodine vapors in a preequilibrated closed chamber, on the bottom of which there are iodine crystals.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**

- PROCEDURE**

**Solution A:** 38 g/L of monobasic sodium phosphate and 2 g/L of anhydrous dibasic sodium phosphate in water. Adjust to a pH of  $5.3 \pm 0.1$ , if necessary.

**Solution B:** Dissolve 400 mg of bromocresol purple in 30 mL of water, add 6.3 mL of 0.1 N sodium hydroxide, and dilute with water to 500 mL.

**Phosphate buffer–bromocresol purple solution:** Mix equal volumes of *Solution A*, *Solution B*, and chloroform, shake in a separator, and discard the chloroform. If ap-

preciable color is extracted, repeat with additional portions of chloroform until no color is extracted.

**Standard stock solution:** 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

**Standard solution:** 40  $\mu$ g/mL of USP Biperiden Hydrochloride RS, prepared as follows: Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask, add 25% of the flask volume of water, and dilute with methanol to volume.

**Sample solution:** Nominal concentration of 40  $\mu$ g/mL of biperiden hydrochloride from NLT 20 Tablets, prepared as follows: Transfer a portion of finely powdered Tablets, to obtain the final nominal concentration, to a suitable volumetric flask; add 25% of the volume of water; and heat on a steam bath for 15 min. Cool, and dilute with methanol to volume.

**Blank:** Methanol and water (3:1)

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank* Transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 20.0 mL of chloroform for 2 min. After the layers have separated, pass each chloroform extract through filter paper (Whatman No. 31 or equivalent) into separate glass-stoppered, 50-mL volumetric flasks. In the same manner, extract the solution in each separator with another 20.0-mL portion of chloroform, filter, and wash each filter with 8 mL of chloroform, collecting each combined filtrate and washing, respectively, in the 50-mL volumetric flask containing the first extract. Dilute each with chloroform to volume. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 408 nm, with a suitable spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of the label claim of  $C_{21}H_{29}NO \cdot HCl$  in the Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Biperiden Hydrochloride RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS**

- DISSOLUTION (711)**

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

[NOTE—Determine the amount of  $C_{21}H_{29}NO \cdot HCl$  dissolved by using the following method.]

**Phosphate buffer–bromocresol purple solution:** Prepare as directed in the Assay.

**Standard stock solution:** 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

**Standard solution:** 2  $\mu$ g/mL of USP Biperiden Hydrochloride RS, prepared as follows: Pipet 5 mL of *Standard stock solution* into a 500-mL volumetric flask, and add 0.01 N hydrochloric acid to volume. Pipet 25 mL of this solution into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Sample solution:** Sample per *Dissolution (711)*. Filter 75 mL of the solution under test, pipet 50 mL of the clear filtrate into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solu-

tion to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Blank:** Water

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Pipet 20.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* into individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 40.0 mL of chloroform for 10 min. After the layers have separated, pass each chloroform extract through filter paper into separate, glass-stoppered containers, discarding the first 10 mL of each filtrate. Determine the amount of  $C_{21}H_{29}NO \cdot HCl$  dissolved from absorbances at the wavelength of maximum absorbance at about 408 nm (10-cm cells) of the extract from the *Sample solution* in comparison with that of the extract from the *Standard solution*, using the *Blank* to set the instrument.

**Tolerances:** NLT 75% (Q) of  $C_{21}H_{29}NO \cdot HCl$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Biperiden Hydrochloride RS

## Biperiden Lactate Injection

$C_{21}H_{29}NO \cdot C_3H_6O_3$  401.54

1-Piperidinepropanol,  $\alpha$ -bicyclo[2.2.1]hept-5-en-2-yl- $\alpha$ -phenyl-, compd. with 2-hydroxypropanoic acid (1:1).  
 $\alpha$ -5-Norbornen-2-yl- $\alpha$ -phenyl-1-piperidinepropanol lactate (salt) [7085-45-2].

» Biperiden Lactate Injection is a sterile solution of biperiden lactate ( $C_{21}H_{29}NO \cdot C_3H_6O_3$ ) in Water for Injection, prepared from Biperiden with the aid of Lactic Acid. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{21}H_{29}NO \cdot C_3H_6O_3$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards (11)**—

USP Biperiden RS  
USP Endotoxin RS

**Identification**—Using a volume of Injection, equivalent to about 50 mg of biperiden lactate, and using a solution of 50 mg of USP Biperiden RS in 25 mL of 0.01 N hydrochloric acid, proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with “Transfer the liquid to a separator”: the Injection meets the requirements of the test.

**Bacterial endotoxins (85)**—It contains not more than 83.3 USP Endotoxin Units per mg of biperiden lactate.

**pH (791):** between 4.8 and 5.8.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Phosphate buffer–bromocresol purple solution*—Prepare as directed in the Assay under *Biperiden Hydrochloride Tablets*.

*Standard preparation*—Transfer about 80 mg of USP Biperiden RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, add 25 mL of water, dilute with methanol to volume, and mix to

obtain a *Standard preparation* having a known concentration of about 40  $\mu$ g per mL.

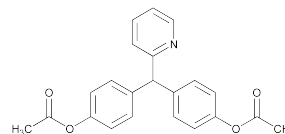
*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of biperiden lactate, to a 100-mL volumetric flask, add 25 mL of water, dilute with methanol to volume, and mix.

*Procedure*—Proceed as directed in the Assay under *Biperiden Hydrochloride Tablets*. Calculate the quantity, in mg, of  $C_{21}H_{29}NO \cdot C_3H_6O_3$  in each mL of the Injection taken by the formula:

$$(401.55 / 311.47)(0.1C / V)(A_U / A_S)$$

in which 401.55 and 311.47 are the molecular weights of biperiden lactate and biperiden, respectively; C is the concentration, in  $\mu$ g per mL, of USP Biperiden RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisacodyl



$C_{22}H_{19}NO_4$  361.39  
Phenol, 4,4'-(2-pyridinylmethylene)bis-, diacetate (ester);  
4,4'-(2-Pyridylmethylene)diphenol diacetate (ester);  
4,4'-(Pyridin-2-ylmethylene)diphenyl diacetate [603-50-9].

**DEFINITION**

Bisacodyl contains NLT 98.0% and NMT 101.0% of  $C_{22}H_{19}NO_4$ , calculated on the dried basis. **[CAUTION—**Avoid inhalation and contact with the eyes, skin, and mucous membranes.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197S)**

Cell: 1.0 mm

**Sample solution:** 5 mg/mL in chloroform, previously dried

- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard stock solution*, as obtained under *Organic Impurities*.

**ASSAY**

- **PROCEDURE**

**Sample solution:** Dissolve 300 mg of Bisacodyl in 60 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 36.14 mg of  $C_{22}H_{19}NO_4$ .

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **HEAVY METALS (231), Method II:** NMT 10 ppm
- **ORGANIC IMPURITIES**

**Buffer:** 1.58 g/L of ammonium formate in water, adjusted with formic acid to a pH of 5.0

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Diluent:** Acetonitrile and water (35:5)

**Standard stock solution:** 1.0 mg/mL of USP Bisacodyl RS in *Diluent*

**Standard solution:** 1.0  $\mu$ g/mL of USP Bisacodyl RS in *Diluent*

**System suitability solution:** 0.8 mg/mL of USP Bisacodyl RS; 2 µg/mL each of USP Bisacodyl Related Compounds A, C, and E RS; and 4 µg/mL of USP Bisacodyl Related Compound B RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Bisacodyl in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 25-cm; 4-µm or 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Run time:** 3.5 times the retention time of bisacodyl

**Injection size:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*. [NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Relative standard deviation:** NMT 5.0% for the bisacodyl peak, *Standard solution*

**Tailing factor:** NMT 2.0 for the bisacodyl peak, *System suitability solution*

**Resolution:** NLT 1.5 between the bisacodyl related compound E and bisacodyl peaks, *System suitability solution*

#### Analysis

**Samples:** *Standard stock solution*, *Standard solution*, and *Sample solution*

[NOTE—Chromatograph the *Standard stock solution* to perform *Identification* test B.]

Calculate the percentage of any individual impurity in the portion of Bisacodyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of bisacodyl from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*. [NOTE—The reporting level for impurities is 0.05%.]

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bisacodyl related compound A <sup>a</sup>	0.20	1.7	0.15
Bisacodyl related compound B <sup>b</sup>	0.40	1.5	0.15
Bisacodyl related compound C <sup>c</sup>	0.45	1.3	0.50
Specified unidentified impurity 1	0.85	1.0	0.20
Bisacodyl related compound E <sup>d</sup>	0.90	1.0	0.50
Bisacodyl	1.0	—	—
Specified unidentified impurity 2	2.6	1.0	0.30

<sup>a</sup> 4,4'-Diphenol impurity.

<sup>b</sup> 2,4'-Diphenol impurity.

<sup>c</sup> Monoacetyl bisacodyl.

<sup>d</sup> 2,4'-Bisacodyl analog.

**Table 1** (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> 4,4'-Diphenol impurity.

<sup>b</sup> 2,4'-Diphenol impurity.

<sup>c</sup> Monoacetyl bisacodyl.

<sup>d</sup> 2,4'-Bisacodyl analog.

#### SPECIFIC TESTS

- **Loss on Drying** (731): Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

#### • USP REFERENCE STANDARDS <11>

USP Bisacodyl RS

USP Bisacodyl Related Compound A RS

4,4'-(Pyridin-2-ylmethylene)diphenol.

C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub> 277.32

USP Bisacodyl Related Compound B RS

2,4'-(Pyridin-2-ylmethylene)diphenol.

C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub> 277.32

USP Bisacodyl Related Compound C RS

4-[(4-Hydroxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.

C<sub>20</sub>H<sub>17</sub>NO<sub>3</sub> 319.35

USP Bisacodyl Related Compound E RS

2-[(4-Acetoxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.

C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> 361.39

## Bisacodyl Suppositories

» Bisacodyl Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>.

**Packaging and storage**—Preserve in well-closed containers at a temperature not exceeding 30°.

#### USP Reference standards <11>—

USP Bisacodyl RS

#### Identification—

**A:** Transfer a quantity of Suppositories, equivalent to about 150 mg of bisacodyl, to a 500-mL conical flask, add 75 mL of solvent hexane, and heat on a steam bath until they are melted. Filter the solution, with the aid of vacuum, through a medium-porosity, sintered-glass funnel, and wash the residue with about 100 mL of warm solvent hexane until it is free from fat. Continue the vacuum until the residue appears dry. Dissolve the residue by rinsing the filter with about 50 mL of warm acetone, collecting the filtrate in a 150-mL beaker, and evaporate the filtrate on a steam bath to a volume of about 5 mL. To the residual liquid add about 75 mL of water, heat on a steam bath for 15 minutes, and cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 minutes: the bisacodyl so obtained melts between 129° and 135°, and responds to *Identification* test A under *Bisacodyl*.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for bisacodyl, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of 0.074 M sodium acetate in water [adjusted with 2.5% (v/v) acetic acid to a pH of 7.4] and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bisacodyl RS in acetonitrile to obtain a *Standard preparation* having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer a number of Suppositories, equivalent to about 100 mg of bisacodyl, to a 500-mL separator, add 150 mL of *n*-hexane, and shake until all the suppositories are dissolved. Add 50 mL of acetonitrile, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 200-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 50-mL portions of acetonitrile, combining the lower layers in the volumetric flask. Dilute the combined extracts in the volumetric flask with acetonitrile to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 265-nm detector, a 3.9-mm × 30-cm column that contains packing L1, and a guard column that contains packing L2. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> in the Suppositories taken by the formula:

$$200C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisacodyl Rectal Suspension

» Bisacodyl Rectal Suspension is a suspension of Bisacodyl in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>.

**Packaging and storage**—Preserve in unit-dose containers at a temperature not exceeding 30°.

**USP Reference standards** <11>—

USP Bisacodyl RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**pH** <791>: between 5.0 and 6.8.

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 0.01 M monobasic potassium phosphate (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Dissolve a suitable quantity of ethylparaben in methanol, and dilute with an equal volume of water to obtain a solution containing about 5.0 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bisacodyl RS in methanol, add an accurately measured volume of *Internal standard solution*, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 67 µg per mL and 250 µg per mL for bisacodyl and ethylparaben, respectively.

**Assay preparation**—Transfer an accurately measured volume of Rectal Suspension, equivalent to 6.7 mg of bisacodyl, to a 100-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 2.0 for bisacodyl and 1.0 for ethylparaben; the resolution, *R<sub>s</sub>*, between bisacodyl and the internal standard is not less than 7.0; the column efficiency, determined for the analyte peak, is not less than 2000 theoretical plates; the tailing factor is not more than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> in the portion of Rectal Suspension taken by the formula:

$$100C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the bisacodyl peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisacodyl Delayed-Release Tablets

» Bisacodyl Delayed-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>. Bisacodyl Delayed-Release Tablets are enteric coated.

**Packaging and storage**—Preserve in well-closed containers at a temperature not exceeding 30°.

**Labeling**—Label the Tablets to indicate that they are enteric coated.

**USP Reference standards** <11>—

USP Bisacodyl RS

**Identification—**

**A:** Macerate a portion of powdered Tablets, equivalent to about 300 mg of bisacodyl, with 100 mL of acetone. Heat on a steam bath to boiling, filter, and evaporate to about 20 mL. Add 200 mL of water, and warm the mixture on the steam bath, passing a stream of nitrogen over the surface to evaporate the acetone. After 30 minutes, cool the mixture, and filter through a sintered-glass funnel. Discard the filtrate, and dissolve the crystals in 50 mL of acetone. Evaporate the solution to about 15 mL, add about 75 mL of water, heat on a steam bath for 15 minutes, and then cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 minutes: the bisacodyl so obtained responds to *Identification* test A under *Bisacodyl*.

**B:** The retention time of the major peak for bisacodyl in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Disintegration** (701)—Proceed as directed for *Delayed-Release (enteric coated) Tablets*: the tablets do not disintegrate after 1 hour of agitation in simulated gastric fluid TS, but then disintegrate within 45 minutes in simulated intestinal fluid TS.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Bisacodyl Suppositories*.

*Assay preparation*—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to 100 mg of bisacodyl, to a 200-mL volumetric flask, add 25 mL of water, and shake by mechanical means for 15 minutes followed by sonication for 15 minutes. Add 100 mL of acetonitrile, and shake by mechanical means for 15 minutes followed by sonication for 15 minutes. Dilute with acetonitrile to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{19}NO_4$  in the Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Milk of Bismuth

» Milk of Bismuth contains bismuth hydroxide and Bismuth Subcarbonate in suspension in water, and yields not less than 5.2 percent and not more than 5.8 percent (w/w) of bismuth trioxide ( $Bi_2O_3$ ).

Bismuth Subnitrate . . . . .	80 g
Nitric Acid . . . . .	120 mL
Ammonium Carbonate . . . . .	10 g
Strong Ammonia Solution,	
Purified Water, each, a sufficient	
quantity, to make . . . . .	1000 mL

Mix the Bismuth Subnitrate with 60 mL of Purified Water and 60 mL of the Nitric Acid in a suitable container, and agitate, warming gently until solution is effected. Pour this solution, with constant stirring, into 5000 mL of Purified Water containing 60 mL of the Nitric Acid. Dilute 160 mL of

Strong Ammonia Solution with 4300 mL of Purified Water in a glazed or glass vessel of at least 12,000-mL capacity. Dissolve the Ammonium Carbonate in this solution, and then pour the bismuth solution quickly into it with constant stirring. Add sufficient 6 N ammonium hydroxide, if necessary, to render the mixture distinctly alkaline, allow to stand until the precipitate has settled, then pour or siphon off the supernatant, and wash the precipitate twice with Purified Water, by decantation. Transfer the magma to a strainer of close texture, so as to provide continuous washing with Purified Water, the outlet tube being elevated to prevent the surface of the magma from becoming dry. When the washings no longer yield a pink color with phenolphthalein TS, drain the moist preparation, transfer to a graduated vessel, add sufficient Purified Water to make 1000 mL, and mix.

NOTE—This method of preparation may be varied, provided the product meets the following requirements.

**Packaging and storage**—Preserve in tight containers, and protect from freezing.

#### Identification—

**A:** It responds to the tests for *Bismuth* (191) and for *Carbonate* (191).

**B:** Add 1 mL of 3 N hydrochloric acid to 1 mL of Milk of Bismuth: a clear solution is produced. Pour the clear solution into 10 volumes of water: a white precipitate is formed.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 100 cfu per mL and the test for *Escherichia coli* is negative.

**Water-soluble substances**—Boil 10 mL with 90 mL of water for 10 minutes, cool, add water to make the total volume 100 mL, mix, and filter. Evaporate 50 mL of the filtrate to dryness, and ignite it gently: the weight of the residue does not exceed 5 mg (0.1%).

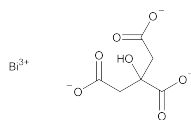
**Arsenic, Method I** (211)—Evaporate 3.75 mL on a steam bath to dryness, add 2 mL of sulfuric acid, and heat until copious fumes of sulfur trioxide are evolved. The limit is 0.8 ppm.

**Lead**—To 5 mL add warm nitric acid, dropwise, until it is just dissolved, and pour the solution into 50 mL of water: a white precipitate may form. Filter, if necessary, evaporate the filtrate on a steam bath to 15 mL, again filter, and to 10 mL of the filtrate add an equal volume of 2 N sulfuric acid: no precipitate is formed.

**Limit of alkalies and alkaline earths**—Dissolve 2.0 mL in 5 mL of hydrochloric acid, dilute with water to 100 mL, add hydrogen sulfide to precipitate the bismuth completely, and filter. To 50 mL of the clear filtrate add 5 drops of sulfuric acid, evaporate to dryness, and ignite: the weight of the residue does not exceed 3 mg (0.3%).

**Assay**—Evaporate an accurately weighed quantity of Milk of Bismuth to dryness, and ignite the residue to constant weight. From the weight of the  $Bi_2O_3$  so obtained determine the percentage in the assay specimen.

## Bismuth Citrate



$\text{BiC}_6\text{H}_5\text{O}_7$  398.08 [813-93-4].

» Bismuth Citrate contains not less than 49 percent and not more than 54 percent of bismuth (Bi).

**Packaging and storage**—Preserve in tight, light-resistant containers, store at controlled room temperature, and prevent exposure to excessive heat.

**USP Reference standards** (11)—  
USP Bismuth Citrate RS

### Identification—

**A:** *Infrared Absorption* (197K): on the undried specimen.

**B:** When strongly heated, the salt chars, and on ignition leaves a more or less blackened residue having a yellow surface. The residue is soluble in warm nitric acid, and this solution, when dropped into a large excess of water, produces a white turbidity.

**C:** Dissolve 1 g in ammonia TS. When treated with hydrogen sulfide in excess, a black precipitate is obtained. Filter this mixture, drive off the excess hydrogen sulfide by heating, and allow to cool. To a portion of this cooled solution add an excess of calcium hydroxide TS, and boil: a white precipitate is formed. Reserve a second portion of the cooled solution for the test for *Limit of nitrate*.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* as follows. Triturate 300 mg with an equal weight of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid: the limit is 10 µg per g.

**Limit of nitrate**—To the second portion of cooled solution reserved from *Identification* test C, add an equal volume of sulfuric acid, mix, and allow to cool. Into the liquid, drop a crystal of ferrous sulfate, and allow to stand for 30 minutes: no brown or brownish black color appears around the crystal.

### Limit of copper, lead, and silver—

**Standard solution**—Prepare a solution containing 1000 µg of copper per mL, a solution containing 1000 µg of lead per mL, and a solution containing 1000 µg of silver per mL. Transfer 3.0 mL of each solution to a 2000-mL volumetric flask, dilute with 1 N nitric acid to volume, and mix. [NOTE—The concentrations of copper, lead, and silver in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]

**Test solution**—Ignite about 3 g of Bismuth Citrate, accurately weighed, in a porcelain crucible, cool, and cautiously add 6 N nitric acid to dissolve the residue. Add 100 mL of water, and mix. A white precipitate forms. Filter this mixture, evaporate on a steam bath to obtain about 15 mL of solution, and filter again. Dilute the filtrate with water to 20.0 mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission lines of 324.7 nm, 217 nm, and 328.1 nm for copper, lead, and silver, respectively, with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with copper, lead, and silver hollow-cathode lamps and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* for each element (10 µg per g).

### Limit of soluble bismuth—

**Standard solution**—Transfer 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask. Add 3 mL of 1.5 N nitric acid, swirl to dissolve, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 N nitric acid, dilute with water to volume, and mix. This solution contains 2.0 µg of bismuth (Bi) per mL. [NOTE—The concentration of bismuth in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]

**Test solution**—Prepare a mixture of 5.0 g of Bismuth Citrate and 100 mL of water, and stir by mechanical means the suspension thus obtained for 2 hours. Pass through filter paper. Pass the filtrate thus obtained through a filter having a 0.1-µm or finer porosity. To 10.0 mL of the filtrate add 0.1 mL of nitric acid.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission line of 223.06 nm for bismuth with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a bismuth hollow-cathode lamp and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* (40 µg per g).

**Assay**—Transfer about 300 mg of Bismuth Citrate, accurately weighed, to a porcelain crucible, and ignite. Allow to cool, add 2 mL of nitric acid to the residue, dropwise, and warm until complete solution has been effected. Add about 60 mL of water and 0.3 mL of xylenol orange TS, and titrate with 0.05 N edetate disodium VS to a yellow endpoint. Each mL of 0.05 N edetate disodium is equivalent to 10.45 mg of bismuth (Bi).

## Bismuth Subcarbonate

» Bismuth Subcarbonate contains not less than 97.6 percent and not more than 100.7 percent of  $(\text{BiO})_2\text{CO}_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**Identification**—It responds to the tests for *Bismuth* (191) and for *Carbonate* (191).

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.

**Chloride** (221)—Mix 5.0 g of it with 10 mL of water, add 20 mL of nitric acid, warm to achieve dissolution, allow to cool, and dilute with water to obtain 100 mL of solution. To 6.6 mL of this stock solution add 4 mL of nitric acid, and dilute with water to obtain 50 mL of solution. A 15.0-mL portion of this test solution shows no more chloride than corresponds to 70 µL of 0.020 N hydrochloric acid (0.05%).

**Limit of alkalis and alkaline earths**—Boil 1.0 g of it with 20 mL of a mixture of acetic acid and water (1:1). After 2 minutes, cool and filter. Collect the filtrate, wash the residue with 20 mL of water, and add the washing to the filtrate. To this solution add 2 mL of 2 N hydrochloric acid and 20 mL of water. Heat to boiling and precipitate the bismuth by adding hydrogen sulfide. Cool the mixture, and filter. Collect the filtrate, wash the residue with water, and add the washing to the filtrate. Evaporate this solution to dryness on a water bath. To the residue add 0.5 mL of sulfuric acid, dry slowly, and cool: the weight of the residue does not exceed 10 mg (1.0%).

### Limit of nitrate—

**Indigo carmine titrant**—Dissolve 4 g of indigo carmine in 900 mL of water, add 2 mL of sulfuric acid, and dilute with water to 1000 mL.

**Standard solution**—Prepare a solution of potassium nitrate in water containing 0.0815 mg per mL (equivalent to 0.05 mg of nitrate per mL). Add 20.0 mL of this solution to a 125-mL conical flask (*Standard solution*).

**Test preparation**—To 250 mg of Bismuth Subcarbonate in a 125-mL conical flask add 20 mL of water, and swirl to suspend.

**Procedure**—To the *Standard solution* and the *Test preparation* add 0.05 mL of *Indigo carmine titrant*. Carefully add 30 mL of sulfuric acid, and immediately titrate with *Indigo carmine titrant* to a stable blue endpoint. The volume of *Indigo carmine titrant* consumed by the *Test preparation* does not exceed that consumed by the *Standard solution* (0.4%).

**Limit of silver**—To 2.0 g of Bismuth Subcarbonate add 1 mL of water and 4 mL of nitric acid. Heat gently to achieve dissolution, add water to obtain 11 mL of solution, and cool. Add 2 mL of 1 N hydrochloric acid, and allow to stand in a dark place for 5 minutes. No more turbidity is produced than corresponds to that produced with 10 mL of a solution containing 7.87 µg of silver nitrate per mL concomitantly treated with 1 mL of nitric acid and 2 mL of 1 N hydrochloric acid (0.0025%).

**Arsenic, Method I** (211)—Prepare the *Test Preparation* by dissolving 600 mg of it in 35 mL of 3 N hydrochloric acid. The limit is 5 µg per g.

#### Limit of copper—

**Standard solution**—To a 100-mL volumetric flask add 1.34 g of cupric chloride, 10 g of ammonium chloride, and 3 mL of sodium metabisulfite solution (275 mg per mL). Dilute with water to volume, and mix. This stock solution contains the equivalent of 5 mg of copper per mL. Dilute an accurately measured volume of this solution quantitatively and stepwise with 2 N nitric acid to obtain a solution containing the equivalent of 10 µg of copper per mL. Mix 0.25 mL of this solution and 9.75 mL of water (*Standard solution*).

**Test solution**—To 5 mL of the stock solution retained from the test for *Chloride* add 2 mL of 6 N ammonium hydroxide, dilute with water to 50 mL, mix, and filter. Use the filtrate as the *Test solution*.

**Procedure**—To 10 mL of the *Standard solution* and the *Test solution* add 1 mL of a solution of sodium diethyldithiocarbamate (1 in 1000): no more color is obtained from the *Test solution* than is obtained from the *Standard solution* (0.005%).

#### Limit of lead—

**Diluent**—Use 6 N nitric acid that is lead-free.

**Standard solutions**—Prepare a solution of lead nitrate in *Diluent* containing 0.1598 mg per mL. This solution contains 100 µg of lead per mL. Dilute an accurately measured volume of this solution, quantitatively and stepwise, with *Diluent* to obtain *Standard solutions* containing 1.0, 2.0, and 3.0 µg of lead per mL.

**Test solution**—Dissolve 12.5 g of Bismuth Subcarbonate in 75 mL of *Diluent*. Heat to boiling for 1 minute, cool, and dilute with water to 100 mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the lead emission line of 283.3 nm with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a lead hollow-cathode lamp and an air-acetylene flame, using a 1:5 dilution of the *Diluent* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg per mL, of lead, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of lead in the *Test solution*. Calculate the percentage of lead

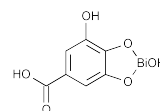
(Pb) in the portion of Bismuth Subcarbonate taken by the formula:

$$C / 1250.$$

The limit is 0.002%.

**Assay**—Dissolve about 500 mg of Bismuth Subcarbonate, accurately weighed, in 3 mL of nitric acid. Dilute with water to 250 mL, add 0.3 mL of xylenol orange TS, and titrate with 0.05 M edetate disodium VS to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 12.75 mg of (BiO)<sub>2</sub>CO<sub>3</sub>.

## Bismuth Subgallate



C<sub>7</sub>H<sub>5</sub>BiO<sub>6</sub> 394.09

Gallic acid bismuth basic salt [99-26-3].

» Bismuth Subgallate is a basic salt which, when dried at 105° for 3 hours, contains the equivalent of not less than 52.0 percent and not more than 57.0 percent of Bi<sub>2</sub>O<sub>3</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### Identification—

**A:** When heated to redness, it at first chars, leaving finally a yellow residue. This residue responds to the tests for *Bismuth* (191).

**B:** Agitate thoroughly about 100 mg with an excess of hydrogen sulfide TS, filter, and boil the filtrate to expel the dissolved gas. Cool, and add 1 drop of ferric chloride TS: a purplish blue mixture is produced.

**Loss on drying** (731)—Dry it at 105° for 3 hours. It loses not more than 7.0% of its weight.

**Limit of nitrate**—Mix about 100 mg with 5 mL of 2 N sulfuric acid and 5 mL of ferrous sulfate TS, filter the mixture, and carefully superimpose the filtrate, without mixing, on 5 mL of sulfuric acid, in a test tube: no reddish brown color appears at the zone of contact of the two liquids.

**Arsenic**—Triturate 400 mg with an equal weight of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid: the solution without further treatment meets the requirements of the test for *Arsenic* (211) (7.5 ppm).

**Copper, Lead, and Silver**—Ignite 3 g in a porcelain crucible, cool, and cautiously add, dropwise, just sufficient nitric acid to dissolve the residue upon warming. Evaporate the solution to dryness, again ignite, and cool. Cautiously dissolve the residue in just sufficient nitric acid with the aid of gentle heat, concentrate the solution to about 4 mL, pour it into 100 mL of water, filter, evaporate the filtrate on a steam bath to 20 mL, again filter, and divide this filtrate into portions of 5 mL each. Using these several portions as the test liquid, proceed as directed for *Copper*, *Lead*, and *Silver* under *Bismuth Subnitrate*. The specified results are obtained.

**Limit of alkalies and alkaline earths**—Boil 1.0 g with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Precipitate the bismuth from the filtrate by the addition of hydrogen sulfide, boil the mixture, and filter. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.5%).



**Free gallic acid**—Shake 1.0 g with 20 mL of alcohol for 1 minute, filter and evaporate the filtrate to dryness on a steam bath, and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 5 mg (0.5%).

**Assay**—Dry about 1 g of Bismuth Subgallate at 105° for 3 hours, then weigh accurately and ignite in a porcelain crucible. Allow it to cool and add nitric acid to the residue, dropwise, warming until complete solution has been effected. Evaporate the solution to dryness and carefully ignite the residue to constant weight. From the weight of the residue so obtained, determine the percentage of Bi<sub>2</sub>O<sub>3</sub> in the portion of Bismuth Subgallate taken.

## Bismuth Subnitrate

Bi<sub>5</sub>O(OH)<sub>9</sub>(NO<sub>3</sub>)<sub>4</sub> 1461.99  
Bismuth hydroxide nitrate oxide Bi<sub>5</sub>O(OH)<sub>9</sub>(NO<sub>3</sub>)<sub>4</sub>.  
Bismuth hydroxide nitrate oxide Bi<sub>5</sub>O(OH)<sub>9</sub>(NO<sub>3</sub>)<sub>4</sub>  
[1304-85-4].

» Bismuth Subnitrate is a basic salt that contains the equivalent of not less than 79.0 percent of bismuth trioxide (Bi<sub>2</sub>O<sub>3</sub>), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—It responds to the tests for *Bismuth* (191) and for *Nitrate* (191).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 3.0% of its weight.

**Carbonate**—Add 3 g to 3 mL of warm nitric acid: no effervescence occurs. Pour the solution into 100 mL of water: a white precipitate forms. Filter, evaporate the filtrate on a steam bath to 30 mL, again filter the liquid, divide the latter filtrate into portions of 5 mL each, and use these several portions in the tests for *Chloride*, *Sulfate*, *Copper*, *Lead*, and *Silver*.

**Chloride** (221)—A 10-mL portion of the test liquid retained in the test for *Carbonate* shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

**Sulfate** (221)—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add 5 drops of barium nitrate TS: no turbidity is produced immediately.

**Limit of ammonium salts**—Boil about 100 mg with 5 mL of 1 N sodium hydroxide: the vapor does not turn moistened red litmus paper blue.

**Arsenic, Method I** (211)—Mix 375 mg with 5 mL of water, cautiously add 2 mL of sulfuric acid, and heat the mixture until fumes of sulfur trioxide are copiously evolved. Cool, cautiously add 10 mL of water, and again evaporate to strong fuming, repeating, if necessary, to remove any trace of nitric acid. The limit is 8 ppm.

**Copper**—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add a slight excess of 6 N ammonium hydroxide: the liquid does not exhibit a bluish color.

**Lead**—Mix a 5-mL portion of the test liquid retained in the test for *Carbonate* with an equal volume of 2 N sulfuric acid: the liquid does not become cloudy.

**Silver**—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add hydrochloric acid, dropwise: no precipitate is formed that is insoluble in a slight excess of hydrochloric acid, but that is soluble in 6 N ammonium hydroxide.

**Limit of alkalis and alkaline earths**—Boil 1.0 g with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Add 2 mL of 3 N hydrochloric acid, precipitate the bismuth by the addition of hydrogen sulfide,

boil the mixture, and filter it. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.5%).

**Assay**—Transfer about 400 mg of Bismuth Subnitrate, accurately weighed, to a 250-mL beaker. Add 5 mL of water, then add 2 mL of nitric acid, and warm, if necessary, to effect solution. Dilute with water to 100 mL, add 0.3 mL of xylenol orange TS, and titrate with 0.05 M edetate disodium VS to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 11.65 mg of Bi<sub>2</sub>O<sub>3</sub>.

## Bismuth Subsalcylate

C<sub>7</sub>H<sub>5</sub>BiO<sub>4</sub> 362.09  
(2-Hydroxybenzoato-O<sup>1</sup>)-oxobismuth;  
2-Hydroxybenzoic acid bismuth (3+) salt, basic  
[14882-18-9].

### DEFINITION

Bismuth Subsalcylate is a basic salt that contains NLT 56.0% and NMT 59.4% of bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. IDENTIFICATION TESTS—GENERAL**, *Bismuth* (191): Meets the requirements

### ASSAY

#### • BISMUTH

**Sample solution:** Transfer an equivalent to 300 mg of Bismuth Subsalcylate, previously dried at 105° for 3 h, to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylenol orange TS.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).

**Acceptance criteria:** 56.0%–59.4% of bismuth (Bi) on the dried basis

#### • TOTAL SALICYLATES

**Solution A:** Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)

**Standard stock solution:** 0.2 mg/mL of USP Salicylic Acid RS in water

**Standard solution:** 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.

**Reacted standard solution:** To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.

**Unreacted standard solution:** To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Sample solution:** Transfer 52 mg of Bismuth Subsalcylate, previously dried at 105° for 3 h, into a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Reacted sample solution:** To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.

**Unreacted sample solution:** To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Blank:** Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5

**Reacted blank solution:** To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.

**Unreacted blank:** To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** 525 nm

#### Analysis

**Samples:** *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank solution*. Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of total salicylates in the portion of dried Bismuth Subsalicylate taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_S/C_U) \times 100$$

$A_{UR}$  = absorbance of the *Reacted sample solution*

$A_{UU}$  = absorbance of the *Unreacted sample solution*

$B$  = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*

$A_{SR}$  = absorbance of the *Reacted standard solution*

$A_{SU}$  = absorbance of the *Unreacted standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bismuth Subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 36.5%–39.3% of total salicylates on the dried basis

#### IMPURITIES

##### • ARSENIC, Method I (211)

**Sample:** 300 mg of Bismuth Subsalicylate with 300 mg of calcium hydroxide

**Analysis:** Triturate the *Sample*, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid.

**Acceptance criteria:** 10 ppm

##### • LIMIT OF COPPER, LEAD, AND SILVER

**Standard stock solution:** Add 3.0 mL each of 1000-µg/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.

**Standard solution:** 1.5 µg/mL of copper, 1.5 µg/mL of lead, and 1.5 µg/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** Ignite 3 g of sample in a porcelain crucible, cool, and cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask, and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver

**Lamps:** Copper, lead, and silver hollow-cathode, and oxidizing flames

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

##### • LIMIT OF SOLUBLE BISMUTH

**Standard solution:** 2 µg/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** 5.0 g of Bismuth Subsalicylate in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1-µm or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 223.06 nm for bismuth

**Lamp:** Bismuth hollow-cathode and an oxidizing flame

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

**Acceptance criteria:** 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

##### • LIMIT OF NITRATE

**Standard solution:** To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100 µg of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.

**Sample solution:** Add 10 mL of water to 0.1 g of Bismuth Subsalicylate. Carefully add 20 mL of sulfuric acid.

**Acceptance criteria:** 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

##### • LIMIT OF FREE SALICYLIC ACID

**Mobile phase:** Methanol and 0.06 M acetic acid (55:45)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Add 260 mg of Bismuth Subsalicylate to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting. Combine the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5-µm or finer pore size, and collect the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Columns**

**Guard:** 3.2-mm × 1.5-cm; 5-μm packing L1  
**Analytical:** 4.6-mm × 30-cm; 5-μm packing L1  
**Flow rate:** 1 mL/min  
**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Bismuth Subsalicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of salicylic acid from the *Sample solution*

$r_S$  = peak area of salicylic acid from the *Standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the Bismuth Subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%

**SPECIFIC TESTS**• **PH** (791)

**Sample solution:** 10 g of Bismuth Subsalicylate in 90 mL of water

**Analysis:** Shake by mechanical means for 10 min, and filter.

**Acceptance criteria:** 2.7–5.0

• **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Bismuth Subsalicylate RS

USP Salicylic Acid RS

**Bismuth Subsalicylate Magma****DEFINITION**

Bismuth Subsalicylate Magma is a suspension of Bismuth Subsalicylate in water that contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ). Bismuth subsalicylate is a basic salt that when dried at 105° for 3 h contains NLT 56.0% and NMT 59.4% bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates.

Dry at 105° for 3 h to determine the solids content and, after determining the solids content, perform all tests on a portion of the dried Magma.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197M)

• **B. IDENTIFICATION TESTS—GENERAL,** *Bismuth* (191): Meets the requirements

**ASSAY**• **BISMUTH**

**Sample solution:** Transfer an equivalent to 300 mg of bismuth subsalicylate, previously dried at 105° for 3 h, to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylene orange TS.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).

**Acceptance criteria:** 56.0%–59.4% of bismuth on the previously dried basis

• **TOTAL SALICYLATES**

**Solution A:** Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)

**Standard stock solution:** 0.2 mg/mL of USP Salicylic Acid RS in water

**Standard solution:** 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.

**Reacted standard solution:** To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.

**Unreacted standard solution:** To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Sample solution:** Transfer an equivalent to 52 mg of bismuth subsalicylate from previously dried Magma at 105° for 3 h to a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Reacted sample solution:** To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.

**Unreacted sample solution:** To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Blank:** Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5

**Reacted blank solution:** To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.

**Unreacted blank:** To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 525 nm

**Analysis**

**Samples:** *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank solution*. Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of total salicylates in the portion of dried Magma taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_S/C_U) \times 100$$

$A_{UR}$  = absorbance of the *Reacted sample solution*

$A_{UU}$  = absorbance of the *Unreacted sample solution*

$B$  = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*

$A_{SR}$  = absorbance of the *Reacted standard solution*

$A_{SU}$  = absorbance of the *Unreacted standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 36.5%–39.3% of total salicylates on the previously dried basis

**IMPURITIES****• LIMIT OF COPPER, LEAD, AND SILVER**

**Standard stock solution:** Add 3.0 mL each of 1000- $\mu$ g/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.

**Standard solution:** 1.5  $\mu$ g/mL of copper, 1.5  $\mu$ g/mL of lead, and 1.5  $\mu$ g/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** Ignite 3 g of sample in a porcelain crucible, cool, cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask, and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of bismuth subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver

**Lamps:** Copper, lead and silver hollow-cathode, and oxidizing flames

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*

**Acceptance criteria:** 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

**• LIMIT OF SOLUBLE BISMUTH**

**Standard solution:** 2  $\mu$ g/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** 5.0 g of bismuth subsalicylate from dried Magma in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1- $\mu$ m or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of bismuth subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 223.06 nm for bismuth

**Lamp:** Bismuth hollow-cathode and an oxidizing flame

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

**Acceptance criteria:** 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

**• LIMIT OF NITRATE**

**Standard solution:** To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100  $\mu$ g of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.

**Sample solution:** Add 10 mL of water to 0.1 g of Magma. Carefully add 20 mL of sulfuric acid, and mix.

**Acceptance criteria:** 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

**• LIMIT OF FREE SALICYLIC ACID**

**Mobile phase:** Methanol and 0.06 M acetic acid (550:450)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Add 260 mg of bismuth subsalicylate from dried Magma to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting, combining the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5- $\mu$ m pore size, collecting the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Columns**

**Guard:** 3.2-mm  $\times$  1.5-cm; 5- $\mu$ m packing L1

**Analytical:** 4.6-mm  $\times$  30-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Magma taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of salicylic acid from the *Sample solution*

$r_S$  = peak area of salicylic acid from of the *Standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the bismuth subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

**• LABELING:** The label states that this article is not intended for direct administration to humans or animals.

**• USP REFERENCE STANDARDS (11)**

USP Bismuth Subsalicylate RS

USP Salicylic Acid RS

## Bismuth Subsalicylate Oral Suspension

### DEFINITION

Bismuth Subsalicylate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ). It may contain one or more suitable buffers, coloring agents, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Bismuth (191):** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Salicylate (191):** Meets the requirements after acidifying with nitric acid

### ASSAY

#### • PROCEDURE

**Standard stock solution:** 2.5 mg/mL of bismuth metal in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid and diluting with 0.01 N nitric acid to volume.

**Standard solution:** 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*

**Sample solution:** Transfer 10 g of Oral Suspension, previously well shaken in its original container to ensure homogeneity, in a 200-mL volumetric flask. Add about 100 mL of 1 N nitric acid, and dilute with 1 N nitric acid to volume. Mix well without shaking, transfer 10.0 mL of this mixture to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge about 20 mL at 4500 rpm for at least 10 min.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV-Vis

**Analytical wavelength:** 463 nm

**Cell:** 1 cm

**Blank:** 1 N nitric acid

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer a measured volume of the *Sample solution* that contains 0.9 mg of bismuth subsalicylate and 10 mL of the *Standard solution* to separate 50-mL volumetric flasks. Add 10.0 mL of 10% ascorbic acid solution and 25.0 mL of 20% potassium iodide solution to each volumetric flask, and dilute with water to volume. Concomitantly determine the absorbances of both solutions, using the *Blank* to set the spectrophotometer. Calculate the percentage of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of bismuth in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bismuth subsalicylate, 362.09

$M_{r2}$  = molecular weight of bismuth, 208.98

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $10^2$  cfu/g, and the total combined molds and yeasts count is NMT  $5 \times 10^1$  cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*.
- **pH (791):** 3.0–5.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from freezing. Avoid excessive heat (over 40°).

## Bismuth Subsalicylate Tablets

### DEFINITION

Bismuth Subsalicylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Bismuth (191):** Meet the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Salicylate (191):** After acidifying with nitric acid, it meets the requirements of the test with ferric chloride TS.

### ASSAY

#### • PROCEDURE

**Standard stock solution:** 2.5 mg/mL of bismuth in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid, and diluting with 0.01 N nitric acid to volume.

**Standard solution:** 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*

**Sample stock solution:** Equivalent to 90 mg of bismuth subsalicylate from finely powdered Tablets in a 200-mL volumetric flask. Add 150 mL of 1 N nitric acid, and sonicate for 2 min. Dilute with 1 N nitric acid to volume.

**Sample solution:** Transfer 20.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge a portion at 4500 rpm for at least 10 min.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV-Vis

**Analytical wavelength:** 463 nm

**Cell:** 1 cm

**Blank:** 10% ascorbic acid solution, 20% potassium iodide solution, and 1 N nitric acid (2:5:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Transfer 10.0 mL of the *Standard solution* and the *Sample solution* to separate 50.0-mL volumetric flasks, and dilute with the *Blank* to volume. Concomitantly determine the absorbance of the solutions at the wavelength of maximum absorbance at 463 nm with a suitable spectrophotometer, using the combined reagent solutions as the blank.

Calculate the percentage of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of bismuth in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bismuth subsalicylate, 362.09

$M_{r2}$  = molecular weight of bismuth, 208.98

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISINTEGRATION <701>

This test does not apply to Tablets labeled as chewable.

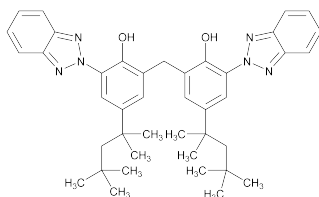
Time: 10 min

Acceptance criteria: Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Avoid excessive heat (over 40°).
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing.

## Bisotrizole



$C_{41}H_{50}N_6O_2$  658.87

Phenol, 2,2'-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]-

2,2'-Methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] [103597-45-1].

» Bisotrizole contains not less than 96.0 percent and not more than 102.0 percent of  $C_{41}H_{50}N_6O_2$ , calculated on the as-is basis.

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

### USP Reference standards <11>—

USP Bisotrizole RS

USP Bisotrizole Related Compound A RS

2-(2*H*-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol.

$C_{20}H_{25}N_3$  323.43

USP Bisotrizole Resolution Mixture RS

A mixture of approximately 1.5% of bisotrizole isomer [phenol, 2,2'-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]] in a matrix of bisotrizole.

### Identification—

**A:** Infrared Absorption <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Heavy metals, Method II <231>:** 0.002%.

### Limit of bisotrizole related compound A and the bisotrizole isomer—

*Diluent, Solution A, Solution B, and Mobile phase*—Proceed as directed in the *Assay*.

*Standard stock solution A*—Dissolve an accurately weighed quantity of USP Bisotrizole RS in tetrahydrofuran to obtain a solution having a known concentration of about 0.65 mg per mL.

*Standard stock solution B*—Dissolve an accurately weighed quantity of USP Bisotrizole Related Compound A RS in tetrahydrofuran to obtain a solution having a known concentration of about 0.40 mg per mL.

*Standard solution*—Quantitatively transfer 5 mL of *Standard stock solution A* and 1.0 mL of *Standard stock solution B* to a 100-mL volumetric flask. Add 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

*Test solution*—Proceed as directed for the *Assay preparation*.

*Chromatographic system*—Proceed as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between bisotrizole and the bisotrizole isomer is not less than 1.5. [NOTE—For identification purposes, the relative retention times are about 0.42 for bisotrizole related compound A and about 1.1 for the bisotrizole isomer.]

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of bisotrizole related compound A taken by the formula:

$$10,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bisotrizole Related Compound A RS;  $W$  is the weight, in mg, of Bisotrizole taken;  $r_U$  is the peak response for bisotrizole related compound A in the *Test solution*; and  $r_S$  is the peak response for bisotrizole related compound A in the *Standard solution*: not more than 0.5% of bisotrizole related compound A is found. Calculate the percentage of the bisotrizole isomer taken by the formula:

$$10,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bisotrizole RS;  $W$  is the weight, in mg, of Bisotrizole taken;  $r_U$  is the peak response for the bisotrizole isomer in the *Test solution*; and  $r_S$  is the peak response for bisotrizole in the *Standard solution*: not more than 4.0% of the bisotrizole isomer is found.

### Related compounds—

*Diluent, Solution A, Solution B, Mobile phase, and Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Proceed as directed for the *Assay preparation*.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Bisotrizole taken by the formula:

$$100(r_i/r_S)$$

in which  $r_i$  is the peak response for each individual impurity, and  $r_S$  is the sum of the responses of all the peaks: not more than 0.1% of any individual impurity, excluding bisotrizole related compound A and the bisotrizole isomer, is found. Not more than 4.0% of total impurities, including bisotrizole related compound A, and the bisotrizole isomer, determined in the test for *Limit of bisotrizole related compound A and the bisotrizole isomer*, is found.

### Assay—

*Diluent*—Prepare a mixture containing tetrahydrofuran and a 0.2% (w/v) aqueous solution of 1-pentane sulfonic acid sodium salt (60:40).

*Solution A*—Prepare a filtered and degassed solution containing 0.4 g of 1-pentane sulfonic acid sodium salt, 800 mL of methanol, 200 mL of water, and 0.5 mL of phosphoric acid.

*Solution B*—Prepare a filtered and degassed solution containing 0.4 g of 1-pentane sulfonic acid sodium salt, 1000 mL of methanol, and 0.5 mL of phosphoric acid.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve an accurately weighed quantity of USP Bisoprolol Resolution Mixture RS in tetrahydrofuran, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 0.8 mg per mL of bisoprolol.

**Standard preparation**—Transfer about 80 mg of USP Bisoprolol RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in 60 mL of tetrahydrofuran, dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 80 mg of Bisoprolol, accurately weighed, to a 100-mL volumetric flask. Dissolve in 60 mL of tetrahydrofuran, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 346-nm detector and a 3.0-mm × 25-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–1	70	30	isocratic
1–11	70→3	30→97	linear gradient
11–27	3	97	isocratic
27–28	3→70	97→30	linear gradient

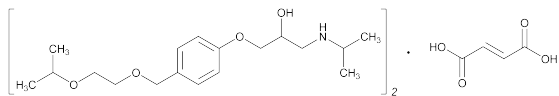
Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for bisoprolol and 1.1 for the bisoprolol isomer; the resolution, *R*, between bisoprolol and the bisoprolol isomer is not less than 1.5; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{41}H_{50}N_6O_2$  in the portion of Bisoprolol taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisoprolol RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisoprolol Fumarate



$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  766.96

2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (E)-2-butenedioate (2:1) (salt).

(±)-1-[[α-(2-isopropoxyethoxy)-p-tolyl]oxy]-3-(isopropylamino)-2-propanol fumarate (2:1) (salt) [104344-23-2].

» Bisoprolol Fumarate contains not less than 97.5 percent and not more than 102.0 percent of

$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at controlled room temperature.

**USP Reference standards** <11>—  
USP Bisoprolol Fumarate RS

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** <781>: between −2° and +2°.

*Test solution*: 10 mg per mL, in methanol.

**Water**, *Method I* <921>: not more than 0.5%.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals**, *Method I* <231>: 0.002%.

**Chromatographic purity**—

*Diluent*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Prepare as directed for *Standard preparation* in the *Assay*.

*Test solution*—Prepare as directed for *Assay preparation* in the *Assay*.

**Procedure**—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of total impurities in the portion of Bisoprolol Fumarate taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the sum of areas for all the peaks, excluding the fumaric acid and bisoprolol peaks; and *r<sub>s</sub>* is the sum of the areas of all the peaks in the chromatogram: not more than 0.5% of total impurities is found.

**Content of fumaric acid**—Transfer about 500 mg of Bisoprolol Fumarate, accurately weighed, to a beaker, and dissolve in 70 mL of dehydrated alcohol. Add 8.0 mL of 0.1 N tetrabutylammonium hydroxide VS, and stir for 2 minutes. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically, using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 5.804 mg of fumaric acid: not less than 14.8% and not more than 15.4% of fumaric acid is found, calculated on the anhydrous basis.

**Assay**—

*Diluent*—Prepare a mixture of water and acetonitrile (65:35).

**Mobile phase**—To a 1-L portion of *Diluent* add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid. Mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Prepare a solution in *Diluent* containing about 0.5 mg of propranolol hydrochloride and 1 mg of Bisoprolol Fumarate per mL.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer about 50 mg of Bisoprolol Fumarate, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 12.5-cm column that contains packing L7.

The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the resolution,  $R$ , between bisoprolol and propranolol is not less than 7.0. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  in the portion of Bisoprolol Fumarate taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisoprolol Fumarate Tablets

### DEFINITION

Bisoprolol Fumarate Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of bisoprolol fumarate  $[(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4]$ .

### IDENTIFICATION

#### • THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST <201>

**Sample solution:** Equivalent to 40 mg of bisoprolol fumarate, from powdered Tablets (NLT 5), in a 50-mL flask. Add about 20 mL of a mixture of dichloromethane and methanol (7:3), shake for 30 min, centrifuge, and use the clear solution.

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Dichloromethane, methanol, and ammonia TS, stronger (70: 10: 0.8)

#### Analysis

**Sample:** *Sample solution*

Proceed as directed in the chapter, except to develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate and to dry the plate in a current of cold air.

### ASSAY

#### • PROCEDURE

**Diluent:** Acetonitrile and water (7:13)

**Mobile phase:** A 1-L portion of *Diluent*. Add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid.

**System suitability solution:** 0.5 mg/mL of propranolol hydrochloride and 1 mg/mL of bisoprolol fumarate in *Diluent*

**Standard solution:** 1 mg/mL of USP Bisoprolol Fumarate RS in *Diluent*

**Sample solution:** Transfer an equivalent of 25 mg of bisoprolol fumarate, from powdered Tablets (NLT 20), to a 25-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Centrifuge for 20 min, and use the clear supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 4.6-mm  $\times$  12.5-cm; packing L7

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 7.0 between bisoprolol and propranolol, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bisoprolol Fumarate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bisoprolol fumarate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–105.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 20 min

Determine the amount of  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  dissolved by using the following method.

**Diluent:** Methanol, triethylamine, phosphoric acid, and water (160: 5: 2.5: 35)

**Mobile phase:** Methanol, triethylamine, and water (34:1:50). Adjust with phosphoric acid to a pH of 4.0  $\pm$  0.1.

**Standard stock solution:** USP Bisoprolol Fumarate RS in water to obtain a solution having a known concentration of about twice the concentration of bisoprolol fumarate in the *Sample solution*

**Standard solution:** *Standard stock solution* and *Diluent* (1:1)

**Sample solution:** Sample per *Dissolution* <711>. Withdraw a portion of the solution under test, filter, and dilute with an equal volume of *Diluent*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 227 nm

**Column:** 4.6-mm  $\times$  33-mm; packing L7

**Flow rate:** 1 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 80% (Q) of the labeled amount of  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.5 M sodium chloride; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 20 min

**Analysis:** Proceed as directed for *Test 1* with the following modifications.

**Diluent:** Prepare a mixture of methanol, 0.1 N hydrochloric acid, triethylamine, and phosphoric acid (160: 35: 5: 2.5). The dimensions of the column are 4.6 mm  $\times$  25 cm.



**Tolerances:** NLT 80% (Q) of the labeled amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Bisoprolol Fumarate RS  
2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy-  
y]methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (E)-  
2-butenedioate (2:1) (salt).  
 $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  766.96

### Bisoprolol Fumarate and Hydrochlorothiazide Tablets

» Bisoprolol Fumarate and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bisoprolol fumarate  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  and hydrochlorothiazide  $(C_7H_8ClN_3O_4S_2)$ .

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at controlled room temperature.

#### USP Reference standards (11)—

USP Bisoprolol Fumarate RS  
USP Chlorothiazide RS  
USP Hydrochlorothiazide RS

#### Identification—

**A:** *Thin-Layer Chromatographic Identification Test (201)*—

**Test solution**—Finely powder 1 Tablet, and transfer the powder to a 5-mL volumetric flask. Dilute with methanol to volume, sonicate for 5 minutes, centrifuge, and use the supernatant.

**Standard solution 1**—Dissolve a suitable quantity of USP Bisoprolol Fumarate RS in methanol to obtain a solution containing 1 mg per mL.

**Standard solution 2**—Dissolve a suitable quantity of USP Hydrochlorothiazide RS in methanol to obtain a solution containing 1 mg per mL.

**Application volume:** 25  $\mu$ L.

**Developing solvent system:** a mixture of methylene chloride, methanol, and 14.5 M ammonium hydroxide solution (43:20:8).

**Procedure**—Locate the spots on the plate under short-wavelength UV light and by exposure to iodine vapors: the  $R_f$  values of the principal spots in the chromatogram obtained from the *Test solution* correspond to those of the principal spots in the chromatograms obtained from *Standard solution 1* and *Standard solution 2*.

**B:** The retention times of the major peaks in the chromatograms of the *Bisoprolol fumarate assay preparation* and the *Hydrochlorothiazide assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the Assay.

#### Dissolution (711)—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 75 rpm.

**Times:** 20 minutes for bisoprolol fumarate; 30 minutes for hydrochlorothiazide.

**Triethylamine solution**—Mix 2 mL of triethylamine with 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and *Triethylamine solution* (1:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solution 1**—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Medium* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Standard stock solution 2**—Transfer about 30 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Medium* to volume, and mix.

**Standard solution**—Dilute accurately measured volumes of *Standard stock solution 1* and *Standard stock solution 2* with *Medium* to obtain a solution having known concentrations of bisoprolol fumarate and hydrochlorothiazide corresponding to those of the solution under test.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a UV detector capable of measuring peak responses at 227 nm and 272 nm, simultaneously, and a 3.9-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the peak areas for bisoprolol at 227 nm and for hydrochlorothiazide at 272 nm. Calculate the quantities, in mg, of bisoprolol fumarate  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  and hydrochlorothiazide  $(C_7H_8ClN_3O_4S_2)$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  is dissolved in 20 minutes and not less than 80% (Q) of the labeled amount of  $C_7H_8ClN_3O_4S_2$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** Meet the requirements with respect to bisoprolol fumarate and to hydrochlorothiazide.

#### Chromatographic purity—

**Diluent, Solution A, Solution B, Mobile phase, and System suitability solution**—Proceed as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Hydrochlorothiazide RS in *Diluent*, and quantitatively dilute with *Diluent*, if necessary, to obtain a solution having a known concentration of about 2  $\mu$ g per mL.

**Test stock solution**—Proceed as directed for Assay stock preparation in the Assay.

**Test solution**—Quantitatively dilute an accurately measured volume of the *Test stock solution* with *Diluent* to obtain a solution having a concentration of about 100  $\mu$ g of bisoprolol fumarate per mL.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the Assay, but use a 260-nm detector. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the re-

sponses for all the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(100/F)(W_B / W_H)(C_S / C_B)(r_i / r_S)$$

in which  $F$  is the response factor, equal to 1.2 for the peak with a relative retention time of 0.69 and 1.4 for the peak with a relative retention time of 1.2, both retention times relative to that of the hydrochlorothiazide peak;  $W_B$  and  $W_H$  are the labeled quantities, in mg, of bisoprolol fumarate and hydrochlorothiazide, respectively, in each Tablet;  $C_S$  is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard solution*;  $C_B$  is the concentration, in mg per mL, of bisoprolol fumarate in the *Test solution*;  $r_i$  is the peak response of each of the two impurities obtained from the *Test solution*; and  $r_S$  is the response for the hydrochlorothiazide peak obtained from the *Standard solution*: not more than 1.0% for the impurity with a relative retention time of 0.69 is found; and not more than 2.0% for the impurity with a relative retention time of 1.2 is found.

#### Assay—

**Diluent**—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of a mixture of water and acetonitrile (1:1).

**Solution A**—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of water.

**Solution B**—Prepare a mixture of acetonitrile and water (3:2). Add 10 mL of 1 M dibutylammonium phosphate per liter, stir vigorously for 2 minutes, filter, and degas.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution of USP Chlorothiazide RS and USP Hydrochlorothiazide RS in *Diluent* containing 40 µg of each per mL.

**Standard preparation**—Dissolve suitable quantities of USP Bisoprolol Fumarate RS and USP Hydrochlorothiazide RS in *Diluent* to obtain a solution having known concentrations of about 100 µg of each per mL. Stir by mechanical means for 1 hour.

**Assay stock preparation**—Weigh 10 Tablets, and transfer to a 100-mL volumetric flask. Add about 50 mL of *Diluent*, sonicate for 10 minutes, and cool. Dilute with *Diluent* to volume, stir by mechanical means for 1 hour, and centrifuge.

**Bisoprolol fumarate assay preparation**—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 100 µg of bisoprolol fumarate per mL.

**Hydrochlorothiazide assay preparation**—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 62.5 µg of hydrochlorothiazide per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with 225-nm detector and an 8-mm × 10-cm column that contains packing L11. The flow rate is about 3 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–9.0	100→40	0→60	linear gradient
9.0–9.1	40→100	60→0	linear gradient
9.1–12.0	100	0	re-equilibration

Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the resolution,  $R$ , between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard preparation*, and re-

cord the peak areas as directed for *Procedure*: the tailing factor for the hydrochlorothiazide peak is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation*, *Bisoprolol fumarate assay preparation*, and *Hydrochlorothiazide assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of bisoprolol fumarate ( $C_{18}H_{31}NO_4$ )<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> and hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Tablets taken by the formula:

$$5000(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS or USP Hydrochlorothiazide RS in the *Standard preparation*, as appropriate;  $V$  is the volume of the *Assay stock preparation* used to prepare the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation*;  $r_U$  is the peak area obtained from the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation*, as appropriate; and  $r_S$  is the corresponding peak area obtained from the *Standard preparation*.

## Bleomycin Sulfate

Bleomycin sulfate (salt).

Bleomycin sulfate (salt) [9041-93-4].

» Bleomycin Sulfate is the sulfate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus*, or produced by other means. It has a potency of not less than 1.5 Bleomycin Units and not more than 2.0 Bleomycin Units per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### USP Reference standards (11)—

USP Bleomycin Sulfate RS

USP Endotoxin RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** It responds to the tests for *Sulfate* (191).

**pH** (791): between 4.5 and 6.0, in a solution containing 10 Bleomycin Units per mL.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

#### Copper—

**Dilute nitric acid**—Dilute 20 mL of nitric acid to 2000 mL with water.

**Copper stock solution**—Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with *Dilute nitric acid* to volume, and mix. Store in a polyethylene bottle. This solution contains 1000 µg of copper per mL.

**Standard preparations**—Transfer 5.0 mL of *Copper stock solution* to a 100-mL volumetric flask, dilute with *Dilute nitric acid* to volume, and mix. Transfer 3.0, 9.0, and 15.0 mL, respectively, of this solution to separate 100-mL volumetric flasks, dilute the contents of each flask with *Dilute nitric acid* to volume, and mix. These *Standard preparations* contain, respectively, 1.5, 4.5, and 7.5 µg of copper per mL.

**Test preparation**—Dissolve about 75 mg of Bleomycin Sulfate, accurately weighed, in 10.0 mL of *Dilute nitric acid*.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the copper emission line at 324.8 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a copper hollow-cathode lamp and an air-acetylene flame, using *Dilute nitric acid* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of copper, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of copper in the *Test preparation*. Calculate the percentage of copper in the portion of Bleomycin Sulfate taken by the formula:

$$C / W$$

in which *W* is the weight, in mg, of Bleomycin Sulfate taken to prepare the *Test preparation*: not more than 0.1% is found.

#### Content of bleomycins—

**Mobile phase**—Dissolve 960 mg of sodium 1-pentane-sulfonate in 1000 mL of deaerated 0.08 N acetic acid, adjust with ammonium hydroxide to a pH of 4.3, filter, and degas. [NOTE—1.86 g of edetate disodium may be included if needed to obtain satisfactory chromatography.] Use a linear gradient of 10% to 40% methanol mixed with this solution, with a gradient mixing time of 60 minutes, and allow chromatography to proceed with the final gradient mixture for a further 20 minutes or until demethylbleomycin A<sub>2</sub> has been eluted.

**Test preparation**—Dissolve Bleomycin Sulfate in deaerated water to obtain a solution having a concentration of about 2.5 Bleomycin Units per mL. Store this solution in a refrigerator until just prior to use.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 250-mm stainless steel column containing packing L1. The flow rate is about 1.2 mL per minute.

**Procedure**—Inject about 10 µL of the *Test preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatogram, and measure the peak responses for all peaks. The elution order is bleomycinic acid, bleomycin A<sub>2</sub> (major peak), bleomycin A<sub>5</sub>, bleomycin B<sub>2</sub> (major peak), bleomycin B<sub>4</sub>, and demethylbleomycin A<sub>2</sub>. Calculate the percentage contents of bleomycin A<sub>2</sub>, bleomycin B<sub>2</sub>, and bleomycin B<sub>4</sub> taken by the formula:

$$100r_i / r_t$$

in which *r<sub>i</sub>* is the peak response corresponding to the particular bleomycin and *r<sub>t</sub>* is the total of the responses of all peaks: the content of bleomycin A<sub>2</sub> is between 55% and 70%; the content of bleomycin B<sub>2</sub> is between 25% and 32%; the content of bleomycin B<sub>4</sub> is not more than 1%; and the combined percentage of bleomycin A<sub>2</sub> and bleomycin B<sub>2</sub> is not less than 90%.

**Other requirements**—Where the label states that Bleomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Bleomycin for Injection*. Where the label states that Bleomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Bleomycin for Injection*.

#### Assay—

**Assay preparation**—Dissolve a suitable quantity of Bleomycin Sulfate, accurately weighed, in *Buffer No. 16*, and quantitatively dilute with *Buffer No. 16* to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with

*Buffer No. 16* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the *Standard*.

## Bleomycin for Injection

» Bleomycin for Injection contains an amount of Bleomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bleomycin.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

#### USP Reference standards (11)—

USP Bleomycin Sulfate RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

#### Identification—

**A: Infrared Absorption** (197K).

**B:** It responds to the tests for *Sulfate* (191).

**Bacterial endotoxins** (85)—It contains not more than 10.0 USP Endotoxin Units per Bleomycin Unit.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the entire contents of each container being used.

**Water, Method 1c** (921): not more than 6.0%. Prepare the specimen for test as follows. Use a dry syringe to inject 4 mL of anhydrous methanol through the stoppers of two tared containers, respectively, and shake to dissolve. Using the same syringe, aspirate the contents of the two containers, transfer to the titration vessel, and titrate. Perform a blank determination on 8 mL of the anhydrous methanol. Determine the weights of the empty containers, and calculate the percentage of water.

**Other requirements**—It meets the requirements for *pH*, *Copper*, and *Content of bleomycins* under *Bleomycin Sulfate*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

#### Assay—

**Assay preparation**—Constitute Bleomycin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer No. 16* to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer No. 16* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the *Standard*.

## Bovine Acellular Dermal Matrix

» Bovine Acellular Dermal Matrix is a remodelable collagen scaffold derived from fetal or neonatal bovine skin. It is presented to the physician as a flat white sheet that is cut to size and hydrated in room temperature sterile saline solution prior to implantation. It is utilized as a structural scaffold in orthopedic, neurosurgical, urogynecological, dermatological, plastic, and other recon-

structive procedures to contribute to the repair, reinforcement, and generation of tissue. The sterile material is surgically secured, onlayed, and/or packed into deficient soft tissues such as skin, tendon, muscle, and dura mater.

The source fetal or neonatal bovine skin is mechanically and chemically processed to isolate the dermis and remove cells and cellular components. To prevent the transmission of infectious disease, the manufacturing process has been validated to inactivate viruses potentially present in the source material. To prevent the spread of transmissible spongiform encephalopathies, the source material is acquired from appropriate geographic locations in accordance with relevant guidelines subject to governmental oversight. The product is inspected and tested to assure the product meets specifications.

**Packaging and storage**—The package is a sealed, foil pouch that provides an effective moisture, light, gas, and sterility barrier. Store in clean, dry conditions between 15° and 30°.

**Labeling**—Label it to indicate that it is derived from bovine origin. The product is labeled to indicate the product's intended clinical use. It is labeled with the dimensions of the product, the expiration date, the required storage conditions, lot number, part number, and the manufacturer's name and address. The label indicates that the product is sterile and nonpyrogenic and is designed for single patient, one-time use. The labeling cautions the user to inspect the packaging for damage and to discard the product if the packaging has been compromised. The labeling also cautions the user to hydrate the product only in room temperature sterile saline solution.

#### USP Reference standards (11)—

USP Endotoxin RS

**USP Authentic Visual References (11)**—*USP Bovine Acellular Dermal Matrix Reference Photomicrographs*. These Photomicrographs show the histological appearance of failed, cell-containing source material (Photomicrographs 1 and 2) and of passing, processed, decellularized material (Photomicrographs 3 and 4). The samples were prepared as directed in the test for *Histological evaluation*.

**Bacterial endotoxins (85)**—It meets the requirements as directed under *Transfusion and Infusion Assemblies and Similar Medical Devices (161)*.

**Sterility (71)**: meets the requirements.

#### Histological evaluation—

##### SOLUTION PREPARATIONS—

**1% Acid alcohol**—To 99 mL of 70% ethyl alcohol add 1 mL of hydrochloric acid (37.5%).

**Potassium alum solution**—Dissolve 100 g of potassium alum in 1000 mL of distilled water with the aid of heat and a magnetic stirrer.

**Hematoxylin-alcohol solution**—Dissolve 5 g of hematoxylin (see *Reagent Specifications* under *Reagents, Indicators, and Solutions*) in 50 mL of 100% ethyl alcohol at room temperature.

**Hematoxylin solution**—Slowly combine the 1000 mL of the *Potassium alum solution* with the 50 mL of the *Hematoxylin-alcohol solution*. Bring to a boil as rapidly as possible. Remove from heat and slowly add 2.5 g of mercuric oxide. Return the solution to heat until it becomes dark purple, remove from heat, and cool in a sink of cold water.

**Eosin solution**—Dissolve 1.0 g of eosin Y, water soluble, in 100 mL of distilled water. Dissolve 1.0 g of phloxine B in

100.0 mL of distilled water. Combine 100 mL of eosin Y solution with 10 mL of phloxine B solution, 780 mL of 95% ethyl alcohol, and 4.0 mL of glacial acetic acid. [NOTE—Filter daily before use.]

**Bluing agent**—Dissolve 1.54 g of lithium carbonate in 100 mL of distilled water.

**10% Neutral buffered formalin**—To 6.5 g of dibasic sodium phosphate (anhydrous) and 4.0 g of monobasic sodium phosphate, add 900 mL of distilled water and 100 mL of formaldehyde (37% to 40%).

**SAMPLE PREPARATION AND STAINING**—Remove a sample of finished product with an 8.0-mm biopsy punch. Place the sample in a labeled tissue cassette, and fix for 24 hours in *10% Neutral buffered formalin*. Dehydrate the sample in sequential soaks of the following: 70% ethyl alcohol (45 minutes), 80% ethyl alcohol (45 minutes), 95% ethyl alcohol (90 minutes), 100% ethyl alcohol (180 minutes), and xylene (90 minutes). Embed the sample in melted paraffin, cool, and cut 5- $\mu$ m thick sections with a microtome. Collect sections on microscope slides. Deparaffinize the slide with xylene and hydrate with distilled water. Stain in *Hematoxylin solution* for 6 to 15 minutes. Wash in running tap water for 2 to 5 minutes. Stain in *Eosin solution* for 1 to 2 minutes. Wash in running tap water for 2 to 5 minutes. Dip two times in *1% Acid alcohol*. Wash briefly in tap water. Place in *Bluing agent* until the sections are bright blue. Wash in running tap water for 10 minutes. Place in 80% ethyl alcohol for 1 to 2 minutes. Dehydrate and clear through two changes each of 95% ethyl alcohol, 100% ethyl alcohol, and xylene, 2 minutes each. Affix a coverslip over the tissue using an appropriate resinous mounting media. The nuclei stains blue, the cytoplasm stains from pink to red, and the collagen fibers stain from pink to red.

**MICROSCOPIC AND MORPHOLOGICAL CHARACTERISTICS**—The collagen fibers of the Bovine Acellular Dermal Matrix stain pink-red, and no evidence of cell nuclei or cytoplasm are apparent in prepared histological sections as shown in the USP Bovine Acellular Dermal Matrix Reference Photomicrographs of products with acceptable histological appearance.

**Protein determination**—Use the Kjeldahl nitrogen (protein) determination method to calculate the percent protein of the final product as directed under *Nitrogen Determination (461)* with the following specifics. Suitable equipment and procedures are readily available.<sup>1</sup>

**Digestion**—Prepare a rack of 15 to 20 Kjeldahl digestion tubes. In each, place 2.0 to 2.2 g of final product, 0.2  $\pm$  0.05 g of ammonium sulfate, a metallic catalyst tablet,<sup>2</sup> and boiling chips.<sup>3</sup> Prepare a blank tube with catalyst tablets and boiling chips (reagent blank). To each tube add 15 mL of concentrated sulfuric acid, and then, very slowly, 3 mL of hydrogen peroxide (30% to 35%). Place the digestion tubes on a digestion block, and heat to 410°. Digest for 60  $\pm$  5 minutes. The mixture in the tubes should be a clear green.

**Distillation**—Add excess base (50% sodium hydroxide). Generally, for each 5 mL of concentrated sulfuric acid used in the digestion, 20 mL of 40% (w/w) sodium hydroxide is required to make the digest strongly alkaline (pH >11). Mix each tube and let cool to room temperature. Distill each tube to collect approximately 125 mL of total distillate in a flask containing 25 mL of 4% boric acid. A reagent blank is run with each set.

**Titration**—Titrate the collected distillate with standardized 0.2 N sulfuric acid to a neutral gray color endpoint. Record the volume of sulfuric acid used.

<sup>1</sup>A suitable device and associated procedures can be obtained from Labconco, 8811 Prospect Ave., Kansas City, MO.

<sup>2</sup>A suitable catalyst is Pro-Pac CT-37, Alfie Packers, 8901 J St., Omaha, NE.

<sup>3</sup>Commonly referred to as Henger granules.

**Calculation**—Calculate the percentage of protein by the formula:

$$\text{Protein \%} = [\text{mL of sulfuric acid} - \text{mL blank}] \times \text{N of sulfuric acid} \times 1.4007 \times 6.25 / \text{weight of sample (g)}$$

where 1.4007 is the milliequivalent weight  $\text{N} \times 100(\%)$ ; and 6.25 is the protein factor for meat. The percentage of protein in 2.0 to 2.2 g of Bovine Acellular Dermal Matrix sample is between 90.0% and 95.0%.

**Lipid analysis**—A standard Soxhlet extraction apparatus is required. Dry flasks in an oven/dessicator and weigh, recording the weight to the nearest 0.0001 g. Grind or cut into small pieces 3.0 to 4.0 g of test material and place into a thimble. Record the weight of the test material to the nearest 0.0001 g. Place the thimble of material and 80 to 90 mL of petroleum ether into an extraction flask, and place into the Soxhlet extraction tube. Reflux for 4 hours. Collect all of the ether into the flask, and evaporate. Weigh the flask, recording weight to the nearest 0.0001 g.

**Calculation**—For the weight of lipid, subtract the weight of the clean flask from the final weight of the flask. Calculate the percent of lipid based on the weight of the starting material. The percentage of lipid in 3.0 to 4.0 g of Bovine Acellular Dermal Matrix sample is between 0% and 1.5%.

**Moisture content**—Proceed as directed under *Loss on Drying* (731) to calculate the moisture content, with the following specifics. Mince approximately 5.0 g of Bovine Acellular Dermal Matrix; place it into an aluminum dish. Dry the sample in an air oven for 16 to 18 hours at 100° to 102°.

**Calculation**—Calculate the percentage of moisture in the sample taken by the formula:

$$\text{Dry matter \%} = [(\text{wt. of dried sample \& pan (g)} - \text{wt. of pan (g)}) / \text{g of sample}] \times 100$$

$$\text{Moisture \%} = 100 - (\text{dry matter \%}).$$

The moisture loss is not less than 10.0% and not more than 12.0% of the original sample weight.

**Ash determination**—Place a sample of the final product, about 5.0 g, in a kiln-dried, porcelain crucible. Record the weight to the nearest 0.0001 g. Place the crucible containing the sample into an oven at 125° for 2 to 4 hours. Then place the crucible containing the sample into a cool muffle furnace. Heat the furnace to 350°, and maintain the temperature until smoking ceases (generally about 20 minutes). Heat the furnace to 550°. Maintain the temperature for 2 hours. Cool the crucible in a desiccator. Weigh the crucible, and record the weight to the nearest 0.0001 g.

**Calculation**—Calculate the percentage of ash by the following formula:

$$\text{Ash \%} = [(\text{wt. of crucible \& residue (g)} - \text{wt. of crucible (g)}) / \text{g of sample}] \times 100$$

The percentage of ash is between 0% and 0.3%.

#### **Carbohydrates**

**Calculation**—The percentage of carbohydrates is determined by the following formula:

$$\text{Carbohydrate \%} = 100\% - (\text{lipid \%} + \text{protein \%} + \text{moisture \%} + \text{ash \%})$$

The percentage of carbohydrates is equal to or less than 0.0%. Because this is a calculated value, influenced by the error inherent in the test methods above (*Lipid analysis*, *Moisture content*, and *Ash determination*), a calculated value less than 0.0% is acceptable.

**Gel electrophoresis**—Use the electrophoresis determination method as directed under *Biotechnology Derived Arti-*

*cles*—*Polyacrylamide Gel Electrophoresis* (1056) with the following specifics.

#### **SOLUTION PREPARATIONS**

**Collagen extraction solution**—Prepare a 0.5 M acetic acid solution containing 2 mM ethylenediaminetetraacetic acid (EDTA).

**2X Tris-glycine sample buffer**—Prepare a 2X solution containing 63 mM Tris-HCl pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.05% 2-mercaptoethanol, and 0.25% bromophenol blue.<sup>4</sup>

**1X Sample buffer**—Prepare a solution containing a mixture of 2X *Tris-glycine sample buffer* and water (1:1).

**SDS-PAGE running buffer**—Prepare a solution containing 25 mM Tris pH 8.3, 192 mM glycine, and 0.1% SDS.<sup>5</sup>

**Polyacrylamide gel**—Prepare a Tris-HCl polyacrylamide gel with a 4% to 20% gradient.<sup>6</sup>

**Molecular weight marker**—Use a suitable molecular weight marker containing protein bands between 10 and 250 kilodaltons (kDa).

**Staining solution**—Prepare a solution containing 0.25% (w/v) Coomassie brilliant blue R-250 (see *Reagent Specifications under Reagents, Indicators, and Solutions*) in 10% acetic acid and 10% *n*-propanol.

**Destain solution**—Prepare a mixture of water, acetic acid, and *n*-propanol (8:1:1).

**COLLAGEN PREPARATIONS**—Mince 0.5 g of Bovine Acellular Dermal Matrix final product. Weigh a sample of minced Bovine Acellular Dermal Matrix, and add to a volume of *Collagen extraction solution* to obtain a concentration of 5 mg per mL (dry weight of Bovine Acellular Dermal Matrix). Extract on a rocking platform at room temperature for 72 hours.

**Procedure**—Dilute acid-extracted collagen samples in 2X *Tris-glycine sample buffer* to a concentration of 0.5 mg per mL, and incubate for 5 minutes at 100°. Load the *Polyacrylamide gel* in the electrophoresis apparatus, and add *SDS-PAGE running buffer* to the top and bottom reservoirs. Load 10 µL of *Molecular weight markers* in the first well of the *Polyacrylamide gel* and 10 µL of 1X *Sample buffer* in the second well. Load 10 µL (5 µg) of each collagen sample into subsequent gel wells. Attach the cathode and anode to the appropriate terminals, and apply 110 V to the gel. Run the gel until the bromophenol blue reaches the bottom of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Staining solution* to cover the gel. Incubate the gel for 3 hours on a rocker at room temperature. Completely remove the *Staining solution* from the tray, cover the gel with *Destain solution*, and slowly rock the gel for 20 minutes. Remove the *Destain solution*, and repeat the destaining procedure three times. Inspect the gel for bands that have migrated from the origin.

**System suitability**—All bands between 20 and 200 kDa are present. The lane containing 1X *Sample buffer* does not contain any bands.

**Data analysis**—Where a protein band appears in the gel, the molecular weight of this protein is determined by comparing the position of the band to that of the known *Molecular weight marker*.

**Specificity**—The lanes of the *Polyacrylamide gel* that correspond to Bovine Acellular Dermal Matrix show four major protein bands. Two bands, when compared to the *Molecular weight marker*, appear at 96 and 94 kDa. These two bands correspond to the monomeric alpha 1 and alpha 2 chains of collagen Type I, respectively. Another two bands appear close together at 200 kDa, which correspond to alpha 1 and alpha 1/alpha 2 collagen dimers.

<sup>4</sup>A suitable sample buffer can be obtained from Invitrogen Corporation, 1600 Faraday Ave., P.O. Box 6482, Carlsbad, CA 92008.

<sup>5</sup>A suitable gel running buffer can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

<sup>6</sup>A suitable precast acrylamide gel can be obtained from Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547.

**Tensile strength**—Cut test specimens 5-mm wide  $\times$  50-mm long from representative pieces from final product lots. Measure the thickness of the specimen. Test the specimens with a commercially available material test system.<sup>7</sup> Mount and align the specimen, gripping 1 cm of the test specimen on both ends to ensure a test specimen gauge length of 3 cm. Pull the grips apart at 30 mm per minute while concurrently measuring the force exerted on the specimen. Record the maximum force (N) measured during the test.

**Calculation**—Calculate the tensile strength by the formula:

$$\text{Tensile strength (N/mm}^2\text{)} = \text{maximum force (N)} / 5 \text{ (mm)} \times \text{thickness (mm)}$$

The measured tensile strength for each lot is not less than 5 N per mm<sup>2</sup>.

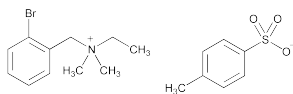
**Suture retention force**—Cut representative 1  $\times$  1 cm test specimens from final product lots. Using an appropriate suture material (e.g., 4-0 polypropylene suture), thread the suture 3 mm from the edge of the sample in the center and pull through. Clamp approximately 5 mm of the opposite, unsutured end of the test specimen in the upper pneumatic grip of a commercially available material test system.<sup>8</sup> The suture tails are hanging freely. Clamp the suture tails to the lower grip. Pull the grips apart at 20 mm per minute while concurrently measuring the force exerted. Record the maximum force (N) measured. The suture retention force measured for each lot is not less than 5 N for a 1  $\times$  1 cm test sample of the Bovine Acellular Dermal Matrix.

**Thermal analysis**—A final product sample of approximately 10 to 20 mg is heated at 2° per minute from 30° to 90°, hydrated with water, and the thermal characteristics of each processed skin is measured with a differential scanning calorimeter as directed under *Thermal Analysis* (891). Bovine Acellular Dermal Matrix displays a single thermal transition peak between 58° and 67°.

**Visual inspection**—Each piece of final product is visually inspected under a white light at a distance of 30 to 45 cm for color, the presence of particulates, and holes. Bovine Acellular Dermal Matrix is white, and neither particulates nor holes are visible.

**Hydration rate**—Cut a sample of finished product lot approximately 1  $\times$  1 cm. The sample fully hydrates, as indicated by a change in color from white to gray, in less than 3 minutes when placed in room temperature saline solution.

## Bretylum Tosylate



C<sub>18</sub>H<sub>24</sub>BrNO<sub>3</sub>S 414.36

Benzenemethanaminium, 2-bromo-N-ethyl-N,N-dimethyl-, salt with 4-methylbenzenesulfonic acid (1:1). (o-Bromobenzyl)ethyldimethylammonium *p*-toluenesulfonate [61-75-6].

» Bretylum Tosylate contains not less than 98.0 percent and not more than 101.0 percent of C<sub>18</sub>H<sub>24</sub>BrNO<sub>3</sub>S, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

<sup>7</sup>A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA.

<sup>8</sup>A suitable material test system is available from Instron Corporation, 825 University Ave., Norwood, MA.

## USP Reference standards (11)—

USP Bretylum Tosylate RS

### Identification—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Related compounds*.

**Loss on drying** (731)—Dry it in vacuum at 75° for 2 hours: it loses not more than 3.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231): 0.002%.

### Related compounds—

**0.01 M Sodium 1-octanesulfonate solution**—Dissolve 1.0814 g of 1-sodium octanesulfonate in 500 mL of water.

**Mobile phase**—Prepare a mixture of 0.01 M Sodium 1-octanesulfonate solution, acetonitrile, glacial acetic acid, and triethylamine (81:19:2:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Bretylum Tosylate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 20 µg per mL.

**Test solution**—Transfer about 200 mg of Bretylum Tosylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L11. The flow rate is about 1.9 mL per minute. Chromatograph the *Standard solution*, record the chromatograms, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 30 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.25, 0.74, 1.0, 1.27, 1.40 for tosylate ion, o-bromobenzylidimethylamine, bretylum, m-bromobenzylidimethylamine, and p-bromobenzylidimethylamine, respectively. The sum of the responses for all the peaks, excluding those of the bretylum and tosylate peaks, from the *Test solution* is not more than two times the bretylum response from the *Standard solution* (2%); and no individual peak response is greater than that of the bretylum peak from the *Standard solution* (1%).

**Assay**—Dissolve about 300 mg of Bretylum Tosylate, accurately weighed, in 50 mL of dioxane in a conical flask. Add 2 drops of crystal violet TS, and titrate with 0.025 N perchloric acid in dioxane to a blue-green endpoint. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.025 N perchloric acid is equivalent to 10.36 mg of C<sub>18</sub>H<sub>24</sub>BrNO<sub>3</sub>S.

## Bretylum Tosylate Injection

» Bretylum Tosylate Injection is a sterile solution of Bretylum Tosylate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>18</sub>H<sub>24</sub>BrNO<sub>3</sub>S.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

## USP Reference standards (11)—

USP Bretylum Tosylate RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of bretylium tosylate.

**pH** (791): between 3.5 and 7.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**pH 3.1 Tetramethylammonium phosphate buffer**—Dissolve 1.38 g of monobasic sodium phosphate and 2.0 mL of 25% tetra-methylammonium hydroxide solution in methanol in 800 mL of water, adjust with phosphoric acid to a pH of 3.1  $\pm$  0.1, dilute with water to 1000 mL, and mix.

**Mobile phase**—Transfer 15 mL of tetrahydrofuran and 75 mL of acetonitrile to a 1000-mL volumetric flask, and dilute with pH 3.1 Tetramethylammonium phosphate buffer to volume.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bretylium Tosylate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for tosylate and 1.0 for bretylium; the resolution, *R*, between the bretylium and tosylate peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.4%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{24}BrNO_3S$  in each mL of the Injection taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the bretylium peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bretylium Tosylate in Dextrose Injection

» Bretylium Tosylate in Dextrose Injection is a sterile solution of Bretylium Tosylate and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of bretylium tosylate ( $C_{18}H_{24}BrNO_3S$ ) and dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ). It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

#### USP Reference standards (11)—

USP Bretylium Tosylate RS

USP Endotoxin RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for bretylium tosylate*.

**B:** It responds to the *Identification* test under *Dextrose*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of bretylium tosylate.

**pH** (791): between 3.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay for bretylium tosylate—

**pH 3.1 Tetramethylammonium phosphate buffer, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay under Bretylium Tosylate Injection*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of bretylium tosylate ( $C_{18}H_{24}BrNO_3S$ ) in each mL of the Injection taken by the formula:

$$50(C/V)(r_U/r_S)$$

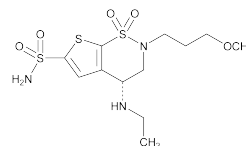
in which *C* is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the bretylium peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

## Brinzolamide



$C_{12}H_{21}N_3O_5S_3$  383.51

2*H*-Thieno[3,2-*e*]-1,2-thiazine-6-sulfonamide, 4-(ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-, 1,1-dioxide, (*R*)-.  
(*R*)-4-(Ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-2*H*-thieno[3,2-*e*]-1,2-thiazine-6-sulfonamide 1,1-dioxide [138890-62-7].

» Brinzolamide contains not less than 98.0 percent and not more than 102.0 percent of  $C_{12}H_{21}N_3O_5S_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Brinzolamide RS

USP Brinzolamide Related Compound A RS

Brinzolamide (*S*)-isomer.

$C_{12}H_{21}N_3O_5S_3$  383.52

USP Brinzolamide Related Compound B RS

(*R*-4-Amino)-2,3-dihydro-2-(3-methoxypropyl)-4*H*-thieno[3,2-*e*]-thiazine-6-sulfonamide-1,1-dioxide ethandioate 1:1.

$C_{10}H_{17}N_3O_5S_3 \cdot C_2H_2O_4$  445.49

**Identification**—

**A:** Infrared Absorption (197K).

**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *System suitability solution*, as obtained in *Test 1* for Related compounds.

**Loss on drying** (731)—Dry it in vacuum at 100° to 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Related compounds**—

TEST 1—

**Mobile phase**—Prepare a filtered and degassed mixture of dehydrated alcohol, chromatographic solvent hexane, methanol, and diethylamine (55:40:5:0.2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve accurately weighed quantities of USP Brinzolamide RS and USP Brinzolamide Related Compound A RS in dehydrated alcohol to obtain a solution having known concentrations of about 0.4 mg per mL and 0.02 mg per mL, respectively.

**Test solution**—Transfer about 25 mg of Brinzolamide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with dehydrated alcohol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L51. The flow rate is about 0.75 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for brinzolamide and 1.2 for brinzolamide related compound A; the resolution, *R*, between brinzolamide and brinzolamide related compound A is not less than 1.8; the column efficiency determined from brinzolamide is not less than 2000 theoretical plates; and the tailing factor for the brinzolamide peak is not more than 1.8.

**Procedure**—Inject about 5  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas for brinzolamide and brinzolamide related compound A. Calculate the percentage of brinzolamide related compound A in the portion of Brinzolamide taken by the formula:

$$100(r_U / r_s)$$

in which  $r_U$  is the peak response for brinzolamide related compound A; and  $r_s$  is the sum of the peak responses for brinzolamide and brinzolamide related compound A: not

more than 0.5% of brinzolamide related compound A is found.

TEST 2—

**Triethylamine phosphate buffer**—Prepare as directed in the *Assay*.

**Mobile phase 1**—Prepare as directed for *Mobile phase* in the *Assay*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Mobile phase 2**—Prepare a filtered and degassed mixture of *Triethylamine phosphate buffer* and acetonitrile (65:35).

**System suitability solution**—Dissolve accurately weighed quantities of USP Brinzolamide RS and USP Brinzolamide Related Compound B RS in *Mobile phase 1* to obtain a solution having known concentrations of about 0.1 mg of each per mL.

**Test solution**—Transfer about 50 mg of Brinzolamide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase 1* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.0 mL per minute. Using *Mobile phase 1*, chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for brinzolamide related compound B and 1.0 for brinzolamide; the resolution, *R*, between brinzolamide and brinzolamide related compound B is not less than 2.0; the column efficiency determined from brinzolamide is not less than 1200 theoretical plates; and the tailing factor for the brinzolamide peak is not more than 2.0.

**Procedure**—Using *Mobile phase 1*, separately inject equal volumes (about 10  $\mu$ L) of *Mobile phase 1* and the *Test solution* into the chromatograph, record the chromatograms, allowing the elution to continue for 20 minutes, and measure the areas for all the peaks, excluding the peaks obtained from *Mobile phase 1*. Calculate the percentage of each impurity in the portion of Brinzolamide taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses for all the peaks: not more than 0.3% of any individual impurity is found.

Equilibrate the system with *Mobile phase 2*, inject the *Test solution* again, record the chromatograms, allowing the elution to continue for 20 minutes, and measure the areas for brinzolamide and all the peaks having a relative retention time greater than 6. Calculate the percentage of each impurity in the portion of Brinzolamide taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses for all the peaks: not more than 0.3% of any individual impurity is found; and not more than 1.0% of total impurities using *Mobile phase 1* and *Mobile phase 2* is found.

**Assay**—

**Triethylamine phosphate buffer**—Add 4.0 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Triethylamine phosphate buffer* and acetonitrile (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Brinzolamide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer about 50 mg of Brinzolamide, accurately weighed, to a 50-mL volumetric flask, dilute with



*Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1200 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for brinzolamide. Calculate the quantity, in mg, of C<sub>12</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub> in the portion of Brinzolamide taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Brinzolamide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Brinzolamide Ophthalmic Suspension

» Brinzolamide Ophthalmic Suspension is a sterile, aqueous suspension of Brinzolamide containing a suitable antimicrobial preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of brinzolamide (C<sub>12</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub>).

**Packaging and storage**—Preserve in tight containers. Store at a temperature between 4° and 30°.

### USP Reference standards <11>—

USP Brinzolamide RS

USP Brinzolamide Related Compound A RS  
Brinzolamide (S)-isomer.

C<sub>12</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub> 383.52

USP Brinzolamide Related Compound B RS

(R-4-Amino)-2,3-dihydro-2-(3-methoxypropyl)-4H-thieno  
[3,2,-e]-thiazine-6-sulfonamide-1,1-dioxide ethandioate  
1:1.

C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub> · C<sub>2</sub>H<sub>2</sub>O<sub>4</sub> 445.49

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** <791>: between 6.5 and 8.5.

### Related compounds—

#### TEST 1—

*Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in *Test 1* for *Related compounds* under *Brinzolamide*.

**Test solution**—Transfer an accurately weighed volume of Ophthalmic Suspension, equivalent to about 10 mg of brinzolamide, to a 25-mL volumetric flask, dilute with alcohol to volume, and mix.

**Procedure**—Proceed as directed in *Test 1* for *Related compounds* under *Brinzolamide*: not more than 1.5% of brinzolamide related compound A is found.

#### TEST 2—

*Buffer solution* and *Mobile phase*—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Brinzolamide Related Compound B RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.5 μg per mL.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system**—Proceed as directed in the *Assay*, and chromatograph the *System suitability preparation*, prepared as directed in the *Assay*, and the *Standard solution*, instead of the *Standard preparation*.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the quantity, in mg, of each impurity in the portion of Ophthalmic Suspension taken by the formula:

$$(356.46/445.49)50C(r_i / r_S)$$

in which 356.46 and 445.49 are the molecular weights of des-ethyl brinzolamide and des-ethyl brinzolamide oxalate, respectively; C is the concentration, in mg per mL, of USP Brinzolamide Related Compound B RS in the *Standard solution*;  $r_i$  is peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the peak response for USP Brinzolamide Related Compound B RS obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

### Assay—

**Buffer solution**—Dissolve 11.75 g of ammonium acetate in about 1000 mL of water. Adjust with acetic acid to a pH of 5.2.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Brinzolamide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**System suitability preparation**—Dissolve an accurately weighed quantity of USP Brinzolamide Related Compound B RS in *Standard preparation* to obtain a solution having a known concentration of about 0.06 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 10 mg of brinzolamide, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are between 0.48 and 0.61 for brinzolamide related compound B and 1.0 for brinzolamide; the resolution,  $R$ , between brinzolamide and brinzolamide related compound B is not less than 4.5; the column efficiency is not less than 2500 theoretical plates; and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

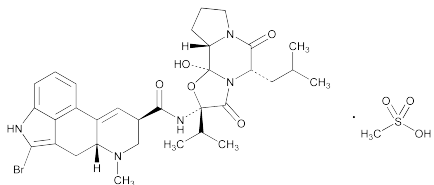
**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

mg, of brinzolamide ( $C_{12}H_{21}N_3O_5S_3$ ) in the portion of Ophthalmic Suspension taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Brinzolamide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bromocriptine Mesylate



$C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$  750.70  
Ergotaman-3',6',18-trione, 2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)-, monomethanesulfonate (salt), (5α)-; 2-Bromoergocryptine monomethanesulfonate (salt) [22260-51-1].

### DEFINITION

Bromocriptine Mesylate contains NLT 98.0% and NMT 102.0% of  $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ , calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197M): Undried
- B. ULTRAVIOLET ABSORPTION** (197U)  
Sample solution: 50 µg/mL in 0.1 M methanolic methanesulfonic acid  
Acceptance criteria: Meets the requirements

### ASSAY

- PROCEDURE**  
Sample solution: 600 mg of Bromocriptine Mesylate  
Analysis: Dissolve with 80 mL of a mixture of acetic anhydride and glacial acetic acid (7:1). Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 75.07 mg of  $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ .  
Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS, Method II** (231): NMT 20 ppm

#### Organic Impurities

- PROCEDURE 1: LIMIT OF METHANESULFONIC ACID CONTENT**  
Sample solution: 400 mg of Bromocriptine Mesylate  
Analysis: Dissolve with 70 mL of methanol. Titrate under nitrogen with 0.1 N methanolic potassium hydroxide VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N methanolic potassium hydroxide is equivalent to 9.61 mg of  $CH_3SO_3H$ .  
Acceptance criteria: NLT 12.5% and NMT 13.4% of  $CH_3SO_3H$  on the dried basis
- PROCEDURE 2**  
Solution A: 0.1 N citric acid solution. Adjust with hydrochloric acid to a pH of 2.0.  
Diluent: Methanol and *Solution A* (1:1)  
Solution B: Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (2:3)

**Solution C:** Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (3:2)

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
18	100	0
30	0	100
40	0	100
41	100	0

**System suitability solution:** 2.0 mg/mL each of α-ergocryptine and Bromocriptine Mesylate in *Diluent*

**Standard stock solution:** 46 µg/mL of USP Bromocriptine Mesylate RS in methanol and *Solution A* (1:1).

[NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]

**Standard solution:** 4.6 µg/mL of USP Bromocriptine Mesylate RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 4.6 mg/mL of Bromocriptine Mesylate in methanol and *Solution A* (1:1). [NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE— The relative retention times for α-ergocryptine and bromocriptine mesylate are 0.46 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 15 between α-ergocryptine and bromocriptine mesylate, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE— The relative retention times for bromocriptine and bromocriptinine are 1.0 and 1.7, respectively.] Calculate the percentage of each impurity in the portion of Bromocriptine Mesylate taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of bromocriptine from the *Standard solution*

$C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bromocriptine Mesylate in the *Sample solution* (mg/mL)

F = relative response factor equal to 1.4 for any peak eluting at a relative retention time of about 0.9 or less, and equal to 1.0 for all other peaks

### Acceptance criteria

**Individual impurities:** NMT 0.4% of bromocriptinine is found; NMT 0.1% of any individual impurity is found.

**Total impurities:** NMT 1.0%

### SPECIFIC TESTS

- COLOR OF SOLUTION** (631)

**Matching solutions:** Prepare three solutions, A, B, and C, containing, respectively, the following parts of cobal-

tous chloride CS, ferric chloride CS, cupric sulfate CS, and dilute hydrochloric acid (1 in 40).

A: 3.0: 3.0: 2.4: 31.6

B: 1.0: 2.4: 0.4: 36.2

C: 0.6: 2.4: 0: 37.0

**Sample solution:** 10 mg/mL of Bromocriptine Mesylate in methanol

**Analysis:** Compare the *Sample solution* with 10-mL portions of the *Matching solutions* in suitable matched tubes.

**Acceptance criteria:** The solution is clear and not darker in color than *Matching solutions A, B, and C*.

• **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 10 mg/mL, in a mixture of methylene chloride and methanol (1:1)

**Acceptance criteria:** +95° to +105°

• **LOSS ON DRYING**

(See *Thermal Analysis* (891).)

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis using 10 mg of Bromocriptine Mesylate. Heat the specimen under test at the rate of 10°/min in an atmosphere of nitrogen at a flow rate of 45 mL/min. Record the thermogram from ambient temperature to 160°.

**Acceptance criteria:** It loses NMT 4.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place.
- **USP REFERENCE STANDARDS (11)**  
USP Bromocriptine Mesylate RS

## Bromocriptine Mesylate Capsules

**DEFINITION**

Bromocriptine Mesylate Capsules contain bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ).

**IDENTIFICATION**

- **A.** The principal spot of the *Sample solution* corresponds, in  $R_f$  value and color, to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

**ASSAY**

• **PROCEDURE**

Conduct this procedure without exposure to daylight and with minimum exposure to artificial light.

**Buffer:** 0.125 g/L of ammonium carbonate in water

**Mobile phase:** Acetonitrile and *Buffer* (3:2)

**Standard solution:** 1.0 mg/mL of bromocriptine from USP Bromocriptine Mesylate RS in dehydrated alcohol. Sonicate as needed.

**Sample solution:** 1.0 mg/mL of bromocriptine in methanol, prepared as follows. Remove, as completely as possible, the contents of NLT 10 Capsules. Weigh and determine the average weight per Capsule. Mix the combined contents, and transfer a weighed quantity of the powder, nominally equivalent to 50 mg of bromocriptine, to a 50-mL volumetric flask. Add 30 mL of dehydrated alcohol, and shake for 15 min. Dilute with dehydrated alcohol to volume, mix, and filter. Use this solution without delay.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4-mm × 25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of bromocriptine, from USP Bromocriptine Mesylate RS, in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISSOLUTION (711)**

**Medium:** 0.1 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 60 min

**Standard solution:** USP Bromocriptine Mesylate RS in *Medium*, at a concentration similar to the *Sample solution*. [NOTE—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to bring the *Standard* into solution before dilution with *Medium*.]

**Sample solution:** Sample per *Dissolution* (711), passed through a glass-fiber filter.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Fluorometry

**Excitation wavelength:** 315 nm

**Emission wavelength:** 445 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) dissolved.

**Tolerances:** NLT 75% (Q) of the labeled amount of bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905)**

**Procedure for content uniformity**

Protect all solutions from light.

**Diluent:** Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.

**Standard solution:** 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent*

**Sample solution:** Transfer the contents of 1 Capsule into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** Maximum absorbance (about 306 nm)

Cell: 1 cm

Blank: Diluent

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bromocriptine, 654.59

$M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70

**Acceptance criteria:** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.

**Standard stock solution:** 2.3 mg/mL of USP Bromocriptine Mesylate RS in methanol, equivalent to 2 mg/mL of bromocriptine

**Standard solution 1:** 0.06 mg/mL (3.0%) of bromocriptine in methanol, from *Standard stock solution*

**Standard solution 2:** 0.04 mg/mL (2.0%) of bromocriptine in methanol, from *Standard stock solution*

**Standard solution 3:** 0.02 mg/mL (1.0%) of bromocriptine in methanol, from *Standard stock solution*

**Standard solution 4:** 0.01 mg/mL (0.50%) of bromocriptine in methanol, from *Standard stock solution*

**Sample solution:** 2.0 mg/mL of bromocriptine in methanol, prepared as follows. Transfer a quantity of the Capsule contents, equivalent to 20 mg of bromocriptine, to a conical flask. Add 10 mL of methanol, and stir by mechanical means for 20 min. Centrifuge the suspension for 10 min at about 3500 rpm. Use the clear supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 50  $\mu$ L as 1.5-cm bands

**Developing solvent:** Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180:15:5:1)

**Spray reagent:** 0.2% o-phthalaldehyde in sulfuric acid

#### Analysis

**Samples:** *Standard stock solution*, *Standard solutions*, and *Sample solution*

Develop under the exclusion of light in a tank lined with filter paper, previously equilibrated for 30 min, using *Developing solvent* until the solvent front has moved a distance of 15 cm on the plate. Dry the plate briefly in a current of cold air. Spray evenly with the *Spray reagent*, and view the plate under long-wave-length UV light.

**Acceptance criteria:** Any major secondary spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot obtained from *Standard solution 1* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** <11>  
USP Bromocriptine Mesylate RS

## Bromocriptine Mesylate Tablets

#### DEFINITION

Bromocriptine Mesylate Tablets contain bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ).

#### IDENTIFICATION

- **A.** The principal spot of the *Sample solution* corresponds, in  $R_f$  value and color, to that of the *Standard stock solution*, as obtained in the test for *Organic Impurities*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 0.01 M ammonium carbonate in water

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** 0.22 mg/mL of USP Bromocriptine Mesylate RS in methanol

**Sample solution:** Transfer a quantity of powdered Tablets (NLT 20), equivalent to 10 mg of bromocriptine, to an appropriate container. Add 40 mL of methanol, and stir for 20 min, protected from light. Quantitatively filter through a fine glass filtering funnel into a 50-mL volumetric flask. Rinse the filter with methanol, adding the rinsing to the filtrate, and dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Coefficient of variation:** NMT 3.0% for 3 replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bromocriptine, 654.59

$M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Test 1:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** 0.1 N hydrochloric acid; 500 mL

**Apparatus 1:** 120 rpm

**Time:** 60 min

**Standard solution:** USP Bromocriptine Mesylate RS at a known concentration in *Medium*

[NOTE—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to dissolve the *Standard* before dilution with *Medium*.]

**Sample solution:** Sample per *Dissolution* <711>, passed through a glass-fiber filter.

**Blank:** *Medium*

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Fluorometry

**Excitation wavelength:** 315 nm

**Emission wavelength:** 445 nm

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 0.01 M ammonium carbonate in water

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** Dissolve USP Bromocriptine Mesylate RS in methanol, and quantitatively dilute with *Medium* to obtain a solution having a known concentration similar to the expected concentration of the *Sample solution*.

**Sample solution:** Sample per *Dissolution* <711>.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*. Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>

#### Procedure for content uniformity

[NOTE—Protect all solutions from light.]

**Diluent:** Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.

**Standard solution:** 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent*

**Sample solution:** Transfer 1 Tablet into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 306 nm

**Cell:** 1 cm

**Blank:** *Diluent*

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bromocriptine, 654.59

$M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70

**Acceptance criteria:** Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

[NOTE—Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.]

**Standard stock solution:** 1.2 mg/mL of USP Bromocriptine Mesylate RS in methanol, equivalent to 1 mg/mL of bromocriptine

**Standard solution 1:** 0.50 mg/mL (5%) of bromocriptine in methanol, from *Standard stock solution*

**Standard solution 2:** 0.30 mg/mL (3%) of bromocriptine in methanol, from *Standard stock solution*

**Standard solution 3:** 0.10 mg/mL (1%) of bromocriptine in methanol, from *Standard stock solution*

**Sample solution:** Transfer an equivalent to 20 mg of bromocriptine, from powdered Tablets, to a conical flask. Add 10 mL of methanol, and mix for 20 min. Centrifuge the suspension for 10 min at 4000 rpm. Use the clear supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

#### Application volumes

**Standard solutions:** 10- $\mu$ L, as 1.5-cm bands

**Sample solution:** 50- $\mu$ L, as 1.5-cm bands

**Developing solvent system:** Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180:15:5:0.1)

**Spray reagent:** 0.2% o-phthalaldehyde in sulfuric acid

#### Analysis

**Samples:** *Standard stock solution*, *Standard solutions*, and *Sample solution*

Proceed as directed in *Chromatography* <621>, *Thin-Layer Chromatography*. Dry the plate for 5 min in a current of cold air. Develop in a tank lined with filter paper, previously equilibrated for 20 min, using *Developing solvent system* until the solvent front has moved a distance of 10 cm on the plate. Dry the plate under vacuum at room temperature for 15 min. Spray evenly with the *Spray reagent*, and view the plate under long-wavelength UV light.

**Acceptance criteria:** Any spot, other than the principal spot, from the *Sample solution* is not greater in size and intensity than the spot from *Standard solution 2* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.

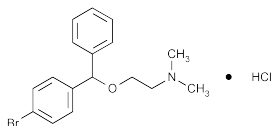
### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.

• **USP REFERENCE STANDARDS** <11>  
USP Bromocriptine Mesylate RS

## Bromodiphenhydramine Hydrochloride



$C_{17}H_{20}BrNO \cdot HCl$  370.71

Ethanamine, 2-(4-bromophenyl)phenylmethoxy-*N,N*-dimethyl-, hydrochloride.

2-(*p*-Bromo- $\alpha$ -phenylbenzyl)oxy-*N,N*-dimethylethylamine hydrochloride [1808-12-4].

» Bromodiphenhydramine Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{20}BrNO \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Bromodiphenhydramine Hydrochloride RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 15  $\mu$ g per mL.

*Medium:* 0.1 N sulfuric acid.

Absorptivities at 228 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 148° and 152°.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Assay**—Dissolve about 700 mg of Bromodiphenhydramine Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, and add 10 mL of benzene and 15 mL of mercuric acetate TS. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.07 mg of  $C_{17}H_{20}BrNO \cdot HCl$ .

## Bromodiphenhydramine Hydrochloride Oral Solution

» Bromodiphenhydramine Hydrochloride Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Bromodiphenhydramine Hydrochloride RS

**Identification, Infrared Absorption** (197K)—

*Test specimen*—Transfer the final solution obtained from the titration in the Assay to a separator, add about 1 mL of 0.1 N sulfuric acid, and shake with 25 mL of ether. (Methyl red enters the ether phase.) Drain the aqueous layer into another separator, add 5 mL of 1 N sodium hydroxide, and shake with 10 mL of chloroform. Drain the chloroform layer into a small flask containing 2 g of anhydrous sodium sulfate, and swirl. Pour the chloroform solution through a small cotton pledget, pre-rinsed with chloroform, into a beaker,

and evaporate to about 5 mL. Apply a few drops of the solution directly to a potassium bromide plate, and completely remove the chloroform by warming for 2 to 3 minutes under an IR lamp.

**Alcohol content, Method I** (611): between 12.0% and 15.0% of  $C_2H_5OH$ .

**Assay**—Evaporate an accurately measured volume of Oral Solution, equivalent to about 250 mg of bromodiphenhydramine hydrochloride, to about half the original volume, using a suitable vacuum evaporator. Transfer the concentrated solution to a 250-mL separator, with the aid of sufficient warm water to bring the volume to the original volume. Add 20 g of sodium chloride, and shake until dissolved. Add 5 mL of 1 N sodium hydroxide, shake with 100 mL of ether, and drain the aqueous layer into a second separator containing 50 mL of ether. Shake, and discard the aqueous layer. Wash the ether solutions with two 20-mL portions of water, shaking each aqueous portion successively in the two separators, and then discard the aqueous solutions. Extract the ether solutions successively with 10.0 mL of 0.1 N sulfuric acid VS, followed by two 5-mL portions of water, and collect the aqueous extracts in a conical flask. Add methyl red TS to the solution in the flask, and titrate the excess acid with 0.02 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N sulfuric acid is equivalent to 37.07 mg of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ).

## Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution

» Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate the alcohol content.

**USP Reference standards** (11)—

USP Bromodiphenhydramine Hydrochloride RS

USP Codeine Phosphate RS

**Identification**—

**A:** Thin-Layer Chromatographic Identification Test (201)—

*Test solution*—Transfer a volume of Oral Solution, equivalent to about 10 mg of codeine phosphate, to a separator, and add 5 mL of water, 5 mL of methylene chloride, and 1 mL of ammonium hydroxide. Shake for 1 minute, allow the layers to separate, and use the clear, lower layer.

*Standard solution*—Prepare a solution of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in methanol containing 10 mg of each per mL.

*Developing solvent system:* a mixture of alcohol and ammonium hydroxide (49:1).

**B:** The retention times of the major peaks in the chromatogram of the Assay preparation correspond to those in the chromatogram of the Standard preparation, as obtained in the Assay.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The to-

tal aerobic microbial count does not exceed 100 cfu per mL, and the total combined molds and yeasts count does not exceed 50 cfu per mL.

**pH** (791): between 4.5 and 6.5.

**Alcohol content**, *Method II* (611): between 4.0% and 6.0% is found.

#### Assay—

**Diluent**—Prepare a mixture of methanol and water (80:20).

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, 0.1 N ammonium hydroxide solution, and 0.1 N ammonium nitrate solution (27:3:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 100 µg per mL and 80 µg per mL, respectively.

**Assay preparation**—Using a pipet calibrated “to contain”, transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of bromodiphenhydramine hydrochloride and 8 mg of codeine phosphate, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30.0-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for bromodiphenhydramine and 1.4 for codeine; the resolution, *R*<sub>s</sub>, between bromodiphenhydramine and codeine is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for bromodiphenhydramine and codeine. Calculate the quantity, in mg, of bromodiphenhydramine hydrochloride (C<sub>17</sub>H<sub>20</sub>BrNO · HCl) in each mL of the Oral Solution taken by the formula:

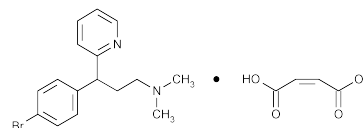
$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bromodiphenhydramine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the bromodiphenhydramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.36)(100C/V)(r_U / r_S)$$

in which 406.37 and 397.36 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the codeine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Brompheniramine Maleate



C<sub>16</sub>H<sub>19</sub>BrN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> 435.31

2-Pyridinepropanamine, γ-(4-bromophenyl)-*N,N*-dimethyl-, (±)-, (*Z*)-2-butenedioate (1:1).

(±)-2-*p*-Bromo-α-2-(dimethylamino)ethylbenzylpyridine maleate (1:1) [980-71-2].

» Brompheniramine Maleate, dried at 105° for 3 hours, contains not less than 98.0 percent and not more than 100.5 percent of C<sub>16</sub>H<sub>19</sub>BrN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Brompheniramine Maleate RS

#### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 35 µg per mL.

*Medium:* methanol.

**Melting range** (741): between 130° and 135°.

**pH** (791): between 4.0 and 5.0, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

#### Related compounds—

**Test preparation**—Dissolve about 200 mg of Brompheniramine Maleate in 5 mL of methylene chloride, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 1.2-m × 4-mm glass column containing 3% phase G3 on support S1AB. The column temperature is maintained at about 190°, and the injection port and detector temperatures are both maintained at about 250°. The carrier gas is dry helium, flowing at a rate adjusted to obtain a retention time of 6 to 7 minutes for the main peak. Chromatograph the *Test preparation*, record the chromatogram, and determine the peak area as directed for *Procedure*: the tailing factor for the brompheniramine maleate peak is not more than 1.8.

**Procedure**—Inject a volume (about 1 µL) of the *Test preparation* into the chromatograph. Record the chromatogram for a total time of not less than twice the retention time of the brompheniramine peak, and measure the areas of the peaks. The total relative area of all extraneous peaks (except that of the solvent peak and maleic acid, if observed) does not exceed 2.0%.

**Assay**—Dissolve about 425 mg of Brompheniramine Maleate, previously dried and accurately weighed, in 50 mL of glacial acetic acid. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.77 mg of C<sub>16</sub>H<sub>19</sub>BrN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>.

## Brompheniramine Maleate Injection

» Brompheniramine Maleate Injection is a sterile solution of Brompheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Brompheniramine Maleate RS  
USP Endotoxin RS

**Identification**—Dilute a volume of Injection, equivalent to about 50 mg of brompheniramine maleate, with dilute hydrochloric acid (1 in 1200) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with “Transfer the liquid to a separator:” the Injection meets the requirements of the test.

**Bacterial endotoxins** (85)—It contains not more than 35.7 USP Endotoxin Units per mg of brompheniramine maleate.

**pH** (791): between 6.3 and 7.3.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* (501), to prepare the solution employed for the determination of the absorbance,  $A_u$ , at 262 nm. For the determination of  $A_s$ , dissolve about 25 mg of USP Brompheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  in each mL of the Injection taken by the formula:

$$(W / V)(A_u / A_s)$$

in which  $W$  is the weight, in mg, of USP Brompheniramine Maleate RS in the *Standard Preparation*, and  $V$  is the volume, in mL, of Injection taken.

## Brompheniramine Maleate Oral Solution

» Brompheniramine Maleate Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Brompheniramine Maleate RS

**Identification**—Transfer a volume of Oral Solution, equivalent to about 50 mg of brompheniramine maleate, to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with two 50-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, and discard the aqueous phase. Filter the combined chloroform extracts into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of dilute hydrochloric acid (1 in 1200), and proceed as directed under *Identification—Organic Nitrogenous Bases* (181),

beginning with “Transfer the liquid to a separator.” The Oral Solution meets the requirements of the test.

**pH** (791): between 2.5 and 3.5.

**Alcohol content, Method I** (611): between 2.7% and 3.3% of  $C_2H_5OH$ .

**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of brompheniramine maleate, to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with ten 10-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, wash the latter with 20 mL of chloroform, and discard the aqueous phase. Quantitatively filter the combined chloroform extracts and washings into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of glacial acetic acid and 5 mL of acetic anhydride, agitate, and allow to stand for about 15 minutes. Add 1 drop of crystal violet TS, and titrate with 0.01 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N perchloric acid is equivalent to 2.177 mg of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ).

## Brompheniramine Maleate Tablets

» Brompheniramine Maleate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight containers.

### USP Reference standards (11)—

USP Brompheniramine Maleate RS

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

### Dissolution (711)—

*Medium*: water; 500 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted with 3 N hydrochloric acid, using 5-cm cuvettes, in comparison with a Standard solution having a known concentration of USP Brompheniramine Maleate RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 160 µg per mL. Transfer 25.0 mL of this solution to a separator containing 25 mL of water, mix, and proceed as directed under *Assay preparation*, beginning with “adjust with sodium hydroxide solution (1 in 10) to a pH of 11.” The concentration of USP Brompheniramine Maleate RS in the *Standard preparation* is about 20 µg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of brompheniramine maleate, mix with 50 mL of water for 10 minutes, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75-mL portions



of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50-mL portions of dilute hydrochloric acid (1 in 120), combining the acid extracts in a 200-mL volumetric flask. Add dilute hydrochloric acid (1 in 120) to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation*, in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$0.2C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Brompheniramine Maleate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution

» Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

### USP Reference standards (11)—

USP Brompheniramine Maleate RS

USP Pseudoephedrine Sulfate RS

### Identification—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** A solution of it meets the requirements of the test for *Sulfate* (191).

**C:** Transfer a volume of Oral Solution, equivalent to about 6 mg of brompheniramine maleate, to a separator, add 0.5 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare separate Standard solutions in methanol containing, respectively, 1.2 mg of USP Brompheniramine Maleate RS and 9 mg of USP Pseudoephedrine Sulfate RS per mL. Separately apply 5  $\mu\text{L}$  of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl ether, methanol, and ammonium hydroxide (16:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wave-length UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the Standard solutions.

### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

### Assay—

**Mobile phase**—Prepare a mixture of water, acetonitrile, methanol, and tetrahydrofuran (550:320:80:50). Transfer 1.0 mL of phosphoric acid, followed by 4.33 g of dodecyl sulfate sodium to this mixture, and mix. Adjust with ammonium hydroxide to a pH of  $3.50 \pm 0.05$ , filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—The pH of the *Mobile phase* is critical and may cause 1 to 4 minutes of differences in the retention times of internal standard and brompheniramine maleate.]

**Internal standard solution**—Transfer about 50 mg of naphazoline hydrochloride to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 6000  $\mu\text{g}$  per mL,  $I$  being the ratio of the labeled amount, in mg, of brompheniramine maleate to the labeled amount, in mg, of pseudoephedrine sulfate per mL (*Solution P*). Transfer about 30 mg of USP Pseudoephedrine Sulfate RS, accurately weighed, to a 25-mL volumetric flask, add 5.0 mL each of *Solution P* and *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 1200  $\mu\text{g}$  of USP Brompheniramine Maleate RS per mL and about 1.2 mg of USP Pseudoephedrine Sulfate RS per mL.

**Assay preparation**—Using a “To contain” pipet transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of pseudoephedrine sulfate, to a 25-mL volumetric flask. Rinse the pipet with about 5 mL of *Mobile phase*, collecting the rinse in the volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

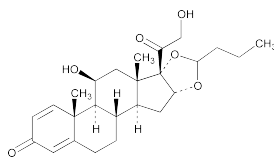
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for pseudoephedrine sulfate, 1.5 for naphazoline hydrochloride, and 2.5 for brompheniramine maleate; the resolution,  $R$ , between the pseudoephedrine sulfate and naphazoline hydrochloride peaks is not less than 3, and between the brompheniramine maleate and naphazoline hydrochloride peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ) in each mL of the Oral Solution taken by the formula:

$$25CV(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Brompheniramine Maleate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for brompheniramine maleate and naphazoline hydrochloride from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of pseudoephedrine sulfate ( $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ) in each mL of the Oral Solution taken by the same formula, changing the terms to refer to pseudoephedrine sulfate.

## Budesonide



$C_{25}H_{34}O_6$  430.53  
 Pregna-1,4-diene-3,20-dione, 16 $\alpha$ ,17-[1*R*-butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxy and pre-gna-1,4-diene-3,20-dione, 16 $\alpha$ ,17-[1*S*-butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxy;  
 (RS)-11 $\beta$ ,16 $\alpha$ ,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with butyraldehyde [51372-29-3; 51372-28-2; 51333-22-3].

### DEFINITION

Budesonide is a mixture of two epimeric forms, epimer A (C-22S) and epimer B (C-22R). It contains NLT 40.0% and NMT 51.0% of epimer A, and the sum of both epimers is NLT 98.0% and NMT 102.0% of  $C_{25}H_{34}O_6$ , calculated on the dried basis.

[NOTE—Protect all solutions containing budesonide from light.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
 Sample solution: 25  $\mu$ g/mL  
 Medium: Methanol  
 Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE

**Buffer:** 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid. The pH is  $3.2 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (32:68)

**Standard solution:** Dissolve a quantity of USP Budesonide RS in acetonitrile, and dilute quantitatively with *Buffer* to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to NMT 30%.

**Sample solution:** Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with *Buffer* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention time for epimer A is 1.1, with respect to epimer B.]

#### Suitability requirements

**Resolution:** NLT 1.5 between the two budesonide epimer peaks

**Column efficiency:** NLT 5500 theoretical plates, determined from the budesonide epimer B peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of epimer A ( $C_{25}H_{34}O_6$ ) in the portion of Budesonide taken:

$$\text{Result} = [r_{UA}/(r_{UA} + r_{UB})] \times 100$$

$r_{UA}$  = peak area of epimer A from the *Sample solution*

$r_{UB}$  = peak area of epimer B from the *Sample solution*

Calculate the percentage of  $C_{25}H_{34}O_6$  in the portion of Budesonide taken:

$$\text{Result} = [(r_{UA} + r_{UB})/(r_{SA} + r_{SB})] \times (C_S/C_U) \times 100$$

$r_{UA}$  = peak area of epimer A from the *Sample solution*

$r_{UB}$  = peak area of epimer B from the *Sample solution*

$r_{SA}$  = peak area of epimer A from the *Standard solution*

$r_{SB}$  = peak area of epimer B from the *Standard solution*

$C_S$  = concentration of USP Budesonide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Budesonide in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Epimer A:** 40.0%–51.0% on the dried basis

**Both epimers:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### • PROCEDURE 1: LIMIT OF 21-ACETATE OF BUDESONIDE

**Buffer:** 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid. The pH is  $3.2 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Standard solution:** Dissolve a quantity of USP Budesonide RS in acetonitrile, and dilute quantitatively with *Buffer* to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to NMT 30%.

**Sample solution:** Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with *Buffer* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the first eluted epimer of the 21-acetate of budesonide, the second eluted epimer of the 21-acetate of budesonide, the first eluted epimer of budesonide (epimer B), and the second eluted epimer of budesonide (epimer A) are 3.1, 3.2, 1.0, and 1.1, respectively.]

#### Suitability requirements

**Column efficiency:** NLT 5500 theoretical plates, determined from the budesonide epimer B peak

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of the 21-acetate of budesonide in the portion of Budesonide taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times 100$$

$r_{T1}$  = sum of the peak areas for the two epimers of the 21-acetate of budesonide

$r_{T2}$  = sum of the peak areas of the two budesonide peaks

**Acceptance criteria:** NMT 0.10% of the 21-acetate of budesonide is found.

#### • PROCEDURE 2: LIMIT OF 11-KETOBUDESONIDE

**Buffer:** 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid. The pH is  $3.2 \pm 0.1$ .

**Mobile phase:** Acetonitrile, isopropanol, and *Buffer* (26:9:65)

**Standard solution:** Dissolve a quantity of USP Budesonide RS in acetonitrile, and dilute quantitatively with *Buffer* to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to NMT 30%.

**Sample solution:** Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with *Buffer* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L1

**Column temperature:** 50°

[NOTE—Preheat the *Mobile phase* to 50°.]

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the two epimers of 11-ketobudesonide are 0.73 and 0.78, respectively; the relative retention times for 21-dehydrobudesonide, 14,15-dehydrobudesonide, and the first eluted epimer of budesonide (epimer B) are 0.68, 0.84, and 1.0, respectively.]

#### Suitability requirements

**Column efficiency:** NLT 5500 theoretical plates, determined from the budesonide epimer B peak

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of 11-ketobudesonide in the portion of Budesonide taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times 100$$

$r_{T1}$  = sum of the peak areas for the two ketobudesonide peaks

$r_{T2}$  = sum of the peak areas of the two budesonide peaks

**Acceptance criteria:** NMT 0.2% of 11-ketobudesonide is found.

#### • PROCEDURE 3

**Buffer:** 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid. The pH is  $3.2 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (32:68)

**Standard solution:** Dissolve a quantity of USP Budesonide RS in acetonitrile, and dilute quantitatively with *Buffer* to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to NMT 30%.

**Sample solution:** Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with *Buffer* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 5500 theoretical plates, determined from the budesonide epimer B peak

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Budesonide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area for each impurity

$r_T$  = sum of the areas of all of the peaks

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16α-Hydroxyprednisolone <sup>a</sup>	0.11	0.2
D-Homobudesonide <sup>b</sup>	0.36	0.10
21-Dehydrobudesonide (epimers) <sup>c</sup>	0.61; 0.66	0.07 <sup>d</sup>
14,15-Dehydrobudesonide <sup>e</sup>	0.86	0.10
Total specified impurities	—	0.4 <sup>f</sup>
Any other individual impurity	—	0.10
Total unspecified impurities	—	0.4

<sup>a</sup> 11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione.

<sup>b</sup> 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β-hydroxy-17-(hydroxymethyl)-D-homoandrosta-1,4-diene-3,17a-dione.

<sup>c</sup> 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al.

<sup>d</sup> Limit includes both epimers.

<sup>e</sup> 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione.

<sup>f</sup> Total specified impurities includes 11-ketobudesonide obtained in the test for *Limit of 11-Ketobudesonide* and the impurities listed above.

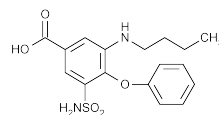
#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count is NMT  $10^3$  cfu/g, and the total combined molds and yeast count is NMT  $10^2$  cfu/g.
- **LOSS ON DRYING** <731>: Dry a sample at 105° to constant weight: it loses NMT 0.3% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Budesonide RS

## Bumetanide



$C_{17}H_{20}N_2O_5S$  364.42

Benzoic acid, 3-(aminosulfonyl)-5-(butylamino)-4-phenoxy-3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid [28395-03-1].

» Bumetanide contains not less than 98.0 percent and not more than 102.0 percent of  $C_{17}H_{20}N_2O_5S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

#### USP Reference standards <11>—

USP Bumetanide RS

USP Bumetanide Related Compound A RS

3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.

$C_{13}H_{12}N_2O_5S$  308.31

USP Bumetanide Related Compound B RS

3-Nitro-4-phenoxy-5-sulfamoylbenzoic acid.

$C_{13}H_{10}N_2O_7S$  338.29

USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS

$C_{21}H_{28}N_2O_5S$  420.53

**Identification—**

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

Solution: 50 µg per mL.

Medium: isopropyl alcohol.

**C:** The principal spot obtained from the chromatogram of the *Test preparation* exhibits an  $R_f$  value corresponding to that in the chromatogram of *Standard solution 1*, as obtained in the test for *Related compounds*.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%, a 1-g specimen being used.

**Heavy metals, Method II** (231): 0.002%.

**Related compounds—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution—**Dissolve an accurately weighed quantity of Bumetanide in methanol to obtain a solution having a concentration of about 25 mg per mL.

**Standard solution 1—**Dissolve an accurately weighed quantity of USP Bumetanide RS in methanol to obtain a solution having a known concentration of about 25 mg per mL.

**Standard solution 2—**Dilute a volume of *Standard solution 1* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

**Standard solution 3—**Dissolve an accurately weighed quantity of USP Bumetanide Related Compound B RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

**Standard solution 4—**Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

**Standard solution 5—**Dissolve an accurately weighed quantity of USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

**Application volume:** 20 µL of each solution.

**Developing solvent system:** a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

**Procedure—**Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After drying the application spots, place the plate in an unlined and unsaturated chromatographic chamber. Examine the plate under short-wavelength UV light. Any secondary spots obtained from the chromatogram of the *Test solution* having  $R_f$  values corresponding to the  $R_f$  values of the principal spots obtained from the chromatograms of *Standard solutions 3, 4, and 5* are not larger or more intense than the principal spots obtained from the chromatograms of *Standard solutions 3, 4, and 5*, respectively: not more than 0.2% of bumetanide related compound B is found; not more than 0.1% of bumetanide related compound A is found; and not more than 0.1% of butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate is found. No other individual secondary spots obtained from the chromatogram of the *Test solution* are larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 2*: not more than 0.2% of any other individual impurity is found; and

not more than 0.4% of the sum of all other impurities is found (excluding bumetanide related compound A, bumetanide related compound B, and butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate).

**Assay—**Dissolve about 1 g of Bumetanide, accurately weighed, in 150 mL of alcohol in a 250-mL conical flask. Add phenol red TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 36.44 mg of  $C_{17}H_{20}N_2O_5S$ .

## Bumetanide Injection

» Bumetanide Injection is a sterile solution of Bumetanide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide ( $C_{17}H_{20}N_2O_5S$ ).

**Packaging and storage—**Preserve in single-dose or multi-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Bumetanide RS

USP Bumetanide Related Compound A RS

3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.

$C_{13}H_{12}N_2O_5S$  308.31

USP Endotoxin RS

**Identification—**

**A:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**B:** The principal spot obtained from the chromatogram of the *Test solution* exhibits an  $R_f$  value corresponding to that of the *Identification solution*, as obtained in the test for *Related compounds*.

**Bacterial endotoxins** (85)—It contains not more than 350 USP Endotoxin Units per mg of bumetanide.

**pH** (791): between 6.8 and 7.8.

**Related compounds—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution—**Pipet a volume of Injection, equivalent to 5 mg of bumetanide, into a 125-mL separator, and adjust with 0.1 N sodium hydroxide to a pH of 12. Extract with two 20-mL portions of ethyl ether, discard the ethyl ether extracts, and adjust the aqueous layer with 1 N acetic acid to a pH of 4. Extract with two 20-mL portions of ethyl ether, passing the extracts through anhydrous sodium sulfate. Wash the sodium sulfate with about 5 mL of ethyl ether. Evaporate the combined ethyl ether extracts with the aid of a stream of nitrogen to dryness, and dissolve the residue in 0.5 mL of methanol.

**Identification solution—**Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 10 mg per mL.

**Standard solutions—**Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.08 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

Standard solution	Dilution	Concentration ( $\mu\text{g}$ of RS per mL)	Percentage (% for comparison with test specimen)
1	undiluted	80	0.8
2	3 in 4	60	0.6
3	1 in 2	40	0.4
4	1 in 4	20	0.2
5	1 in 8	10	0.1

**Standard solution 6**—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.02 mg per mL.

**Application volume:** 50  $\mu\text{L}$ .

**Developing solvent system:** a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate under short-wavelength UV light. Any secondary spot obtained from the chromatogram of the *Test solution* having an  $R_f$  value corresponding to the  $R_f$  value of the principal spot obtained from the chromatogram of *Standard solution 6* is not larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 6*: not more than 0.2% of bumetanide related compound A is found. For all other secondary spots obtained from the chromatogram of the *Test solution*, compare the intensity of each spot with the principal spots obtained from the chromatograms of *Standard solutions 1* through *5*: not more than 0.2% of any individual other impurity is found; and not more than 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, tetrahydrofuran, and glacial acetic acid (50:45:5:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 50 mg of 4-ethylbenzaldehyde to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 10.0 mL of tetrahydrofuran and 4.0 mL of glacial acetic acid, dilute with methanol to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bumetanide RS in *Internal standard solution*, and quantitatively dilute with *Internal standard solution* to obtain a solution having a known concentration of about 250  $\mu\text{g}$  per mL. Transfer 5.0 mL of the resulting solution to a 10-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 125  $\mu\text{g}$  of USP Bumetanide RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 0.25 mg of bumetanide, to a flask. Add an equal volume of *Internal standard solution*, accurately measured, insert the stopper, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for 4-ethylbenzaldehyde and 1.0 for bumetanide; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 1.5, the tailing factor for the analyte peak is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$  in each mL of the Injection taken by the formula:

$$(2C / V)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bumetanide RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bumetanide Tablets

» Bumetanide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide ( $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Bumetanide RS

USP Bumetanide Related Compound A RS

3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.

$\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_5\text{S}$  308.31

#### Identification—

**A:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** The principal spot obtained from the chromatogram of the *Test solution* exhibits an  $R_f$  value corresponding to that of the *Identification solution*, as obtained in the test for *Related compounds*.

#### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**pH 2.9 Glycine buffer**—Dissolve 7.505 g of glycine and 5.85 g of sodium chloride in water to make 1000 mL (stock solution). Dilute 80.0 mL of the stock solution and 20.0 mL of 0.1 N hydrochloric acid with water to 1000 mL. Adjust, if necessary, with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 2.9.

**Procedure**—Determine the amount of  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$  dissolved, by employing a suitable fluorometer having an excitation wavelength of about 350 nm and a fluorescence emission of about 450 nm on filtered portions of the solution under test, suitably diluted with *pH 2.9 Glycine buffer*, in comparison with a *Standard solution* having a known concentration of USP Bumetanide RS in the same *Medium*.

**Tolerances**—Not less than 85% (Q) of the labeled amount of  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Related compounds—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution**—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to 10 mg of bumetanide, to a 50-mL centrifuge tube, add 20 mL of acetone (spectrophotometric or HPLC quality), and shake by mechanical means for 10 minutes. Centrifuge for 10 minutes,

decant the supernatant into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.

**Identification solution**—Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 20 mg per mL.

**Standard solutions**—Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.16 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

Standard solution	Dilution	Concentration ( $\mu\text{g}$ of RS per mL)	Percentage (%, for comparison with test speci- men)
1	undiluted	160	0.8
2	3 in 4	120	0.6
3	1 in 2	80	0.4
4	1 in 4	40	0.2
5	1 in 8	20	0.1

**Standard solution 6**—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.04 mg per mL.

**Application volume:** 25  $\mu\text{L}$ .

**Developing solvent system:** a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate under short-wavelength UV light. Any secondary spot obtained from the chromatogram of the *Test solution* having an  $R_f$  value corresponding to the  $R_f$  value of the principal spot obtained from the chromatogram of *Standard solution 6* is not larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 6*: not more than 0.2% of bumetanide related compound A is found. For all other secondary spots obtained from the chromatogram of the *Test solution*, compare the intensity of each spot with the principal spots obtained from the chromatograms of *Standard solutions 1* through *5*: not more than 0.2% of any individual other impurity is found; and not more than 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

#### Assay—

**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Bumetanide Injection*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.5 mg of bumetanide, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and sonicate for 5 minutes. Add 2.0 mL of water, and mix. Cool, and filter, discarding the first 1 mL of the filtrate.

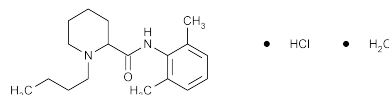
**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for 4-ethylbenzaldehyde and 1.0 for bumetanide. Calculate the quantity, in mg, of bumetanide ( $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$ ) in the portion of Tablets taken by the formula:

$$4C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Bumetanide RS in the *Standard preparation*; and  $R_U$  and  $R_S$

are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bupivacaine Hydrochloride



$\text{C}_{18}\text{H}_{28}\text{N}_2\text{O} \cdot \text{HCl} \cdot \text{H}_2\text{O}$  342.90

2-Piperidinecarboxamide, 1-butyl-N-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate, ( $\pm$ )-.

( $\pm$ )-1-Butyl-2',6'-pipecoloxylidide monohydrochloride, monohydrate [73360-54-0].

Anhydrous 324.90 [18010-40-7].

» Bupivacaine Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of  $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O} \cdot \text{HCl}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Bupivacaine Hydrochloride RS

#### Identification—

**A: Infrared Absorption** (197S)—

**Solution**—Dissolve about 230 mg in 15 mL of water in a separator, add 1 mL of 6 N ammonium hydroxide, and extract with three 30-mL portions of chloroform. Evaporate the chloroform at room temperature with the aid of a stream of nitrogen, and dry the residue in vacuum. Add 2 mL of chloroform to the residue, and dissolve.

**B: Ultraviolet Absorption** (197U)—

**Solution:** 500  $\mu\text{g}$  per mL.

**Medium:** 0.1 N hydrochloric acid.

Absorptivities at 271 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**C:** Dissolve about 50 mg in 10 mL of water in a small separator, render alkaline with 6 N ammonium hydroxide, and extract with 10 mL of ether: the aqueous layer meets the requirements of the tests for *Chloride* (191).

**pH** (791): between 4.5 and 6.0, in a solution (1 in 100).

**Water, Method I** (921): between 4.0% and 6.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 0.001%.

#### Limit of residual solvents—

**Alcohol standard solution**—Pipet 2 mL of dehydrated alcohol into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.08% of alcohol.

**Isopropyl alcohol standard solution**—Pipet 2 mL of isopropyl alcohol into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.004% of isopropyl alcohol.

**Test solution**—Transfer 1.0 g of Bupivacaine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system**—Under typical conditions, the instrument is equipped with a flame-ionization detector and a 4-mm  $\times$  2-m column that contains packing S3. The carrier gas is nitrogen, flowing at a rate of about 40 mL per min-

ute. The column temperature is maintained at about 175°, the injection port temperature is maintained at about 200°, and the detector temperature is maintained at about 280°.

**Procedure**—Inject equal volumes (about 5 µL) of the *Test solution*, the *Alcohol standard solution*, and the *Isopropyl alcohol standard solution* successively into the gas chromatograph. Measure the responses of the alcohol peak and the isopropyl alcohol peak in each chromatogram. Determine the percentage of alcohol taken by the formula:

$$2(r_U / r_S)$$

and determine the percentage of isopropyl alcohol taken by the formula:

$$0.1(r_U / r_S)$$

in which  $r_U$  and  $r_S$  are the responses of the respective analytes in the *Test solution* and of the corresponding analytes in the *Alcohol standard solution* and the *Isopropyl alcohol standard solution*, respectively. The sum of the content of alcohol and the content of isopropyl alcohol does not exceed 2%.

#### Chromatographic purity—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Solvent:** a mixture of chloroform and isopropylamine (99:1).

**Test solution**—Dissolve a suitable quantity of Bupivacaine Hydrochloride in *Solvent* to obtain a solution containing 20.0 mg per mL.

**Standard solution**—Dissolve a suitable quantity of USP Bupivacaine Hydrochloride RS, accurately weighed, in *Solvent* to obtain a solution containing 20.0 mg per mL.

**Diluted standard solution**—Quantitatively dilute a portion of the *Standard solution* in *Solvent* to obtain a solution having a concentration of 100 µg per mL.

**Developing solvent system:** a mixture of hexanes and isopropylamine (97:3).

**Procedure**—Apply separate 10-µL portions of the *Test Solution*, the *Standard solution*, and the *Diluted standard solution* on the starting line of suitable thin-layer chromatographic plate as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Develop the chromatogram in a suitable chamber until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry it in warm air. Place the plate in a closed chamber with a dish containing 1 g of iodine in a shallow layer, and allow to remain for about 5 minutes. Remove the plate from the chamber, spray it with 7 N sulfuric acid, and examine the chromatogram: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*, and the estimated size and intensity of any other spot obtained from the *Test solution* does not exceed that of the principal spot obtained from the *Diluted standard solution* (0.5%); and the total of the estimated sizes and intensities of all of the other spots obtained from the *Test solution* does not exceed four times that of the principal spot obtained from the *Diluted standard solution* (2.0%).

**Assay**—Transfer about 600 mg of Bupivacaine Hydrochloride, accurately weighed, to a 250-mL conical flask, and dissolve in 20 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.49 mg of  $C_{18}H_{28}N_2O \cdot HCl$ .

## Bupivacaine Hydrochloride Injection

» Bupivacaine Hydrochloride Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{28}N_2O \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.

#### USP Reference standards (11)—

USP Bupivacaine Hydrochloride RS

USP Endotoxin RS

#### Identification—

**A:** Dilute a volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, with 0.01 N hydrochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator." The Injection meets the requirements of the test.

**B:** The retention time of the bupivacaine peak in the chromatogram of the *Assay preparation* corresponds to that of the bupivacaine peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 2.5 USP Endotoxin Units per mg of bupivacaine hydrochloride.

**pH** (791): between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**pH 6.8 Phosphate buffer**—Dissolve 1.94 g of monobasic potassium phosphate and 2.48 g of dibasic potassium phosphate in 1000 mL of water. Adjust, if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.

**Mobile phase**—Prepare a fresh solution of acetonitrile and pH 6.8 Phosphate buffer (65:35). Adjust, if necessary, with 1 M phosphoric acid to a pH of  $7.7 \pm 0.2$ . Filter the solution through a membrane filter of 1-µm or finer porosity, and degas.

**Internal standard solution**—Prepare a solution of dibutyl phthalate in methanol containing about 1.3 mg per mL.

**Standard preparation**—Dissolve about 50 mg of USP Bupivacaine Hydrochloride RS, accurately weighed, in 10.0 mL of water, using sonication if necessary, in a 100-mL volumetric flask. Add 10 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 263-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation of the ratios of the bupivacaine hydrochloride peak to the dibutyl phthalate peak is not more than 1.0%, and the resolution  $R$ , factor, between bupivacaine hydrochloride and dibutyl phthalate is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.2 for dibutyl phthalate and 1.0 for bupivacaine hydrochloride. Calculate the quantity, in mg, of

$C_{18}H_{28}N_2O \cdot HCl$  in the volume of Injection taken by the formula:

$$W(R_U / R_S)$$

in which  $W$  is the weight, in mg, of USP Bupivacaine Hydrochloride RS, calculated on the anhydrous basis, in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of the peak responses of bupivacaine hydrochloride to those of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bupivacaine Hydrochloride in Dextrose Injection

» Bupivacaine Hydrochloride in Dextrose Injection is a sterile solution of Bupivacaine Hydrochloride and Dextrose in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amounts of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ) and dextrose ( $C_6H_{12}O_6$ ). It contains no preservative.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

### USP Reference standards <11>—

USP Bupivacaine Hydrochloride RS

USP Dextrose RS

USP Endotoxin RS

### Identification—

**A:** *Thin-Layer Chromatographic Identification Test* <201>—

**Adsorbent:** chromatographic silica gel mixture; 0.25 mm.

**Developing solvent:** mixture of butyl alcohol, water, dehydrated alcohol, and glacial acetic acid (6:2:1:1).

**Test preparation:** Bupivacaine Hydrochloride in Dextrose Injection.

**Standard preparations A, B, and C**—Separately prepare (A) a solution of USP Bupivacaine Hydrochloride RS in water, (B) a solution of USP Dextrose RS in water, and (C) a solution of USP Bupivacaine Hydrochloride RS in (B) to obtain solutions having concentrations corresponding to the labeled concentrations of bupivacaine hydrochloride and dextrose in the Injection.

**Naphthalenediol reagent**—Dissolve 20 mg of 1,3-naphthalenediol in 10 mL of dehydrated alcohol containing 0.2 mL of sulfuric acid.

**Iodoplatinate reagent**—Mix equal volumes of platinic chloride solution (3 in 1000) and potassium iodide solution (6 in 100).

**Procedure**—Separately apply 10  $\mu$ L each of the *Test preparation* and *Standard preparations A* and *C* to a portion of the chromatographic plate, and separately apply 1  $\mu$ L each of the *Test preparation* and *Standard preparation B* to the remaining portion of the plate. Dry the applications in a current of warm air, develop the chromatograms, remove the plate from the developing chamber, and mark the solvent front. Dry the plate in warm circulating air, and examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to the spots obtained from the adjacent chromatograms of *Standard preparations A* and *C*. Spray the plate with *Naphthalenediol reagent*, heat at 90° for 5 minutes, and examine the plate: the  $R_f$  value of the principal blue-purple spot obtained from the *Test preparation* corresponds to that obtained in the adjacent chromatogram of *Standard preparation B*. Cool the plate, spray it with *Iodoplatinate reagent*,

and examine the plate: bupivacaine appears as a blue-purple spot on a salmon-colored background, and the dextrose spots fade slightly: the  $R_f$  value of the bupivacaine spot obtained from the *Test preparation* corresponds to those obtained from the adjacent chromatograms of *Standard preparations A* and *C*.

**B:** It responds to *Identification test B* under *Bupivacaine Hydrochloride Injection*.

**Bacterial endotoxins** <85>—It contains not more than 1.8 USP Endotoxin Units per mg of bupivacaine hydrochloride.

**pH** <791>: between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay for bupivacaine hydrochloride—

**pH 6.8 Phosphate buffer, Mobile phase, Internal standard solution, Standard preparation, Chromatographic system, and Procedure**—Proceed as directed in the *Assay under Bupivacaine Hydrochloride Injection*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Assay for dextrose**—Determine the angular rotation of Injection in a suitable polarimeter tube (see *Optical Rotation* <781>). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6$ ) in the portion of Injection taken by the formula:

$$(100/52.9)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

## Bupivacaine Hydrochloride and Epinephrine Injection

» Bupivacaine Hydrochloride and Epinephrine Injection is a sterile solution of Bupivacaine Hydrochloride and Epinephrine or Epinephrine Bitartrate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ). The content of epinephrine ( $C_9H_{13}NO_3$ ) does not exceed 0.001 percent (1 in 100,000). It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.

**Labeling**—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

### USP Reference standards <11>—

USP Bupivacaine Hydrochloride RS

USP Epinephrine Bitartrate RS

USP Endotoxin RS

**Color and clarity**—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.



**Identification—**

**A:** It responds to the *Identification* tests under *Bupivacaine Hydrochloride Injection*.

**B:** Pipet a volume of Injection, equivalent to about 50 µg of epinephrine, into a suitable container, add 0.1 mL of *Ferro-citrate solution* and 2.0 mL of *Buffer solution* (prepared as directed under *Epinephrine Assay* (391)), mix, and allow the solution to stand for 10 minutes. Filter the solution: the filtrate is violet in color and may turn brownish.

**Bacterial endotoxins** (85)—It contains not more than 1.6 USP Endotoxin Units per mg of bupivacaine hydrochloride.

**pH** (791): between 3.3 and 5.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for bupivacaine hydrochloride—**

*pH 6.8 Phosphate buffer, Mobile phase, Internal standard solution, Standard preparation, Chromatographic system, and Procedure*—Proceed as directed in the Assay under *Bupivacaine Hydrochloride Injection*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Assay for epinephrine—**

*Mobile phase*—Prepare a suitably filtered and degassed mixture of water, methanol, and 2M monobasic sodium phosphate (900:50:50), containing in each 1000 mL, 40 mg of edetate disodium, 0.4 mL of phosphoric acid, and 0.4 g of sodium 1-octanesulfonate. Make adjustments, if necessary, to obtain a retention time of not less than 11 minutes for the epinephrine peak (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase* to obtain a solution having a concentration of about 2 µg per mL.

*Resolution solution*—Dissolve suitable quantities of epinephrine bitartrate and dopamine hydrochloride in *Mobile phase* to obtain a solution containing about 2 µg of each per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 25 µg of epinephrine, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

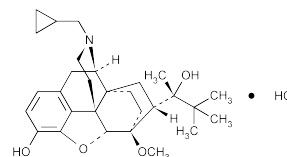
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with an electrochemical detector held at a potential of +0.75 volt and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the epinephrine and dopamine peaks is not less than 6.0; and the relative retention times are about 2 for dopamine and 1.0 for epinephrine. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of epinephrine (C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub>) in each mL of the Injection taken by the formula:

$$(183.21 / 333.30)(25)(C / V)(r_U / r_S)$$

in which 183.21 and 333.30 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; *C* is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; *V* is the volume, in mL, of

Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Buprenorphine Hydrochloride**

C<sub>29</sub>H<sub>41</sub>NO<sub>4</sub> · HCl 504.10

6,14-Ethenomorphinan-7-methanol, 17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-, hydrochloride, [5α, 7α(S)]-

21-Cyclopropyl-7α-[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine hydrochloride [53152-21-9].

» Buprenorphine Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of C<sub>29</sub>H<sub>41</sub>NO<sub>4</sub> · HCl, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Buprenorphine Hydrochloride RS

USP Buprenorphine Related Compound A RS

21-[3-(1-Propenyl)]-7α-[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine. C<sub>29</sub>H<sub>41</sub>NO<sub>4</sub> 467.65

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** To 0.5 mL of a solution of buprenorphine hydrochloride in methanol containing 50 mg per mL add 0.2 mL of a freshly prepared solution (1 in 10) of potassium ferricyanide TS and 0.5 mL of ferric chloride TS: a blue color appears immediately.

**C:** A solution (1 in 100) meets the requirements of the tests for *Chloride* (191).

**Specific rotation** (781S): between −92° and −98°.

*Test solution:* 20 mg per mL, in methanol.

**pH** (791): between 4.0 and 6.0 in a solution containing 10 mg per mL.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity—**

*Mobile phase*—Prepare a filtered and degassed mixture of methanol, 1% solution of ammonium acetate, and glacial acetic acid (60:10:0.01). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve accurately weighed quantities of USP Buprenorphine Hydrochloride RS and USP Buprenorphine Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of 12.5 µg of each Reference Standard per mL.

*Test solution*—Dissolve an accurately weighed quantity of about 50 mg of Buprenorphine Hydrochloride in 10.0 mL of *Mobile phase* to obtain a solution having a concentration of about 5 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 288-nm detector and a 4.6-mm × 25-cm column containing packing L1. The

flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between buprenorphine hydrochloride and buprenorphine related compound A is not less than 3.0; the column efficiency is not less than 6500 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

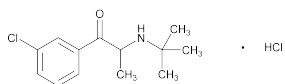
**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than two times the retention time of buprenorphine hydrochloride. Record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Buprenorphine Hydrochloride taken by the formula:

$$100(r_i / r_s)(C_s / C_T)$$

in which  $r_i$  is the peak response for each impurity obtained from the *Test solution*;  $r_s$  is the peak response of buprenorphine hydrochloride obtained from the *Standard solution*;  $C_s$  is the concentration, in mg per mL, of USP Buprenorphine Hydrochloride RS in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of Buprenorphine Hydrochloride in the *Test solution*: not more than 0.25% of any individual impurity is found, and the sum of all impurities is not more than 0.65%.

**Assay**—Dissolve about 0.8 g of Buprenorphine Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 50.41 mg of  $C_{29}H_{41}NO_4 \cdot HCl$ .

## Bupropion Hydrochloride



$C_{13}H_{18}ClNO \cdot HCl$  276.20  
1-Propanone, 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-, hydrochloride, ( $\pm$ );  
( $\pm$ )-2-(*tert*-Butylamino)-3'-chloropropiophenone hydrochloride [31677-93-7].

### DEFINITION

Bupropion Hydrochloride contains NLT 98.0% and NMT 102.0% of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Chloride** <191>  
**Sample solution:** 1 mg/mL of Bupropion Hydrochloride  
**Acceptance criteria:** Meets the requirements for the silver nitrate precipitate test

### ASSAY

#### Change to read:

#### PROCEDURE

**Diluent:** Methanol and water (1:1)

**Buffer:** 3.4 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 7.0.

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer*  $\Delta$ (39:11:50)  $\Delta$  USP36

**Standard solution:** 1 mg/mL of USP Bupropion Hydrochloride RS and 2  $\mu$ g/mL each of USP Bupropion Hydrochloride Related Compound A RS and USP Bupropion Hydrochloride Related Compound B RS in *Diluent*

**Sample solution:** 1 mg/mL of Bupropion Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 3.9-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for bupropion hydrochloride related compound A, bupropion,  $\Delta$  USP36 and bupropion hydrochloride related compound B are about 0.92, 1.0, and 1.14, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound A and bupropion;  $\Delta$ NLT 1.3 between bupropion and bupropion hydrochloride related compound B  $\Delta$  USP36

**Relative standard deviation:** NMT 2.0% determined from bupropion;  $\Delta$  USP36 NMT 5.0% determined from bupropion hydrochloride related compound B

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Change to read:

#### • LIMIT OF *m*-CHLOROBENZOIC ACID

**Mobile phase:** Acetonitrile, trifluoroacetic acid, and water (35:0.1:65)

**System suitability solution:** 1.0 mg/mL of USP Bupropion Hydrochloride RS and 4  $\mu$ g/mL of *m*-chlorobenzoic acid in *Mobile phase*

**Sample solution:** 2 mg/mL of Bupropion Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** 2.7 times the retention time of *m*-chlorobenzoic acid

**System suitability****Sample:** *System suitability solution*[NOTE—The relative retention times for bupropion <sup>▲</sup><sub>USP36</sub> and *m*-chlorobenzoic acid are 1.0 and 2.0, respectively.]**Suitability requirements**<sup>▲</sup><sub>USP36</sub>**Relative standard deviation:** NMT 5.0% for the *m*-chlorobenzoic acid peak**Analysis****Sample:** *Sample solution*Calculate the percentage of *m*-chlorobenzoic acid in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100 \quad \text{▲USP36}$$

 $r_U$  = peak response of *m*-chlorobenzoic acid from the *Sample solution* $r_T$  = sum of the responses of all the peaks from the *Sample solution*<sup>▲</sup> $F$  = relative response factor for *m*-chlorobenzoic acid, 2.4 <sup>▲</sup><sub>USP36</sub>**Acceptance criteria***m*-Chlorobenzoic acid: NMT 0.2%**Change to read:**• **▲ORGANIC IMPURITIES** <sup>▲</sup><sub>USP36</sub>**Diluent, Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response for each impurity from the *Sample solution* $r_S$  = peak response for bupropion <sup>▲</sup><sub>USP36</sub> from the *Standard solution* $C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor for each impurity relative to bupropion <sup>▲</sup>(see Table 1) <sup>▲</sup><sub>USP36</sub>**Acceptance criteria:** <sup>▲</sup>See Table 1. <sup>▲</sup><sub>USP36</sub>**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
<sup>▲</sup> Deschloro-bupropion <sup>a</sup> <sup>▲</sup> <sub>USP36</sub>	0.38	1.5	0.5
<sup>▲</sup> Bupropion dione derivative <sup>b</sup> <sup>▲</sup> <sub>USP36</sub>	0.58	1.0	0.2
<sup>▲</sup> <i>o</i> -Bupropion <sup>c</sup> <sup>▲</sup> <sub>USP36</sub>	0.71	0.45	0.1
<sup>▲</sup> Chloropropiophenone <sup>d</sup> <sup>▲</sup> <sub>USP36</sub>	0.78	1.2	0.1
Bupropion hydrochloride related compound A	0.92	1.4	0.2
Bupropion	1.0	—	—
Bupropion hydrochloride related compound B	1.14	<sup>▲</sup> 0.81 <sup>▲</sup> <sub>USP36</sub>	0.2
<sup>▲</sup> Bromochloropropiophenone <sup>e</sup> <sup>▲</sup> <sub>USP36</sub>	1.63	0.88	0.1
<sup>▲</sup> 4-Chlorobupropion <sup>f</sup> <sup>▲</sup> <sub>USP36</sub>	2.30	1.1	0.2
<sup>▲</sup> 5-Chlorobupropion <sup>g</sup> <sup>▲</sup> <sub>USP36</sub>	2.74	0.69	0.2
Any individual impurity	—	1.0	0.1
<sup>▲</sup> Total impurities <sup>h</sup>	—	—	1.0 <sup>▲</sup> <sub>USP36</sub>

<sup>a</sup> 2-(*tert*-Butylamino)-1-phenylpropan-1-one; also known as 2-(*tert*-butylamino)propiphenone.<sup>b</sup> 1-(3-Chlorophenyl)propane-1,2-dione; also known as 1-(3-chlorophenyl)-1,2-propanedione.<sup>c</sup> 2-(*tert*-Butylamino)-1-(2-chlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-2'-chloropropiophenone.<sup>d</sup> 1-(3-Chlorophenyl)propan-1-one; also known as 3'-chloropropiophenone.<sup>e</sup> 2-Bromo-1-(3-chlorophenyl)propan-1-one; also known as 2-bromo-3'-chloropropiophenone.<sup>f</sup> 2-(*tert*-Butylamino)-1-(3,4-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',4'-dichloropropiophenone.<sup>g</sup> 2-(*tert*-Butylamino)-1-(3,5-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',5'-dichloropropiophenone.<sup>h</sup> Sum of all impurities found in the test for *Limit of m-Chlorobenzoic Acid* and in the test for *Organic Impurities*. <sup>▲</sup><sub>USP36</sub>**SPECIFIC TESTS**• **WATER DETERMINATION, Method I** (921): NMT 0.5%**ADDITIONAL REQUIREMENTS****Change to read:**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. <sup>▲</sup>Store at room temperature. <sup>▲</sup><sub>USP36</sub>• **USP REFERENCE STANDARDS** (11)

USP Bupropion Hydrochloride RS

USP Bupropion Hydrochloride Related Compound A RS  
2-(*tert*-Butylamino)-4'-chloropropiophenone hydrochloride. $C_{13}H_{18}ClNO \cdot HCl$  276.20USP Bupropion Hydrochloride Related Compound B RS  
2-(*tert*-Butylamino)-3'-bromopropiophenone hydrochloride. $C_{13}H_{18}BrNO \cdot HCl$  320.66

## Bupropion Hydrochloride Tablets

### DEFINITION

Bupropion Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

**Acceptance criteria:** The *Sample* shows strong bands at about 1690, 1560, and 1240  $cm^{-1}$  and a weaker band at about 740  $cm^{-1}$ , similar to the reference preparation.

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate and 1.164 g/L of sodium hydroxide in water

**Mobile phase:** Methanol and *Buffer* (65:35)

**Diluent:** Methanol and water (65:35)

**Standard solution:** 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent*

**Sample stock solution:** Nominally 3.0 mg/mL of bupropion hydrochloride in *Diluent* prepared as follows.

Transfer an appropriate number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*, and shake by mechanical means until the Tablets have disintegrated (30–60 min). Sonicate for 5 min, dilute with *Diluent* to volume, and mix. Allow to stand for at least 30 min. Use the supernatant.

**Sample solution:** Nominally 0.6 mg/mL of bupropion hydrochloride from the *Sample stock solution* in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m base-deactivated packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupropion hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** USP Bupropion Hydrochloride RS at a known concentration in 0.1 N hydrochloric acid

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with 0.1 N hydrochloric acid, if necessary.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** 252 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 80% (Q) of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in tight containers.

#### • USP REFERENCE STANDARDS (11)

USP Bupropion Hydrochloride RS

## Bupropion Hydrochloride Extended-Release Tablets

### DEFINITION

Bupropion Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

**Acceptance criteria:** The *Sample* shows strong bands at about 1690, 1560, and 1240  $cm^{-1}$  and a weaker band at about 740  $cm^{-1}$ , similar to the reference preparation.

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent 1:** Methanol and 0.001 N hydrochloric acid (20:80)

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (10:0.04:90)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (95:0.03:5)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

**System suitability stock solution A:** 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS and 0.2 mg/mL of USP Bupropion Hydrochloride Related Compound F RS in methanol

**System suitability solution A:** 0.0018 mg/mL of USP Bupropion Hydrochloride Related Compound C RS and 0.018 mg/mL of USP Bupropion Hydrochloride Related Compound F RS from *System suitability stock solution A* and *Diluent 1*

**System suitability stock solution B:** 0.09 mg/mL of *m*-chlorobenzoic acid in water

**System suitability solution B:** 0.0018 mg/mL of *m*-chlorobenzoic acid from *System suitability stock solution B* and *Diluent 1*

**Standard solution:** 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent 1*

**Sample stock solution A:** Transfer a number of Tablets intact or crushed, to a suitable homogenizer vessel containing sufficient methanol to obtain a concentration of 3.0 mg/mL of bupropion hydrochloride. Immediately homogenize the sample for 30 s at 20,000 rpm. Allow extraction for 3 min, and follow by two additional 10-s pulses, each at 20,000 rpm, pausing 3 min between these pulses to ensure complete extraction. Pass a portion of the solution through a nylon filter of 0.45-μm pore size, discarding the first 2–4 mL of the filtrate.

**Sample solution A:** Nominally 0.6 mg/mL of *Sample stock solution A* in 0.001 N hydrochloric acid. Alternatively, the *Sample solution* can be prepared as follows:

**Buffer:** Dissolve 100 g of anhydrous disodium hydrogen phosphate in 1 L of water. Add 50 mL of phosphoric acid, stir or sonicate until dissolved, and mix. Adjust with phosphoric acid to a pH of 3.0.

**Diluent 2:** Methanol and *Buffer* (1:4)

**Sample stock solution B:** Weigh and grind NLT 20 Tablets to prepare a solution of nominal concentration of 3 mg/mL. Initially add *Diluent 2* (75% of the volume of the flask), stir for 30 min, and sonicate for 15 min, and then dilute to volume. Mix, centrifuge a portion, and use the supernatant.

**Sample solution B:** Nominally 0.6 mg/mL of *Sample stock solution B* in *Diluent 2*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 5 μL

#### System suitability

**Samples:** *System suitability solution A*, *System suitability solution B*, and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between bupropion hydrochloride related compound C and bupropion hydrochloride related compound F, *System suitability solution A*

**Tailing factor:** NMT 1.9, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Relative response factor:** Between 0.22 and 0.26 for *m*-chlorobenzoic acid. [NOTE—Use the responses from *System suitability solution B* and the *Standard solution*.]

#### Analysis

**Samples:** *Standard solution* and *Sample solution A* or *Sample solution B*

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bupropion hydrochloride from *Sample solution A* or *Sample solution B*

$r_S$  = peak response of bupropion hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

For products labeled for dosing every 12 h

##### Test 1

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 4, and 8 h

**Standard solution:** USP Bupropion Hydrochloride RS at a known concentration in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

##### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Cell:** 1.0 cm

**Blank:** *Medium*

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Amount Dissolved (%)
1	25–45
4	60–85
8	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of  $1.5 \pm 0.05$ ); 900 mL, deaerated

**Apparatus 1:** 50 rpm

**Times:** 1, 2, 4, and 6 h

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved by using the following method.

**Buffer:** 3.45 g of monobasic sodium phosphate monohydrate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of  $2.80 \pm 0.05$ .

**Mobile phase:** Methanol and *Buffer* (7:13)

**Standard solution:** USP Bupropion Hydrochloride RS in *Medium* at a known concentration similar to the one expected in the *Sample solution*

**Sample solution:** Use portions of the solution under test, and pass through a nylon filter of 0.45-μm pore size.

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 298 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** See Table 3.

**Table 3**

Time (h)	Amount Dissolved (%)
1	25–50
2	40–65
4	65–90
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and Analysis:** Proceed as directed for *Test 1*, except the wavelength is about 250 nm, and use wire coil sinkers, if necessary.

**Times:** 1, 2, 4, and 6 h

**Tolerances:** See Table 4.

**Table 4**

Time (h)	Amount Dissolved (%)
1	30–55
2	50–75
4	70–90
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium and Analysis:** Proceed as directed for *Test 1*.

**Times:** 1, 3, and 6 h

**Instrumental conditions:** Proceed as directed for *Test 1*, except use a 0.5-cm cell.

**Tolerances:** See Table 5.

**Table 5**

Time (h)	Amount Dissolved (%)
1	35–55
3	65–85
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

**Medium, Apparatus 1, and Times:** Proceed as directed for *Test 2*, including the quantitative chromat-

ographic method, but using as the *Mobile phase* a mixture of *Buffer* with methanol (55:45).

**Tolerances:** See Table 6.

**Table 6**

Time (h)	Amount Dissolved (%)
1	25–50
2	45–70
4	NLT 70
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 9:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

**Medium:** 0.1 N hydrochloric acid, pH 1.5 (prepared as directed for *Test 2*); 900 mL

**Apparatus 1:** 50 rpm

**Times:** 1, 2, 4, and 8 h

**Standard solution:** ( $L/1000$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the Tablet label claim, in mg

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Instrumental conditions:** Proceed as directed for *Test 1*.

**Tolerances:** See Table 7.

**Table 7**

Time (h)	Amount Dissolved (%)
1	20–45
2	35–55
4	55–85
8	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**For products labeled for dosing every 24 h**

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** 0.1 N hydrochloric acid; 900 mL, deaerated

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 16 h

**Standard solution:** USP Bupropion Hydrochloride RS at a known concentration in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Analytical wavelength:** 252 nm

**Cell:** 1.0 cm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** See Table 8.

**Table 8**

Time (h)	Amount Dissolved (%)
2	NMT 20
4	20–45
8	65–90
16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium and Apparatus 1:** Proceed as directed for *Test 4*.

**Times:** 1, 2, 4, 8, and 12 h

**Standard solution:** USP Bupropion Hydrochloride RS at a known concentration in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Cell:** 1.0 cm

**Blank:** *Medium*

**Tolerances:** See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
1	15–35
2	25–50
4	40–65
8	65–90
12	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 8:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 16 h

**Standard solution**

**For Tablets labeled to contain 150 mg:** USP Bupropion Hydrochloride RS dissolved in *Medium* (about 0.167 mg/mL)

**For Tablets labeled to contain 300 mg:** USP Bupropion Hydrochloride RS dissolved in *Medium* (about 0.333 mg/mL)

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Blank:** *Medium*

**Tolerances:** See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
2	NMT 10
4	10–35
8	45–75
16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### Procedure for content uniformity

**Standard solution:** 0.33 mg/mL of USP Bupropion Hydrochloride RS in water

**Sample solution:** Transfer 1 Tablet to a suitable homogenizer vessel containing a volume of water to obtain a concentration of about 0.33 mg of bupropion hydrochloride per mL. Immediately homogenize the sample using single 30-s pulses each at 5000, 10,000, and 15,000 rpm, and follow by two pulses each at 20,000 rpm. After the homogenate has settled, mix at 5000 rpm for an additional 30 s. Pass a portion of the solution through a nylon filter of 0.45- $\mu$ m pore size, discarding the first 4 mL of the filtrate.

**Analysis:** Proceed as directed for the appropriate *Dissolution* procedure, using a 0.5-cm cell, and correct for dilution.

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Sample solution A or Sample solution B, and Chromatographic system:** Proceed as directed in the *Assay*.

##### Analysis

**Samples:** *Standard solution* and *Sample solution A* or *Sample solution B*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response of each impurity from *Sample solution A* or *Sample solution B*

$r_S$  = peak response of bupropion hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

$F$  = relative response factor for each impurity (see *Table 11* for values)

**Acceptance criteria:** See *Table 11*.

Table 11

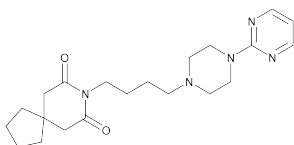
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)	
			100 mg or less	150 mg or greater
2-Amino-1-(3-chlorophenyl)-1-propanone	0.38	0.80	0.3	0.3
(3 <i>S</i> ,5 <i>S</i> ,6 <i>S</i> )-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	0.56	0.86	1.0	1.5
(3 <i>S</i> ,5 <i>R</i> ,6 <i>R</i> )-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid	0.78	0.88	0.5	0.4

Table 11 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)	
			100 mg or less	150 mg or greater
Bupropion	1.0	—	—	—
Bupropion related compound F	1.71	0.55	1.2	2.3
Bupropion related compound C	1.75	0.59	0.3	0.3
<i>m</i> -Chloro-benzoic acid	1.80	0.24	0.3	0.3
1-(3-Chlorophenyl)-1,2-propanedione	2.25	1.00	0.4	0.4
Any unspecified impurity	—	1.00	0.2	0.2
Total impurities	—	—	3.2	3.3

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Bupropion Hydrochloride RS
  - USP Bupropion Hydrochloride Related Compound C RS
  - 1-(3-Chlorophenyl)-2-hydroxy-1-propanone.  
C<sub>9</sub>H<sub>9</sub>O<sub>2</sub>Cl 184.62
  - USP Bupropion Hydrochloride Related Compound F RS
  - 1-(3-Chlorophenyl)-1-hydroxy-2-propanone.  
C<sub>9</sub>H<sub>9</sub>O<sub>2</sub>Cl 184.62

**Buspirone Hydrochloride**

C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> · HCl 421.96  
 8-Azaspiro[4,5]decane-7,9-dione, 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-, monohydrochloride;  
*N*-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-1,1-cyclopentanediacetamide monohydrochloride  
 [33386-08-2; 36505-84-7].

**DEFINITION**

Buspirone Hydrochloride contains NLT 97.5% and NMT 102.5% of C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> · HCl, calculated on the as-is basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

**Sample solution:** 10 mg/mL in water

**ASSAY****PROCEDURE**

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water. Adjust the solution with 10% sodium hydroxide (w/v) to a pH of 7.5, and filter.

**Mobile phase:** Acetonitrile and *Buffer* (2:3)

**Internal standard stock solution:** 2.5 mg/mL of propylparaben in methanol

**Internal standard solution:** 0.125 mg/mL of propylparaben from the *Internal standard stock solution* in water

**Standard stock solution:** Dissolve 50 mg of USP Buspirone Hydrochloride RS in 25 mL of 1 N hydrochloric acid in a 100-mL volumetric flask, and dilute with water to volume.

**Standard solution:** To 10.0 mL of *Standard stock solution* in a 50.0-mL volumetric flask add 10.0 mL of *Internal standard solution*, and dilute with water to volume.

**Sample stock solution:** Dissolve 50 mg of Buspirone Hydrochloride in 25 mL of 1 N hydrochloric acid in a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** 0.1 mg/mL of Buspirone Hydrochloride from the *Sample stock solution* and 0.025 mg/mL of propylparaben from the *Internal standard solution* in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 25 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for propylparaben and buspirone hydrochloride are about 0.55 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4 between buspirone hydrochloride and the internal standard

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> · HCl in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of buspirone hydrochloride to propylparaben from the *Sample solution*

$R_S$  = peak response ratio of buspirone hydrochloride to propylparaben from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.5% on the as-is basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.5%
- **HEAVY METALS, Method II** (231): NMT 20 ppm

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** (921): NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at controlled room temperature.



- **USP REFERENCE STANDARDS** (11)  
USP Buspirone Hydrochloride RS

## Buspirone Hydrochloride Tablets

» Buspirone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, at controlled room temperature.

**USP Reference standards** (11)—  
USP Buspirone Hydrochloride RS

### Identification—

**A:** *Infrared Absorption* (197K)—

*Test specimen*—Grind 20 Tablets to a fine powder, add 50 mL of chloroform, stir for 3 to 5 minutes, and filter into a 250-mL evaporating flask. Evaporate the solution with the aid of a rotary evaporator to dryness at low heat. Use the residue.

**B:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{31}N_5O_2 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbances at about 235 nm on filtered portions of the solution under test, suitably diluted with 0.01 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Buspirone Hydrochloride RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{21}H_{31}N_5O_2 \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Buffer solution, Mobile phase, Internal standard solution, Standard stock solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Buspirone Hydrochloride*.

*Assay preparation*—Transfer a number of Tablets, equivalent to about 100 mg of buspirone hydrochloride, to a 200-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and shake for 15 minutes. Add about 100 mL of water, and shake for 30 minutes. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Pipet 10.0 mL of the filtrate and 10.0 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with water to volume, and mix.

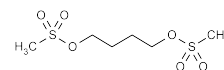
*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Buspirone Hydrochloride*. Calculate the quantity, in mg, of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ) in the Tablets taken by the formula:

$$(L / D)C(R_U / R_S)$$

in which *L* is the labeled amount, in mg, of buspirone hydrochloride in each Tablet; *D* is the concentration, in mg per mL, of buspirone hydrochloride in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; *C* is the concentration, in mg per mL, of USP Buspirone Hydrochloride RS in the *Standard preparation*; and *R<sub>U</sub>*

and *R<sub>S</sub>* are the peak response ratios of buspirone hydrochloride to propylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Busulfan



$C_6H_{14}O_6S_2$  246.30

1,4-Butanediol, dimethanesulfonate.

1,4-Butanediol dimethanesulfonate [55-98-1].

» Busulfan contains not less than 98.0 percent and not more than 100.5 percent of  $C_6H_{14}O_6S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label bears a warning that great care should be taken to prevent inhaling particles of Busulfan and exposing the skin to it.

### Identification—

**A:** Fuse about 100 mg with about 100 mg of potassium nitrate and a pellet of potassium hydroxide weighing approximately 250 mg. Cool, dissolve the residue in water, acidify with 3 N hydrochloric acid, and add a few drops of barium chloride TS: a white precipitate is formed.

**B:** To 100 mg add 10 mL of water and 5 mL of 1 N sodium hydroxide. Heat until a clear solution is obtained: an odor characteristic of methanesulfonic acid is perceptible.

**C:** Cool the solution obtained in *Identification* test *B*, and divide it into two equal portions. To one portion add 1 drop of potassium permanganate TS: the purple color changes to violet, then to blue, and finally to emerald-green. Acidify the second portion of the solution with 2 N sulfuric acid, and add 1 drop of potassium permanganate TS: the color of the permanganate is not discharged.

**Melting range** (741): between 115° and 118°.

**Loss on drying** (731)—Dry it in vacuum at 60° to constant weight: it loses not more than 2.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Assay**—Transfer about 80 mg of Busulfan, accurately weighed, to a 250-mL conical flask. Add about 30 mL of water, swirl, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Connect the flask to a reflux air condenser, and boil the mixture gently for not less than 30 minutes, adding water occasionally to maintain the volume. Cool to room temperature, add phenolphthalein TS, and titrate with 0.05 N sodium hydroxide VS. Each mL of 0.05 N sodium hydroxide is equivalent to 6.158 mg of  $C_6H_{14}O_6S_2$ .

## Busulfan Tablets

» Busulfan Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_6H_{14}O_6S_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Pulverize a suitable number of Tablets, and extract the powder with several portions of acetone. Evaporate the combined acetone extracts, with the aid of a cur-

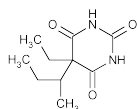
rent of air, on a steam bath: the dry residue responds to the *Identification* tests under *Busulfan*, and melts at about 115°.

**Disintegration** (701): 30 minutes, the use of disks being omitted.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Weigh and finely powder not fewer than 40 Tablets. [Caution—Guard against accidental inhalation of fine powder.] Weigh accurately a portion of the powder, equivalent to about 80 mg of busulfan, and transfer to a 100-mL beaker. Extract with four 20-mL portions of acetone, each time stirring the mixture well, then allowing the insoluble matter to settle, and finally decanting the supernatant through a sintered-glass filter into a 250-mL conical flask. Evaporate the combined acetone extracts to about 10 mL, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Evaporate to dryness, add about 30 mL of water, and proceed as directed in the *Assay* under *Busulfan*, beginning with "Connect the flask." Each mL of 0.05 N sodium hydroxide is equivalent to 6.158 mg of  $C_6H_{14}O_6S_2$ .

## Butabarbital



$C_{10}H_{16}N_2O_3$  212.25  
2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-  
5-(1-methylpropyl)-.  
5-sec-Butyl-5-ethylbarbituric acid [125-40-6].

» Butabarbital contains not less than 98.5 percent and not more than 101.0 percent of  $C_{10}H_{16}N_2O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Butabarbital RS

**Identification**, *Infrared Absorption* (197M).

**Melting range**, *Class Ia* (741): between 164° and 167°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—

**Standard solutions**—Dissolve a quantity of USP Butabarbital RS in a mixture of chloroform and methanol (1:1) to obtain a solution having a concentration of 4.0 mg per mL (*Standard solution A*). Dilute 1.0 mL of *Standard solution A* with a mixture of chloroform and methanol (1:1) to 10.0 mL, and mix (*Standard solution B*).

**Test solution**—Dissolve a quantity of Butabarbital in a mixture of chloroform and methanol (1:1) to obtain a solution having a concentration of 40 mg per mL.

**Procedure**—Proceed as directed for *Procedure* in the test for *Chromatographic purity* under *Butabarbital Sodium*.

**Assay**—

**Internal standard solution**—Transfer about 400 mg of tetracosane to a 200-mL volumetric flask, add chloroform to volume, and mix.

**Standard preparation**—Transfer about 200 mg of USP Butabarbital RS, accurately weighed, to a 100-mL volumetric flask, add chloroform to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and mix to obtain a

solution having a known concentration of about 1.0 mg of USP Butabarbital RS per mL.

**Assay preparation**—Transfer about 200 mg of Butabarbital, accurately weighed, to a 100-mL volumetric flask, add chloroform to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m column packed with 10% phase G37 on support S1AB. The column temperature is maintained at about 260°, the injection port at about 260°, and the detector block at about 300°. Dry nitrogen is used as the carrier gas at a flow rate of about 50 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factors for the analyte and internal standard peaks are not more than 1.3 and 1.2, respectively; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for butabarbital and 1.0 for tetracosane. Calculate the quantity, in mg, of  $C_{10}H_{16}N_2O_3$  in the portion of Butabarbital taken by the formula:

$$200C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Butabarbital RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butabarbital Sodium

$C_{10}H_{15}N_2NaO_3$  234.23  
2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-  
5-(1-methylpropyl)-, monosodium salt.  
Sodium 5-sec-butyl-5-ethylbarbiturate [143-81-7].

» Butabarbital Sodium contains not less than 98.2 percent and not more than 100.5 percent of  $C_{10}H_{15}N_2NaO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Butabarbital RS

**Completeness of solution**—Dissolve 1.0 g in 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

**Identification**—

**A: Infrared Absorption** (197K)—Prepare the test specimen as follows. Transfer about 150 mg to a suitable separator, dissolve in 10 mL of water, and add 15 mL of 3 N hydrochloric acid. Extract with three 20-mL portions of chloroform, filter the extracts through anhydrous sodium sulfate, and collect the extracts in a suitable beaker. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 hours.

**B: Ultraviolet Absorption** (197U)—

**Solution:** 10 µg per mL.

**Medium:** pH 9.6 alkaline borate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*).

Absorptivities at 240 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Ignite about 100 mg; the residue responds to the tests for *Sodium* (191).

**pH** (791): between 10.0 and 11.2, in the solution prepared for the test for *Completeness of solution*.

**Loss on drying** (731)—Dry it at 150° to constant weight; it loses not more than 5.0% of its weight.

**Heavy metals, Method II** (231): 0.003%.

#### Chromatographic purity—

**Standard solutions**—Dissolve a quantity of USP Butabarbital RS in a mixture of chloroform and methanol (1:1) to obtain a solution having a final concentration of 4.0 mg per mL (*Standard solution A*). Dilute 1.0 mL of *Standard solution A* with a mixture of chloroform and methanol (1:1) to 10.0 mL, and mix (*Standard solution B*).

**Test solution**—Dissolve a quantity of Butabarbital Sodium in a mixture of chloroform and methanol (1:1) to obtain a solution having a final concentration of 44 mg per mL.

**Procedure**—Apply 10 µL of the *Test solution* and 10 µL each of *Standard solution A* and *Standard solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of acetone, methylene chloride, methanol, and ammonium hydroxide (5:3:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry the plate in a current of air. Spray the plate with a solution of mercurous nitrate dihydrate in 0.15 N nitric acid (1 in 100), and immediately estimate the intensities of any spots in the chromatogram of the *Test solution*, other than the principal spot, in comparison with *Standard solution B*: the  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from *Standard solution A*; and the sum of the intensities of any secondary spots observed in the chromatogram of the *Test solution* is not greater than the intensity of the principal spot produced by *Standard solution B*, corresponding to not more than a total of 1% of impurities.

#### Assay—

**Standard preparation**—Transfer about 25 mg of USP Butabarbital RS, accurately weighed, to a 200-mL volumetric flask, dissolve in pH 9.6 alkaline borate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and dilute with the same solvent to volume.

**Assay preparation**—Transfer about 28 mg of Butabarbital Sodium, accurately weighed, to a 200-mL volumetric flask, dissolve in pH 9.6 alkaline borate buffer to volume, and dilute with the same solvent to volume.

**Procedure**—Transfer 10.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 100-mL volumetric flasks, dilute each with pH 9.6 alkaline borate buffer to volume, and mix. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using pH 9.6 alkaline borate buffer as the blank. Calculate the quantity, in mg, of  $C_{10}H_{15}N_2NaO_3$  in the portion of Butabarbital Sodium taken by the formula:

$$(234.23 / 212.25)(0.2C)(A_U / A_S)$$

in which 234.23 and 212.25 are the molecular weights of butabarbital sodium and butabarbital, respectively; C is the concentration, in µg per mL, of USP Butabarbital RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Butabarbital Sodium Oral Solution

» Butabarbital Sodium Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Butabarbital RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Place a volume of Oral Solution, equivalent to about 150 mg of butabarbital sodium, in a separator, render it distinctly alkaline by the addition of 1 N sodium hydroxide, and saturate it with sodium chloride. Extract the mixture with two 15-mL portions of ether, and discard the ether. Acidify the solution with hydrochloric acid, and render it just alkaline to litmus by adding small portions of sodium bicarbonate (carbonate-free). Extract the liberated acid barbiturate, using five 20-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water acidified with 1 drop of hydrochloric acid, then extract the water with 10 mL of chloroform, adding the latter to the main chloroform solution. Filter the chloroform solution through a pledget of cotton or other suitable filter, previously washed with chloroform, into a tared beaker, and finally wash the separator and the filter with three 5-mL portions of chloroform. Evaporate the combined chloroform solution and washings on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 hours.

**Alcohol content, Method II** (611): between 95.0% and 115.0% of the labeled amount of  $C_2H_5OH$ .

#### Assay—

**Internal standard solution**—Dissolve an accurately weighed quantity of secobarbital in chloroform, and quantitatively dilute with chloroform to obtain a solution having a known concentration of about 0.7 mg per mL.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butabarbital RS and secobarbital in chloroform, and quantitatively dilute with chloroform to obtain a solution that contains, in each mL, known amounts of about 1 mg of USP Butabarbital RS and about 1.4 mg of secobarbital.

**Assay preparation**—[NOTE—This preparation includes a bromination step for elimination of parabens and a carbonate-chloroform extraction for elimination of benzoic acid.] Transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of butabarbital sodium, to a separator, add 1 mL of bromine water (prepared by dissolving 2.0 mL of bromine and 10 g of potassium bromide in 60 mL of water), and swirl. Allow to stand for 5 minutes, add 1 mL of sodium metabisulfite solution (1 in 10), and swirl. Add 300 mg of sodium bicarbonate in small portions, with mixing, and extract with four 10-mL portions of chloroform. Filter the extracts through about 15 g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrates in a 50-mL volumetric flask, wash the sodium sulfate with 5 mL of chloroform, collecting the washing with the filtrate, dilute with chloroform to volume, and mix. Combine 2.0 mL of this solution with 2.0 mL of *Internal standard solution* in a suitable container, and reduce the volume to about 1 mL by evaporation, with the aid of a stream of dry nitrogen, at room temperature.

**Chromatographic system and System suitability**—Proceed as directed for *Chromatographic System* and *System Suitability* under *Barbiturate Assay* (361), the resolution,  $R$ , between butabarbital and secobarbital being not less than 2.4.

[NOTE—Relative retention times are approximately 0.6 for butabarbital and 1.0 for secobarbital.]

**Procedure**—Proceed as directed for *Procedure* under *Barbiturate Assay* (361). Calculate the quantity, in mg, of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) in each mL of the Oral Solution taken by the formula:

$$(234.23 / 212.25)(50)(R_U)(Q_S)(C_i) / V(R_S)$$

in which 234.23 and 212.25 are the molecular weights of butabarbital sodium and butabarbital, respectively;  $V$  is the volume, in mL, of Oral Solution taken; and the other terms are as defined therein.

## Butabarbital Sodium Tablets

» Butabarbital Sodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Butabarbital RS

**Identification, Infrared Absorption** (197K)—

**Test specimen**—Mix a quantity of ground Tablets, equivalent to about 150 mg of butabarbital sodium, with 1 mL of dimethyl sulfoxide and 1 mL of water, add hydrochloric acid dropwise until the solution is just acid to litmus, and mix. Add 3 g of chromatographic siliceous earth, and mix. Proceed as directed for *Column Partition Chromatography* under *Chromatography* (621), packing the chromatographic tube as follows. The lower layer consists of 4 g of chromatographic siliceous earth mixed with 3 mL of sodium carbonate solution (1 in 10), and the upper layer is the test specimen. Wash the column with 75 mL of a water-saturated mixture of isooctane and ether (4:1), and discard the washing. Elute the butabarbital with 200 mL of water-saturated ether, collecting the eluate in a suitable vessel. Evaporate the eluate to dryness on a steam bath under a current of air, and dry the residue at 105° for 2 hours.

**Dissolution** (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{10}H_{15}N_2NaO_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 239 nm on filtered portions of the solution under test, mixed with sufficient ammonium hydroxide to provide a concentration of 0.5 N ammonium hydroxide, and suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Butabarbital RS in the same medium.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{10}H_{15}N_2NaO_3$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Acid-methanol mixture**—Prepare a mixture of methanol and 1 N hydrochloric acid (9:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Butabarbital RS in *Acid-methanol mixture* to obtain a solution having a known concentration of about 0.45 mg per mL.

**Test preparation**—Transfer 1 finely powdered Tablet to a 25-mL volumetric flask, add *Acid-methanol mixture* to volume, and mix. Filter, discarding the first 5 mL of the filtrate, and dilute the subsequent filtrate, quantitatively and stepwise if necessary, with *Acid-methanol mixture* to obtain a solution containing 0.5 to 0.6 mg of butabarbital sodium per mL.

**Procedure**—Transfer 2.0 mL each of the *Standard preparation* and the *Test preparation* to separate 100-mL volumetric flasks, and transfer 2.0 mL of *Acid-methanol mixture* to a third volumetric flask to provide a blank. Dilute each flask with pH 9.6 alkaline borate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_{10}H_{15}N_2NaO_3$  in the Tablet taken by the formula:

$$(234.23/212.25)/(TC/D)(A_U / A_S)$$

in which 234.23 and 212.25 are the molecular weights of butabarbital sodium and butabarbital, respectively;  $T$  is the labeled quantity, in mg, of butabarbital sodium in the Tablet;  $C$  is the concentration, in mg per mL, of USP Butabarbital RS in the *Standard preparation*;  $D$  is the concentration, in mg per mL, of butabarbital sodium in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay**—

**Internal standard solution**—Dissolve an accurately weighed quantity of secobarbital in chloroform, and dilute quantitatively with chloroform to obtain a solution having a known concentration of about 1.2 mg per mL.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butabarbital RS and secobarbital in chloroform, and dilute quantitatively with chloroform to obtain a solution that contains, in each mL, known amounts of about 0.8 mg of USP Butabarbital RS and about 1 mg of secobarbital.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of butabarbital sodium, to a 50-mL volumetric flask, add 35 mL of dilute ammonium hydroxide (1 in 25), dilute with water to volume, and mix. Filter, if necessary, discarding the first 15 mL of the filtrate, and transfer 25.0 mL of the clear solution to a separator. Add 2 mL of hydrochloric acid, and extract with three 25-mL portions of chloroform. Filter the extracts through about 15 g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrate in a 100-mL volumetric flask, wash the sodium sulfate with 15 mL of chloroform, collecting the washing with the filtrate, dilute with chloroform to volume, and mix. Combine 4.0 mL of this solution with 1.0 mL of *Internal standard solution* in a suitable container, and reduce the volume to about 1 mL by evaporation, with the aid of a stream of dry nitrogen, at room temperature.

**Chromatographic system and System suitability**—Proceed as directed for *Chromatographic System* and *System Suitability* under *Barbiturate Assay* (361). The resolution,  $R$ , between butabarbital and secobarbital is not less than 2.4; and the relative retention times are approximately 0.6 for butabarbital and 1.0 for secobarbital.

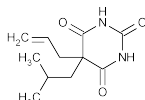
**Procedure**—Proceed as directed for *Procedure* under *Barbiturate Assay* (361). Calculate the quantity, in mg, of

$C_{10}H_{15}N_2NaO_3$  in the portion of Tablets taken by the formula:

$$(234.23 / 212.25)(50)(R_U)(Q_3)(C_i) / (R_3)$$

in which 234.23 and 212.25 are the molecular weights of butabarbital sodium and butabarbital, respectively; and the other terms are as defined therein.

## Butalbital



$C_{11}H_{16}N_2O_3$  224.26  
2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-(2-methylpropyl)-  
5-(2-propenyl)-.  
5-Allyl-5-isobutylbarbituric acid [77-26-9].

» Butalbital contains not less than 98.0 percent and not more than 102.0 percent of  $C_{11}H_{16}N_2O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Butalbital RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 15 µg per mL.

*Medium:* 0.1 N sodium hydroxide.

Absorptivities at 246 nm, calculated on the dried basis, do not differ by more than 2.5%.

**Melting range** (741): between 138° and 141°.

**Loss on drying** (731)—Dry it in vacuum at room temperature to constant weight: it loses not more than 0.2% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, Method II (231): 0.002%.

### Chromatographic purity—

*Chloroform-methanol*—Mix equal volumes of chloroform and methanol.

*Standard preparations*—Dissolve a quantity of USP Butalbital RS in *Chloroform-methanol* to obtain a solution having a concentration of 40 mg per mL (*Solution A*). Dilute 1.0 mL of *Solution A* with *Chloroform-methanol* to 100 mL, and mix (*Solution B*); mix 5.0 mL of *Solution B* with 5.0 mL of *Chloroform-methanol* (*Solution C*); and mix 5.0 mL of *Solution C* with 5.0 mL of *Chloroform-methanol* (*Solution D*).

*Test preparation*—Dissolve a quantity of Butalbital in *Chloroform-methanol* to obtain a solution having a concentration of 40 mg per mL.

*Procedure*—In a suitable chromatographic chamber, arranged for thin-layer chromatography and lined with filter paper, place a volume of a developing solvent consisting of a mixture of acetone, dichloromethane, methanol, and ammonium hydroxide (50:30:10:10) sufficient to develop the chromatogram. Cover the chamber, and allow it to equilibrate for 30 minutes. Apply 10 µL each of the *Test preparation* and *Solutions A, B, C, and D* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram until the solvent front has

moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate in a current of air. Spray the plate with a reagent prepared by dissolving 5 g of potassium hydroxide in a mixture of 25 mL of water and 75 mL of alcohol. Allow the plate to dry in warm air for 10 minutes, and examine the chromatograms under UV light: the chromatograms show principal spots at about the same  $R_f$  value; and the sum of the intensities of any secondary spots, if present in the chromatogram from the *Test preparation*, is not greater than 1% of that of the principal spot from *Solution A*. [NOTE—The relative intensities of the principal spots from the *Standard preparations* are: *Solution A*, 1; *Solution B*, 0.01; *Solution C*, 0.005; and *Solution D*, 0.0025.]

**Assay**—Dissolve about 180 mg of Butalbital, accurately weighed, in a mixture of 25 mL of alcohol and 25 mL of sodium carbonate solution (3 in 100), and titrate with 0.1 N silver nitrate VS, determining the endpoint electrometrically, using a silver electrode, either with a suitable reference electrode containing a saturated aqueous solution of potassium nitrate, or a combination electrode in which the reference portion of the electrode contains a saturated aqueous solution of potassium nitrate. Each mL of 0.1 N silver nitrate is equivalent to 22.43 mg of  $C_{11}H_{16}N_2O_3$ .

## Butalbital, Acetaminophen, and Caffeine Capsules

» Butalbital, Acetaminophen, and Caffeine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Butalbital RS

USP Caffeine RS

**Identification**—The retention times of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Assay preparation* correspond to those of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 60 minutes.

*Mobile phase and Chromatographic system*—Prepare as directed in the *Assay* under *Butalbital, Acetaminophen, and Caffeine Tablets*.

*Standard preparation*—Prepare a solution in methanol having known concentrations of about 0.02A mg of USP Acetaminophen RS per mL, 0.02B mg of USP Butalbital RS per mL, and 0.02C mg of USP Caffeine RS per mL, in which A, B, and C are the labeled amounts, in mg of acetaminophen, butalbital, and caffeine, respectively, per Capsule. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Pass a portion of the solution under test through a filter of 10-µm or finer porosity. Separately inject equal volumes (about 20 µL) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acet-

aminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the same formula:

$$900C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amounts of  $C_{11}H_{16}N_2O_3$ ,  $C_8H_9NO_2$ , and  $C_8H_{10}N_4O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Internal standard solution, Butalbital standard stock solution, Caffeine standard stock solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Butalbital, Acetaminophen, and Caffeine Tablets.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about the weight of the contents of 1 Capsule, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a filter of 0.5  $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Capsules taken by the formula:

$$500D(R_U / R_S)$$

in which  $D$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butalbital, Acetaminophen, and Caffeine Tablets

» Butalbital, Acetaminophen, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Acetaminophen RS

USP Butalbital RS

USP Caffeine RS

**Identification**—The retention times of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Assay preparation* correspond to those of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Standard preparation*, as obtained in the Assay.

#### Dissolution, Procedure for a Pooled Sample (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

*Mobile phase and Chromatographic system*—Prepare as directed in the Assay.

*Standard preparation*—Prepare a solution in methanol having known concentrations of about 0.02A mg of USP Acetaminophen RS per mL, 0.02B mg of USP Butalbital RS per mL, and 0.02C mg of USP Caffeine RS per mL, in which A, B, and C are the labeled amounts, in mg, of acetaminophen, butalbital, and caffeine, respectively, per Tablet. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Pass a portion of the solution under test through a suitable filter having a 10- $\mu$ m or finer porosity. Separately inject equal volumes (about 20  $\mu$ L) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the same formula:

$$900C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amounts of  $C_{11}H_{16}N_2O_3$ ,  $C_8H_9NO_2$ , and  $C_8H_{10}N_4O_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase*—Transfer 800 mg of monobasic potassium phosphate to a 2000-mL volumetric flask. Dissolve in 1100 mL of water, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5- $\mu$ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of phenacetin in methanol containing 0.65 mg per mL.

*Butalbital standard stock solution*—Dissolve an accurately weighed quantity of USP Butalbital RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01B mg per mL, B being the labeled amount, in mg, of butalbital per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.

*Caffeine standard stock solution*—Dissolve an accurately weighed quantity of USP Caffeine RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01C mg per mL, C being the labeled amount, in mg, of caffeine per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.

*Standard preparation*—Transfer to a 50-mL volumetric flask about 0.1A mg of USP Acetaminophen RS, A being the labeled amount, in mg, of acetaminophen per Tablet, 10.0 mL of *Butalbital standard stock solution*, and 10.0 mL of *Caffeine standard stock solution*, sonicate for 5 minutes, dilute with water to volume, and mix. This solution contains about 0.002B mg of butalbital, 0.002A mg of acetaminophen, and 0.002C mg of caffeine per mL. Pass a portion of this solution through a suitable filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Standard preparation*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 average Tablet weight, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow

to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.5- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 216-nm detector and a 4-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.16 for acetaminophen, 0.33 for caffeine, 0.77 for phenacetin, and 1.0 for butalbital; the resolution,  $R$ , between any two peaks is not less than 1.2; the column efficiency, calculated from the butalbital peak, is not less than 1000 theoretical plates; and the relative standard deviations of the acetaminophen, caffeine, and butalbital responses for replicate injections are not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken by the same formula:

$$500D(R_U / R_S)$$

in which  $D$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butalbital and Aspirin Tablets

» Butalbital and Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Aspirin RS

USP Butalbital RS

USP Salicylic Acid RS

**Identification**—The retention times of the butalbital peak and the aspirin peak in the chromatogram of the *Assay preparation* correspond to those of the butalbital peak and the aspirin peak in the chromatograms of the *Butalbital and salicylic acid standard preparation* and the *Aspirin standard preparation*, as obtained in the *Assay for butalbital and aspirin and limit of free salicylic acid*.

**Dissolution** <711>—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 60 minutes.

**Determination of dissolved butalbital**—

**Mobile phase**—Prepare a suitable filtered and degassed mixture of water, acetonitrile, and phosphoric acid (3100:725:4). Adjust the ratio as necessary.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butalbital RS and of salicylic acid in *Mobile phase* to obtain a solution containing known concentrations of about 1  $\mu$ g of butalbital per mL for each mg of the labeled amount per Tablet and about 30  $\mu$ g of salicylic acid per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the butalbital and salicylic acid peaks is not less than 3.0; and the relative standard deviation of butalbital responses for replicate injections is not more than 3.0%.

**Procedure**—Filter a portion of the solution under test through a 0.5- $\mu$ m filter. Separately inject equal volumes (about 10 to 25  $\mu$ L) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for aspirin, 0.85 for salicylic acid, and 1.0 for butalbital. [NOTE—After use, the column may be regenerated by passing through it at least 50 mL of a mixture of acetonitrile, methanol, and water (1:1:1), followed by 50 mL of a mixture of acetonitrile and water (1:1).] Calculate the amount, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ) dissolved by the formula:

$$0.9C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Butalbital RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the butalbital peak responses obtained from the solution under test and the *Standard preparation*, respectively.

**Determination of dissolved aspirin**—

**pH 4.5 Buffer**—Dissolve 5.98 g of sodium acetate trihydrate in 500 mL of water, add 2.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix. Adjust this solution with glacial acetic acid to a pH of  $4.5 \pm 0.05$ , and mix.

**Procedure**—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, diluted with 4 volumes of *pH 4.5 Buffer*, in comparison with a *Standard solution* having a known concentration of USP Aspirin RS in the same medium. [NOTE—Prepare the *Standard solution* at the time of use. An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the *Reference Standard* into solution prior to dilution first with water and then with 4 volumes of *pH 4.5 Buffer*.]

**Tolerances**—Not less than 75% (Q) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and aspirin ( $C_9H_8O_4$ ) are dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to butalbital and for *Weight Variation* with respect to aspirin.

**Assay for butalbital and aspirin and limit of free salicylic acid**—

**Mobile phase**—Prepare a suitable filtered and degassed mixture of water, acetonitrile, and phosphoric acid (3100:725:4). Adjust the ratio as necessary.

**Solvent mixture**—Mix 40 mL of formic acid and 4000 mL of acetonitrile.

**Butalbital standard stock solution**—Dissolve an accurately weighed quantity of USP Butalbital RS in *Solvent mixture* to obtain a solution having a known concentration of about 3250  $\mu$ g per mL,  $J$  being the ratio of the labeled amount, in mg, of butalbital to the labeled amount, in mg, of aspirin per tablet.

**Salicylic acid standard stock solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 200  $\mu$ g per mL.

**Butalbital and salicylic acid standard preparation**—Transfer 25.0 mL of *Butalbital standard stock solution* and 3.0 mL of *Salicylic acid standard stock solution* to a 250-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This

solution contains about 325  $\mu\text{g}$  of butalbital and 2.4  $\mu\text{g}$  of salicylic acid per mL.

**Aspirin standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Solvent mixture* to obtain a solution having a known concentration of about 325  $\mu\text{g}$  per mL.

**Resolution solution**—Transfer 4.0 mL of *Butalbital standard stock solution* and 3.0 mL of *Salicylic acid standard stock solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 80 mg of aspirin, to a 250-mL volumetric flask, dilute with *Solvent mixture* to volume, sonicate for 15 minutes, and mix. Pass a portion of this solution through a 0.5- $\mu\text{m}$  porosity filter before use.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm  $\times$  30-cm column that contains 10- $\mu\text{m}$  packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Butalbital and salicylic acid standard preparation*, the *Aspirin standard preparation*, and the *Resolution solution* as directed for *Procedure*: the resolution,  $R$ , between the butalbital and salicylic acid peaks is not less than 3.0; and the relative standard deviation for replicate injections of the Standard preparations is not more than 3.0% for butalbital and aspirin, and not more than 6.0% for salicylic acid.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparations* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks, and for the minor peak corresponding to salicylic acid. The relative retention times are about 0.6 for aspirin, 0.85 for salicylic acid, and 1.0 for butalbital. [NOTE—After use, the column may be regenerated by passing through it at least 50 mL of a mixture of acetonitrile, methanol, and water (1:1:1), followed by a mixture of acetonitrile and water (1:1).] Calculate the quantity, in mg, of butalbital ( $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_3$ ) in the portion of Tablets taken by the formula:

$$0.25C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Butalbital RS in the *Butalbital and salicylic acid standard preparation*; and  $r_U$  and  $r_S$  are the butalbital peak responses obtained from the *Assay preparation* and the *Butalbital and salicylic acid standard preparation*, respectively. Calculate the quantity, in mg, of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) in the portion of Tablets taken by the formula:

$$0.25C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Aspirin RS in the *Aspirin standard preparation*; and  $r_U$  and  $r_S$  are the aspirin peak responses obtained from the *Assay preparation* and the *Aspirin standard preparation*, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$25(C / a)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of the USP Salicylic Acid RS in the *Butalbital and salicylic acid standard preparation*;  $a$  is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $r_U$  and  $r_S$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Butalbital and salicylic acid standard preparation*, respectively: not more than 3.0% is found.

## Butalbital, Aspirin, and Caffeine Capsules

» Butalbital, Aspirin, and Caffeine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_3$ ), aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ), and caffeine ( $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS  
USP Butalbital RS  
USP Caffeine RS  
USP Salicylic Acid RS

**Identification**—The retention times of the butalbital, aspirin, and caffeine peaks in the chromatogram of the *Assay preparation* correspond to those of the butalbital, aspirin, and caffeine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: water; 1000 mL.

*Apparatus 2*: 50 rpm.

*Time*: 60 minutes.

Determine the amounts of butalbital, aspirin, and caffeine dissolved employing the procedure set forth in the *Assay*, making any necessary modifications.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of butalbital ( $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_3$ ), caffeine ( $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ ), and aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—[NOTE—Use glassware in this test.]

**Solvent**—Add 1 mL of phosphoric acid to 1000 mL of methanol, and mix.

**Standard preparation**—Prepare a solution of USP Salicylic Acid RS in *Solvent* having a known concentration of about 0.0012 mg per mL. Use this solution promptly.

**Test preparation**—[NOTE—Perform this test on the same day the powder is removed from the Capsules.] Transfer an accurately weighed portion of the powder remaining from the preparation of the *Assay preparation* in the *Assay*, equivalent to about 65 mg of aspirin, to a 200-mL flask, add 100.0 mL of *Solvent*, and shake for about 1 minute. Promptly filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate as the *Test preparation*. Use this solution within 20 minutes after the addition of the *Solvent*.

**Procedure**—Immediately place the cell containing the solution under test in the cell compartment of a spectrophotofluorimeter, and allow to equilibrate for 2 minutes. Concomitantly measure the intensities of the fluorescence of the *Test preparation* and the *Standard preparation* at 444 nm, using an excitation wavelength of 305 nm. Calculate the percentage of salicylic acid in the portion of Capsules taken by the formula:

$$10,000(C/a)(I_U / I_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*;  $a$  is the quantity, in mg, of aspirin in the portion of Capsules taken to prepare the *Test preparation*, based on the labeled amount; and  $I_U$  and  $I_S$  are the fluorescence intensity readings obtained from the *Test preparation* and the *Standard preparation*, respectively. [NOTE—If the intensity of the *Test preparation* greatly exceeds that of the *Standard preparation*, immediately transfer 5.0 mL of the *Test preparation* to a 50-mL volumetric



flask, dilute with *Solvent* to volume, and mix. Immediately determine the intensity of this solution, and calculate the percentage of salicylic acid in the portion of Capsules taken by the formula:

$$100,000(C/a)(I_U / I_S).$$

Not more than 2.5% is found.

#### Assay—

**0.01 M Phosphate buffer**—Dissolve 1.361 g of monobasic potassium phosphate in 1000 mL of water, and mix.

**Mobile phase**—Prepare a suitable filtered and degassed mixture of 0.01 M Phosphate buffer and methanol (550:450), and adjust with phosphoric acid to a pH of 3.9. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**pH 2.5 Buffer**—Prepare a mixture of 0.01 M Phosphate buffer and methanol (550:450), and adjust with phosphoric acid to a pH of 2.5 ± 0.05.

**Aspirin standard stock solution**—Dissolve an accurately weighed quantity of USP Aspirin RS in pH 2.5 Buffer to obtain a solution having a known concentration of about 1.6 mg per mL, sonicating and shaking the solution, if necessary, to achieve complete dissolution. Use this solution within 24 hours.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butalbital RS and USP Caffeine RS quantitatively in *Aspirin standard stock solution* to obtain a solution having known concentrations of about 1.6J mg of USP Butalbital RS and 1.6J' mg of USP Caffeine RS per mL, J and J' being the ratios of the respective labeled amounts, in mg, of butalbital and caffeine to the labeled amount, in mg, of aspirin per Capsule, sonicating and shaking the solution, if necessary, to achieve complete dissolution. Use this solution within 24 hours.

**Salicylic acid solution**—Prepare a solution of salicylic acid in pH 2.5 Buffer containing about 0.1 mg per mL. Pass this solution through a suitable filter of 0.5-μm or finer porosity.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 200-mL volumetric flask, dilute with pH 2.5 Buffer to volume, sonicate for about 30 minutes, and mix. Pass a portion of this solution through a suitable filter of 0.5-μm or finer porosity, and use the filtrate as the *Assay preparation*. Use this solution within 24 hours. [NOTE—Reserve the remaining portion of the powder for the test for *Limit of free salicylic acid*.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector set at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm and a 210-nm detector, and a 3.9-mm × 30-cm column that contains packing L1 and is maintained at 35 ± 1°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.45 for caffeine, 0.6 for aspirin, and 1.0 for butalbital; the resolution, *R<sub>s</sub>*, between the caffeine and aspirin peaks is not less than 2.0; the column efficiency determined from the butalbital peak is not less than 2000 theoretical plates; and the relative standard deviations of the caffeine, aspirin, and butalbital responses for replicate injections are not more than 2.0%. Inject 10 μL of the *Salicylic acid solution*, and record the peak response as directed for *Procedure*: the salicylic acid peak has the same retention time as that of the aspirin peak obtained in the chromatogram of the *Standard preparation*. [NOTE—If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. The retention time of the salicylic acid peak decreases

about 0.3 minute for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, using the 277-nm detector to record the caffeine and aspirin peak responses and the 210-nm detector to record the butalbital peak responses, and measure the areas for the major peaks. Calculate the quantities, in mg, of caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>), aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>), and butalbital (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>) in the portion of Capsules taken by the same formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively. Correct the amount of aspirin obtained for the amount of salicylic acid present by the formula:

$$A - (0.01FA)$$

in which A is the quantity, in mg, of aspirin in the portion of Capsules taken to prepare the *Assay preparation*; and F is the percentage of salicylic acid obtained in the test for *Limit of free salicylic acid*.

## Butalbital, Aspirin, and Caffeine Tablets

» Butalbital, Aspirin, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>), aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>), and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aspirin RS  
USP Butalbital RS  
USP Caffeine RS  
USP Salicylic Acid RS

**Identification**—The retention times of the butalbital, aspirin, and caffeine peaks in the chromatogram of the *Assay preparation* correspond to those of the butalbital, aspirin, and caffeine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 60 minutes.

Determine the amounts of butalbital (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>), aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>), and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) dissolved by employing the procedure set forth in the *Assay* under *Butalbital, Aspirin, and Caffeine Capsules*, making any necessary modifications.

**Tolerances**—Not less than 80% (Q) of the labeled amounts of butalbital (C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>), caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>), and aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—[NOTE—Use glassware in this test.]

**Solvent**—Add 1 mL of phosphoric acid to 1000 mL of methanol, and mix.

**Standard preparation**—Prepare a solution of USP Salicylic Acid RS in *Solvent* having a known concentration of about 0.0012 mg per mL. Use this solution promptly.

**Test preparation**—[NOTE—Perform this test on the same day the tablets are powdered.] Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 65 mg of aspirin, to a 200-mL flask, add 100.0 mL of *Solvent*, and shake by mechanical means for 15 minutes. Filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate as the *Test preparation*. Use this solution within 20 minutes after the addition of the *Solvent*.

**Procedure**—Immediately place the cell containing the solution under test in the cell compartment of a spectrophotofluorimeter, and allow to equilibrate for 2 minutes. Concomitantly measure the intensities of the fluorescence of the *Test preparation* and the *Standard preparation* at 444 nm, using an excitation wavelength of 305 nm. Calculate the percentage of salicylic acid in the portion of Tablets taken by the formula:

$$10,000(C/a)(I_U/I_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*; *a* is the quantity, in mg, of aspirin in the portion of Tablets taken to prepare the *Test preparation*, based on the labeled amount; and *I<sub>U</sub>* and *I<sub>S</sub>* are the fluorescence intensity readings obtained from the *Test preparation* and the *Standard preparation*, respectively. [NOTE—If the intensity of the *Test preparation* greatly exceeds that of the *Standard preparation*, immediately transfer 5.0 mL of the *Test preparation* to a 50-mL volumetric flask, dilute with *Solvent* to volume, and mix. Immediately determine the intensity of this solution, and calculate the percentage of salicylic acid in the portion of Tablets taken by the formula:  $100,000(C/a)(I_U/I_S)$ .] Not more than 3.0% is found.

#### Assay—

0.01 M Phosphate buffer, Mobile phase, pH 2.5 Buffer, Aspirin standard stock solution, Standard preparation, Salicylic acid solution, and Chromatographic system—Proceed as directed in the Assay under Butalbital, Aspirin, and Caffeine Capsules.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 200-mL volumetric flask, dilute with pH 2.5 buffer to volume, and sonicate for 30 minutes. Pass a portion of this solution through a suitable filter of 0.5 µm or finer porosity, and use the filtrate as the *Assay preparation*. Use this solution within 24 hours.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, using the 277-nm detector to record the caffeine and aspirin peak areas and the 210-nm detector to record the butalbital peak areas, and measure the peak areas for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken by the same formula:

$$200C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively. Correct the amount of as-

pirin obtained for the amount of salicylic acid present taken by the formula:

$$A - (0.01FA)$$

in which *A* is the quantity, in mg, of aspirin in the portion of Tablets taken to prepare the *Assay preparation*; and *F* is the percentage of salicylic acid obtained in the test for Limit of free salicylic acid.

## Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules

» Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Aspirin RS  
USP Butalbital RS  
USP Caffeine RS  
USP Codeine Phosphate RS  
USP Salicylic Acid RS

**Identification**—The retention times of the butalbital, aspirin, caffeine, and codeine peaks in the chromatograms of the *Assay preparation* correspond to those of the butalbital, aspirin, caffeine, and codeine peaks in the chromatograms of the *Standard preparation*, as obtained in the Assay.

#### Dissolution (711)—

Medium: water; 1000 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

0.01 M Phosphate buffer, pH 2.5 Buffer, Mobile phase, and Salicylic acid solution—Prepare as directed in the Assay.

**Dilute salicylic acid solution**—Transfer 5.0 mL of Salicylic acid solution, prepared as directed in the Assay, to a 50-mL volumetric flask, dilute with pH 2.5 Buffer to volume, and mix. Pass through a suitable filter of 0.5 µm or finer porosity.

**Aspirin standard stock solution**—Prepare a solution of USP Aspirin RS in a mixture of pH 2.5 Buffer and Dissolution Medium (1:1) containing a known concentration of about 0.16 mg per mL. Use this solution within 24 hours.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butalbital RS, USP Caffeine RS, and USP Codeine Phosphate RS quantitatively in *Aspirin standard stock solution* to obtain a solution having known concentrations of about 0.16/ mg of USP Butalbital RS, 0.16/ mg of USP Caffeine RS, and 0.16/ mg of USP Codeine Phosphate RS per mL, *J*, *J'*, and *J''* being the ratios of the respective labeled amounts, in mg, of butalbital, caffeine, and codeine phosphate to the labeled amount, in mg, of aspirin per Capsule, sonicating and shaking the solution, if necessary to achieve complete dissolution. Pass a portion of this solution through a suitable filter of 0.5 µm or finer porosity. Use this solution within 24 hours.

**Chromatographic system**—Proceed as directed for Chromatographic system in the Assay, except to inject 100 µL, instead of 10 µL, into the chromatograph, and to use Dilute salicylic acid solution instead of Salicylic acid solution.

**Procedure**—Pass about 20 mL of the solution under test through a suitable filter of 0.5-µm or finer porosity, discard-

ing the first 2 mL of the filtrate. Mix 5.0 mL of the filtrate and 5.0 mL of *pH 2.5 Buffer* to obtain the *Test preparation*. Separately inject equal volumes (about 100  $\mu$ L) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, using the 277-nm detector to record the caffeine and aspirin peaks and the 210-nm detector to record the butalbital and codeine responses, and measure the areas for the major peaks. Calculate the quantities, in mg, of caffeine ( $C_8H_{10}N_4O_2$ ), aspirin ( $C_9H_8O_4$ ), and butalbital ( $C_{11}H_{16}N_2O_3$ ) dissolved by the same formula:

$$2000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Test preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved by the formula:

$$(406.37 / 397.37)(2000C)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the codeine peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (*Q*) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) are dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—[NOTE—Use glassware in this test.]

**Solvent**—Add 1 mL of phosphoric acid to 1000 mL of methanol, and mix.

**Standard preparation**—Prepare a solution of USP Salicylic Acid RS in *Solvent* having a known concentration of about 0.0012 mg per mL. Use this solution promptly.

**Test preparation**—[NOTE—Perform this test on the same day the powder is removed from the Capsules.] Transfer an accurately weighed portion of the powder remaining from the preparation of the *Assay preparation* in the *Assay*, equivalent to about 65 mg of aspirin, to a 200-mL flask, add 100.0 mL of *Solvent*, and shake for about 1 minute. Filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate as the *Test preparation*. Use this solution within 20 minutes after the addition of the *Solvent*.

**Procedure**—Immediately place the cell containing the solution under test in the cell compartment of a spectrophotofluorimeter, and allow to equilibrate for 2 minutes. Concomitantly measure the intensities of the fluorescence of the *Test preparation* and the *Standard preparation* at 444 nm using an excitation wavelength of 305 nm. Calculate the percentage of salicylic acid in the portion of Capsules taken by the formula:

$$10,000(C / a)(I_U / I_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*; *a* is the quantity, in mg, of aspirin in the portion of Capsules taken to prepare the *Test preparation*, based on the labeled amount; and  $I_U$  and  $I_S$  are the fluorescence intensity readings obtained from the *Test preparation* and the *Standard preparation*, respectively. [NOTE—If the intensity of the *Test preparation* greatly exceeds that of the *Standard preparation*, immediately transfer 5.0 mL of the *Test preparation* to a 50-mL volumetric

flask, dilute with *Solvent* to volume, and mix. Immediately determine the intensity of this solution, and calculate the percentage of salicylic acid in the portion of Capsules taken by the formula:

$$100,000(C / a)(I_U / I_S)]$$

Not more than 3.0% is found.

#### Assay—

**0.01 M Phosphate buffer**—Dissolve 1.361 g of monobasic potassium phosphate in 1000 mL of water, and mix.

**Mobile phase**—Prepare a suitable mixture of 0.01 M Phosphate buffer and methanol (550:450), and adjust with phosphoric acid to a pH of 3.9. Pass through a suitable filter of 0.5- $\mu$ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**pH 2.5 Buffer**—Prepare a mixture of 0.01 M Phosphate buffer and methanol (550:450), and adjust with phosphoric acid to a pH of 2.5  $\pm$  0.05.

**Aspirin standard stock solution**—Dissolve an accurately weighed quantity of USP Aspirin RS in *pH 2.5 Buffer* to obtain a solution having a known concentration of about 1.6 mg per mL, sonicating and shaking the solution, if necessary, to achieve complete dissolution. Use this solution within 24 hours.

**Standard preparation**—Dissolve accurately weighed quantities of USP Butalbital RS, USP Caffeine RS, and USP Codeine Phosphate RS quantitatively in *Aspirin standard stock solution* to obtain a solution having known concentrations of about 1.6J mg of USP Butalbital RS, 1.6J' mg of USP Caffeine RS, and 1.6J'' mg of USP Codeine Phosphate RS per mL, *J*, *J'*, and *J''* being the ratios of the respective labeled amounts, in mg, of butalbital, caffeine, and codeine phosphate to the labeled amount, in mg, of aspirin per Capsule, sonicating and shaking the solution, if necessary, to achieve complete dissolution.

**Salicylic acid solution**—Prepare a solution of salicylic acid in *pH 2.5 Buffer* containing about 0.1 mg per mL. Pass this solution through a suitable filter of 0.5  $\mu$ m or finer porosity.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 200-mL volumetric flask, dilute with *pH 2.5 Buffer* to volume, sonicate for about 30 minutes, and mix. Pass a portion of this solution through a suitable filter of 0.5  $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*. Use this solution within 24 hours. [NOTE—Reserve the remaining portion of the powder for the test for *Limit of free salicylic acid*.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector set at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm and a 210-nm detector, and a 3.9-mm  $\times$  30-cm column that contains packing L1 and is maintained at 35  $\pm$  1°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for codeine, 0.45 for caffeine, 0.6 for aspirin, and 1.0 for butalbital; the resolution, *R*, between the caffeine and aspirin peaks is not less than 2.0; the column efficiency determined from the butalbital peak is not less than 2000 theoretical plates; and the relative standard deviations of the codeine, caffeine, aspirin, and butalbital responses for replicate injections are not more than 2.0%. Inject 10  $\mu$ L of the *Salicylic acid solution*, and record the peak response as directed for *Procedure*: the salicylic acid peak has the same retention time as that of the aspirin peak obtained in the chromatogram of the *Standard preparation*. [NOTE—If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. The retention time of the salicylic acid

peak decreases about 0.3 minute for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, using the 277-nm detector to record the caffeine and aspirin peak responses and the 210-nm detector to measure the codeine and butalbital responses, and measure the areas for the major peaks. Calculate the quantities, in mg, of caffeine ( $C_8H_{10}N_4O_2$ ), aspirin ( $C_9H_8O_4$ ), and butalbital ( $C_{11}H_{16}N_2O_3$ ) in the portion of Capsules taken by the same formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Capsules taken by the formula:

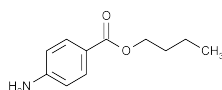
$$(406.37 / 397.37)(200C)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the codeine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Correct the amount of aspirin obtained for the amount of salicylic acid present by the formula:

$$A - (0.01FA)$$

in which  $A$  is the quantity, in mg, of aspirin in the portion of Capsules taken to prepare the *Assay preparation*; and  $F$  is the percentage of salicylic acid obtained in the test for *Limit of free salicylic acid*.

## Butamben



$C_{11}H_{15}NO_2$  193.24  
Benzoic acid, 4-amino-, butyl ester.  
Butyl *p*-aminobenzoate [94-25-7].

» Butamben, dried over phosphorus pentoxide for 3 hours, contains not less than 98.0 percent and not more than 101.0 percent of  $C_{11}H_{15}NO_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Butamben RS

**Completeness and color of solution**—One g dissolves completely in 30 mL of alcohol and in 30 mL of ether, and the solutions are colorless.

**Identification, Infrared Absorption** (197K).

**Melting range, Class I** (741): between 57° and 59°.

**Reaction**—Dissolve 1 g in 10 mL of neutralized alcohol: a clear solution results. Dilute this solution with 10 mL of water, and add 2 drops of phenolphthalein TS and 1 drop of 0.1 N sodium hydroxide: a red color is produced.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Chloride**—To a solution of 200 mg in 10 mL of alcohol add 1 mL of 2 N nitric acid and a few drops of silver nitrate TS: no opalescence is produced.

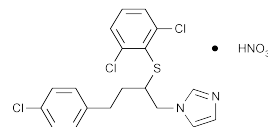
**Heavy metals, Method I** (231)—Dissolve 2 g in 2 mL of 1 N acetic acid and sufficient alcohol to make 25 mL: the limit is 0.001%.

**Assay**—

*Ferrocypen indicator solution*—Dissolve, without warming, 0.5 g of ferrocypen in 50 mL of sulfuric acid.

**Procedure**—Dissolve about 400 mg of Butamben, previously dried and accurately weighed, in a mixture of 100 mL of water and 20 mL of hydrochloric acid. Add 1 mL of *Ferrocypen indicator solution*. Cool the solution in an ice bath to about 10°, and titrate with 0.1 M sodium nitrite VS to a violet endpoint that is stable for not less than three minutes. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 19.32 mg of  $C_{11}H_{15}NO_2$ .

## Butoconazole Nitrate



$C_{19}H_{17}Cl_3N_2S \cdot HNO_3$  474.79  
1H-Imidazole, 1-[4-(4-chlorophenyl)-2-[(2,6-dichlorophenyl)thio]butyl-, mononitrate, ( $\pm$ )-.  
( $\pm$ )-1-[4-(*p*-Chlorophenyl)-2-[(2,6-dichlorophenyl)thio]butyl]imidazole mononitrate [64872-77-1].

» Butoconazole Nitrate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—  
USP Butoconazole Nitrate RS

**Identification, Infrared Absorption** (197K).

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Ordinary impurities** (466)—

*Test solution*: a mixture of methylene chloride and methanol (2:1).

*Standard solutions*: a mixture of methylene chloride and methanol (2:1).

*Adsorbent*: a 0.25-mm layer of chromatographic silica gel.

*Eluant*: a mixture of chloroform, tetrahydrofuran, cyclohexane, and ammonium hydroxide (18:18:13:1).

*Visualization*: 22.

**Assay**—

*Phosphate buffer*—Dissolve 2.18 g of monobasic potassium phosphate and 4.18 g of dibasic potassium phosphate in 900 mL of water, dilute with water to 1000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *Phosphate buffer* (3:1), making adjustments if

necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Butoconazole Nitrate RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer about 20 mg of Butoconazole Nitrate, accurately weighed, to a 100-mL volumetric flask, and dissolve in *Mobile phase*. Dilute with *Mobile phase* to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L1 and is maintained at 40°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 2800 theoretical plates; the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$  in the portion of Butoconazole Nitrate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Butoconazole Nitrate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butoconazole Nitrate Vaginal Cream

» Butoconazole Nitrate Vaginal Cream is Butoconazole Nitrate in a suitable cream base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of butoconazole nitrate ( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ).

**Packaging and storage**—Preserve in collapsible tubes or tight containers. Avoid excessive heat and avoid freezing.

**USP Reference standards** (11)—

USP Butoconazole Nitrate RS

**Identification**—Prepare a mixture of the *Standard preparation* and the *Assay preparation* (1:1), prepared as directed in the *Assay*, and chromatograph as directed in the *Assay*: the chromatogram so obtained exhibits two main peaks, corresponding to butoconazole nitrate and the internal standard.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Acetate buffer**—Dissolve 1.4 g of potassium acetate in 980 mL of water, adjust with about 2 mL of glacial acetic acid to a pH of  $4.3 \pm 0.1$ , dilute with water to 1000 mL, and mix. Adjust the buffer molarity (0.018–0.072 M) as necessary to obtain suitable chromatographic performance. Increased retention time may be achieved by a decrease in the buffer molarity.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Acetate buffer* (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluent**—Prepare a mixture of methanol and *Acetate buffer* (6:4).

**Internal standard solution**—Dissolve 1-benzylimidazole in methanol to obtain a solution containing about 1.6 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Butoconazole Nitrate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a stock solution having a known concentration of about 400 µg per mL. Transfer 2.0 mL of this stock solution and 3.0 mL of *Internal standard solution* to a 50-mL flask, add 35.0 mL of *Diluent*, and mix.

**Assay preparation**—Place about 200 mL of methanol in a 250-mL volumetric flask. Transfer to the flask an accurately weighed quantity of Vaginal Cream, equivalent to about 100 mg of butoconazole nitrate, and sonicate until the Vaginal Cream is dissolved completely. Cool to room temperature, dilute with methanol to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL flask, add 3.0 mL of *Internal standard solution* and 35.0 mL of *Diluent*, and mix. Allow the precipitated excipients which form to rise to the top of the solution, remove them by aspiration, and discard. Centrifuge or filter the remaining solution.

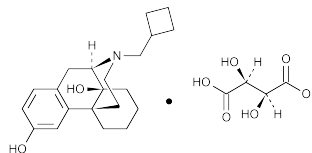
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L9 which has been converted to the potassium form by the use of 0.555 M potassium acetate solution. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for butoconazole nitrate and 1.0 for 1-benzylimidazole; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 4.0; the column efficiency determined from the analyte peak is not less than 1100 theoretical plates; the tailing factor for the analyte peak is not more than 2.1; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of butoconazole nitrate ( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ) in the portion of Vaginal Cream taken by the formula:

$$0.25C(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Butoconazole Nitrate RS in the stock solution used to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of butoconazole nitrate to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butorphanol Tartrate



$C_{21}H_{29}NO_2 \cdot C_4H_6O_6$  477.55  
Morphinan-3,14-diol, 17-(cyclobutylmethyl)-, (-)-, [5-( $R^*$ ,  $R^*$ )]-2,3-dihydroxybutanedioate (1:1) (salt).  
(-)-17-(Cyclobutylmethyl)morphinan-3,14-diol D-(-)-tartrate (1:1) (salt) [58786-99-5].

» Butorphanol Tartrate contains not less than 98.0 percent and not more than 102.0 percent of

$C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Butorphanol Tartrate RS

**Identification**—

A: *Infrared Absorption* (197K).

B: The  $R_f$  value of the principal spot in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained for test A in the *Chromatographic purity* test.

**Specific rotation** (781S): between  $-60^\circ$  and  $-66^\circ$ .

*Test solution*: 4 mg per mL, in methanol.

**Water**, *Method I* (921): not more than 2.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.003%.

**Chromatographic purity**—

METHOD A (Thin-Layer Chromatography)—

*Standard solution*—Prepare a solution in methanol containing 1 mg of USP Butorphanol Tartrate RS per mL.

*Test solution*—Transfer 100 mg of Butorphanol Tartrate to a 10-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix.

*Iodoplatinate spray reagent*—Prepare a 1 in 10 solution of chloroplatinic acid in water. To 0.5 mL of this solution add 33 mL of water and 1 g of potassium iodide to obtain the spray reagent. Prepare fresh daily.

*Procedure*—Apply 50  $\mu$ L of the *Test solution*, containing 500  $\mu$ g of butorphanol tartrate, and 5  $\mu$ L and 10  $\mu$ L of the *Standard solution*, containing 5  $\mu$ g and 10  $\mu$ g of USP Butorphanol Tartrate RS, respectively, about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing, and equilibrated with, a mixture of chloroform, methanol, benzene, and ammonium hydroxide (85:25:20:5). Develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Iodoplatinate spray reagent*. Estimate the percentage of the impurities present in the *Test solution* by comparing the intensities of secondary spots, if present, with the intensities of the principal spots obtained from the chromatograms of the *Standard solution*. The sum of the impurities observed is not greater than 2.0%.

METHOD B (Gas Chromatography)—Dissolve a suitable quantity of Butorphanol Tartrate in methanol to obtain a solution containing about 10 mg per mL. Inject 1  $\mu$ L of this solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m  $\times$  4-mm glass column containing 3% liquid phase G3 on support S1AB. The temperatures of the injection port, column, and detector are maintained at about 280°, 250°, and 290°, respectively. The carrier gas is nitrogen. Record a 30-minute chromatogram. Preferably using an electronic integrator, determine the areas of all peaks in the chromatogram excluding the area of the solvent. In a suitable chromatogram, the retention time for the alpha isomer of butorphanol tartrate is 1.2 relative to 1.0 for butorphanol tartrate; and the retention time of butorphanol tartrate is not less than 15 minutes. Calculate the percentage of synthesis precursors in the test specimen by the formula:

$$100A_v / A_s$$

in which  $A_v$  is the sum of the areas of all minor peaks; and  $A_s$  is the sum of the areas of the major and minor peaks. The limit is 2.0%.

**Assay**—Dissolve about 500 mg of Butorphanol Tartrate, accurately weighed, in 75 mL of glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.76 mg of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ .

## Butorphanol Tartrate Injection

» Butorphanol Tartrate Injection is a sterile solution of Butorphanol Tartrate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ . It may contain a suitable preservative and a buffer.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Butorphanol Tartrate RS

USP Endotoxin RS

**Identification**—Apply 10- $\mu$ L portions of the Injection and a Standard solution of USP Butorphanol Tartrate RS having the same concentration about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing a mixture of chloroform, ethyl acetate, and methanol (40:10:9), and develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution. Benzethonium chloride, if present, is observed as a streaked zone near the point of application. Visualize the butorphanol spots by lightly spraying the plate with a 1 in 250 solution of bromocresol purple in dehydrated alcohol: butorphanol appears as a blue spot against a light yellow background.

**pH** (791): between 3.0 and 5.5.

**Bacterial endotoxins** (85)—It contains not more than 88.0 USP Endotoxin Units per mg of butorphanol tartrate.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*—Prepare a mixture of 0.05 M ammonium acetate and acetonitrile (3:1) adjusted by the addition of glacial acetic acid to a pH of 4.1. The mixture is appropriately filtered and degassed.

*Internal standard solution*—Dissolve about 50 mg of propylparaben in 5.0 mL of methanol contained in a 250-mL volumetric flask. Add water to volume, and mix.

*Standard preparation*—Transfer about 50 mg of USP Butorphanol Tartrate RS, accurately weighed, to a 25-mL volumetric flask containing 1.0 mL of 1 N sulfuric acid. Swirl the flask to dissolve the powder completely, add water to volume, and mix. Pipet 5 mL of the resulting solution into a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*. Add water to volume, mix, and filter through a microporous filter, discarding the first 5 mL of the filtrate and collecting the remainder in a suitable container.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of butorphanol tartrate, to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, mix, add water to volume, and mix.

Filter through a microporous filter, discarding the first 5 mL of the filtrate and collecting the remainder in a suitable container.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.5%, and the capacity factor for butorphanol tartrate is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, adjusting the flow rate and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.7 for propylparaben and 1.0 for butorphanol tartrate. Calculate the quantity, in mg, of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$  in each mL of the Injection taken by the formula:

$$50(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Butorphanol Tartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $R_U$  and  $R_S$  are the peak response ratios of the butorphanol tartrate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butorphanol Tartrate Nasal Solution

» Butorphanol Tartrate Nasal Solution is an aqueous solution of butorphanol tartrate for administration as a metered spray to the nasal mucosa. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ).

**Packaging and storage**—Preserve in tight containers at controlled room temperature. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** <11>—  
USP Butorphanol Tartrate RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** *Thin-Layer Chromatographic Identification Test* <201>—

**Test solution**—Prepare a composite solution by pooling the contents of three containers of Nasal Solution into a suitable vessel. Transfer 1.0 mL of pooled sample to a 10-mL volumetric flask, and dilute with methanol to volume.

**Standard solution**—Dissolve an accurately weighed quantity of USP Butorphanol Tartrate RS in methanol to obtain a solution having a known concentration of about 1.0 mg per mL.

**Developing solvent system**—Prepare a mixture of chloroform, methanol, benzene, and ammonium hydroxide (17:5:4:1). Mix thoroughly. [Caution—Prepare in a hood while wearing appropriate safety gloves, lab coat, and protective eyewear.]

**Spray reagent**—Prepare a 1 in 10 solution of chloroplatinic acid in water. To 0.5 mL of this solution, add 33 mL of water and 1 g of potassium iodide. Prepare fresh daily.

**Procedure**—Proceed as directed in the chapter, except to spray the plate with *Spray reagent*. The typical  $R_f$  value is about 0.7 for butorphanol tartrate.

**Osmolality** <785>: between 252 and 292 mOsmol per kg.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—The total aerobic microbial count does not exceed 1000 cfu per g or mL, and the total combined molds and yeasts count does not exceed 100 cfu per g or mL. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**pH** <791>: between 4.0 and 6.0.

### Related compounds—

**Phosphate buffer** (0.025 M)—Prepare as directed in the *Assay*.

**Mobile phase**—Prepare a filtered and degassed mixture of phosphate buffer, acetonitrile, and triethylamine (85:15:5.1). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of  $3.0 \pm 0.1$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Dissolve an accurately weighed quantity of USP Butorphanol Tartrate RS in water, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.005 mg per mL.

**Sensitivity solution**—Transfer 2.5 mL of the *Standard solution* to a 50-mL volumetric flask, dilute with water to volume, and mix. Do not filter.

**Test solution**—Prepare a composite solution by pooling a minimum of four containers of Nasal Solution into a suitable glass vessel. Transfer the equivalent of 50 mg of butorphanol tartrate to a 50-mL volumetric flask. Dilute with water to volume, and mix. Do not filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector, a 4.6-mm × 25-cm column that contains 5-µm packing L11, and a 4.6-mm × 1-cm guard column packed with 5-µm packing L11. The column temperature is maintained at 40°. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for six replicate injections is not more than 10.0%. Chromatograph the *Sensitivity solution*, and record the peak responses as directed for *Procedure*: the peak height for butorphanol tartrate is greater than or equal to three times the baseline noise.

**Procedure**—Separately inject equal volumes (about 60 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the butorphanol tartrate peak in the *Standard solution* and for all known and unknown related compounds in the *Test solution*. The chromatographic run time is about 40 minutes. Calculate the percentage of each related compound (see *Table 1*) and each unknown impurity in the portion of Nasal Solution taken by the formula:

$$5000(C/VLA)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Butorphanol Tartrate RS in the *Standard solution*; V is the volume of sample taken; LA is the labeled amount, in mg per mL, of butorphanol tartrate in the sample;  $r_i$  is the peak response of each known or unknown related compound in the *Test solution*; and  $r_s$  is the peak response of butorphanol tartrate in the *Standard solution*: the impurities meet the requirements specified in *Table 1*.

Table 1

Compound	Relative Retention Time	Limit (%)
3,14-Dihydroxymorphinan	0.3	0.3
$\Delta^6$ -Butorphanol	0.7	0.5
Butorphanol tartrate	1.0	—
Unknown impurity	—	0.3
Total impurities	—	1.0

**Assay—**

*Phosphate buffer (0.025 M)*—Transfer 3.4 g of monobasic potassium phosphate into a 1000-mL volumetric flask. Dilute with water to volume, and filter.

*Mobile phase*—Prepare a filtered and degassed mixture of phosphate buffer, acetonitrile, and triethylamine (85:15:2). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of  $3.0 \pm 0.1$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Butorphanol Tartrate RS in *Mobile phase* to obtain a solution having a known concentration of 0.2 mg per mL. Mix, and filter, discarding the first 2 mL of the filtrate. The *Standard preparation* is stable for at least 108 hours.

*Assay preparation*—Prepare a composite solution by pooling a minimum of four containers of Nasal Solution into a

suitable glass vessel. Transfer the equivalent of 20 mg of butorphanol tartrate to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 2 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector, a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L11, and a 4.6-mm  $\times$  1-cm guard column that contains 5- $\mu$ m packing L11. The column temperature is maintained at 30°. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the butorphanol tartrate peak is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%.

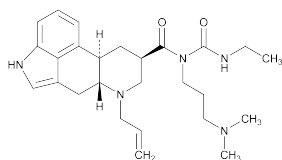
*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the butorphanol tartrate peak. Calculate the quantity, in mg, of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ) in the portion of Nasal Solution taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Butorphanol Tartrate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for butorphanol tartrate obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Cabergoline



$C_{26}H_{37}N_5O_2$  451.60  
 Ergoline-8 $\beta$ -carboxamide, *N*-[3-(dimethylamino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)-; 1-[(6-Allylergolin-8 $\beta$ -yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea [81409-90-7].

### DEFINITION

Cabergoline contains NLT 98.0% and NMT 102.0% of the labeled amount of  $C_{26}H_{37}N_5O_2$ , calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Prepare solutions immediately before use and protect from light.]

**Buffer:** Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute to 1 L. Add 0.2 mL of triethylamine to the resulting solution and mix.

**Mobile phase:** Acetonitrile and *Buffer* (4:21)

**Standard solution:** 0.25 mg/mL of USP Cabergoline RS in *Mobile phase*. [NOTE—Sonicate if needed.]

**Sample solution:** 0.25 mg/mL of Cabergoline in *Mobile phase*. [NOTE—Sonicate if needed.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm  $\times$  25-cm; 10  $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2.0% for five replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{26}H_{37}N_5O_2$  in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cabergoline in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS**, *Method II* (231): 20 ppm

### Organic Impurities

#### PROCEDURE

[NOTE— Prepare solutions immediately before use, and protect from light.]

**Buffer and Mobile phase:** Proceed as directed in the *Assay*.

**System suitability solution:** To 10 mL of 0.1 M sodium hydroxide add 50 mg of Cabergoline and stir for about 15 min. To 1 mL of the suspension add 1 mL of 0.1 M hydrochloric acid, and dilute with *Mobile phase* to 10.0 mL. Sonicate until dissolution is complete. [NOTE— The main degradation product obtained is cabergoline related compound A.]

**Sample solution:** 0.25 mg/mL of Cabergoline in *Mobile phase*. [NOTE—Sonicate if needed.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm  $\times$  25-cm; 10  $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between cabergoline and cabergoline related compound A

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of the peak responses for all peaks from the *Sample solution*

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.8%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline related compound D <sup>a</sup>	0.3	0.1
Cabergoline related compound B <sup>b</sup>	0.6	0.1
Cabergoline related compound A <sup>c</sup>	0.8	0.3
Cabergoline	1.0	—
Cabergoline related compound C <sup>d</sup>	2.9	0.3
Any other individual, unidentified impurity	—	0.10

<sup>a</sup> (6a*R*,9*R*,10a*R*)-*N*-[3-(Dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide.

<sup>b</sup> (6a*R*,9*R*,10a*R*)-*N*<sup>9</sup>-[3-(Dimethylamino)propyl]-*N*<sup>4</sup>-ethyl-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide.

<sup>c</sup> (6a*R*,9*R*,10a*R*)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid.

<sup>d</sup> (6a*R*,9*R*,10a*R*)-*N*<sup>9</sup>-[3-(Dimethylamino)propyl]-*N*<sup>4</sup>-ethyl-*N*<sup>9</sup>-(ethyl-carbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide.

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S):  $-77^{\circ}$  to  $-83^{\circ}$   
*Sample solution*: 1 mg/mL in alcohol, on the anhydrous basis
- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers, protected from light.
- **USP REFERENCE STANDARDS** (11)  
 USP Cabergoline RS

**Cabergoline Tablets**

» Cabergoline Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cabergoline ( $C_{26}H_{37}N_5O_2$ ).

**Packaging and storage**—Preserve in light-resistant, tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—  
 USP Cabergoline RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: 0.1 N hydrochloric acid; 500 mL, degassed with helium.

*Apparatus 2*: 50 rpm.

*Time*: 15 minutes.

Determine the amount of  $C_{26}H_{37}N_5O_2$  dissolved by employing the following procedure.

*Mobile phase and Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Cabergoline RS in *Medium* to obtain a solution having a known concentration of about 1  $\mu$ g per mL.

*Test solution*—Pass the solution under test through a suitable filter, discarding the first few mL.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of  $C_{26}H_{37}N_5O_2$  dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times L}$$

in which  $r_U$  and  $r_S$  are the responses for the cabergoline peak obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration of USP Cabergoline RS, in mg per mL, in the *Standard solution*; 500 is the volume of *Medium*; and  $L$  is the Tablet label claim, in mg per Tablet.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{26}H_{37}N_5O_2$  is dissolved in 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Chromatographic purity**—[NOTE—Prepare solutions immediately before use, and protect from light.]

*Mobile phase*—Prepare as directed in the *Assay*.

*Resolution solution*—To 10 mL of 0.1 M sodium hydroxide add 50 mg of cabergoline. Stir for about 15 minutes. To 1 mL of the suspension add 1 mL of 0.1 M hydrochloric acid, and dilute with *Mobile phase* to 10 mL. Sonicate until dissolution is complete. The main degradation product obtained is cabergoline related compound A.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph (about 20  $\mu$ L) of the *Resolution solution*, and record the peak responses as directed for *Procedure*. Identify the peaks due to cabergoline related compound A and cabergoline using the relative retention times (RRT) given in *Table 1*: the resolution,  $R$ , between cabergoline and cabergoline related compound A is not less than 3.0.

*Procedure*—Inject a volume (about 100  $\mu$ L) of the *Test solution* into the chromatograph, and record the chromatogram. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area of each impurity obtained from the *Test solution*; and  $r_s$  is the sum of the peak areas of all the impurities and the main peak due to cabergoline obtained from the *Test solution*. Calculate the percentage of total impurities in the portion of Tablets taken by the formula:

$$100(r_t / r_s)$$

in which  $r_t$  is the sum of the peak areas of all the impurities obtained from the *Test solution*; and  $r_s$  is the sum of the peak areas of all the impurities and the main peak due to cabergoline obtained from the *Test solution*. The relative retention times and limits for cabergoline related compounds A and cabergoline N-oxide are given in *Table 1*.

**Table 1**

Name	RRT	Limit (%)
Cabergoline related compound A <sup>1</sup>	0.8	NMT 2.0
Cabergoline	1.0	—
Cabergoline N-oxide <sup>2</sup>	1.4	NMT 1.0
Any unspecified degradation product	—	NMT 0.5
Total	—	NMT 2.5

<sup>1</sup>(6aR,9R,10aR)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxylic acid.

<sup>2</sup>(6aR,9R,10aR)-7-Allyl-N-(3-(dimethylazino)propyl)-N-(ethylcarbamoyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxamide.

**Assay**—[NOTE—Prepare solutions immediately before use, and protect from light.]

*Buffer*—Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, add 0.2 mL of triethylamine, and mix well.

*Mobile phase*—Prepare a mixture of *Buffer* and acetonitrile (84:16), and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cabergoline RS in *Mobile phase* to obtain a solution having a known concentration of about 0.25 mg per mL. [NOTE—Sonication may be used to aid in the dissolution of cabergoline.]

*Assay preparation*—Grind not fewer than 20 Tablets into a fine powder. Transfer an accurately weighed portion of the

powder, equivalent to about 2.5 mg of cabergoline based on the label claim, to a 10-mL volumetric flask. Dilute with *Mobile phase* to volume, and sonicate until completely dissolved. This solution has a nominal concentration of about 0.25 mg per mL of cabergoline, based on the label claim. [NOTE—The *Assay preparation* may be passed through a PVDF type filter with a pore size of 0.45 µm prior to analysis.]

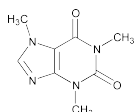
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 25-cm column that contains 10-µm packing L1. The flow rate is about 1.3 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of the label claim of C<sub>26</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub> in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration of USP Cabergoline RS, in mg per mL, in the *Standard preparation*;  $C_U$  is the nominal concentration of cabergoline, in mg per mL, in the *Assay preparation*, based on the label claim; and  $r_U$  and  $r_S$  are the peak responses for cabergoline obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Caffeine



C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> · H <sub>2</sub> O	212.21
C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	194.19
1 <i>H</i> -Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-; 1,3,7-Trimethylxanthine [58-08-2].	
Monohydrate [5743-12-4].	

### DEFINITION

Caffeine is anhydrous or contains one molecule of water of hydration. It contains NLT 98.5% and NMT 101.0% of C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>, calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197M>
- **B.** The retention time of the caffeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 0.82 g/L of anhydrous sodium acetate

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (25:20:955). Adjust with glacial acetic acid to a pH of 4.5.

**System suitability solution:** 0.02 mg/mL of theophylline in *Mobile phase*. Shake, and sonicate if necessary, to dissolve.

**Standard solution:** Transfer 5.0 mg of USP Caffeine RS to a 25-mL volumetric flask. Add 5.0 mL of the *System suitability solution* and 10 mL of *Mobile phase*. Shake, and sonicate if necessary. Dilute with *Mobile phase* to volume, and filter.

**Sample solution:** 0.2 mg/mL of Caffeine in *Mobile phase*. [NOTE—Shake, and sonicate if necessary, to dissolve.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for theophylline and caffeine are 0.69 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6.0 between theophylline and caffeine

**Tailing factor:** NMT 2.0 for theophylline and caffeine peaks

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Caffeine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of caffeine from the *Sample solution*

$r_S$  = peak response of caffeine from the *Standard solution*

$C_S$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Caffeine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–101.0% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS, Method II** <231>: NMT 10 ppm
- **ORGANIC IMPURITIES**

**Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Caffeine taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_T$  = sum of the responses of all the peaks from the *Sample solution*

#### Acceptance criteria

**Individual impurities:** NMT 0.1%

**Total impurities:** NMT 0.1%

### SPECIFIC TESTS

- **WATER DETERMINATION, Method III** <921>: Dry a sample at 80° for 4 h: the anhydrous form loses NMT 0.5% of its weight, and the hydrous form loses NMT 8.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve hydrous Caffeine in tight containers. Preserve anhydrous Caffeine in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or hydrous.
- **USP REFERENCE STANDARDS** <11>  
USP Caffeine RS

**Caffeine Citrate Injection**

» Caffeine Citrate Injection is a sterile solution containing Caffeine and citric acid in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ). It contains no bacteriostat or other preservative.

**Packaging and storage**—Preserve in single-dose, tight containers of Type I glass, and store at a temperature between 15° and 30°.

**USP Reference standards** <11>—

USP Caffeine RS  
USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** It meets the requirements of the test for *Citrate* (191).

**C:** Transfer about 4 g of potassium iodide to a 100-mL volumetric flask. Add 10 mL of water, and shake until the potassium iodide is dissolved. Transfer 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume, and mix. Transfer 5 drops of the solution so obtained to a 25-mL centrifuge tube containing 5.0 mL of the Injection, and mix. Add 0.5 mL of 2.0 M hydrochloric acid solution, and mix: a brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.

**Color and clarity**—Transfer a suitable portion of the Injection to a clear glass test tube, and visually examine the solution in a well-lighted area: the solution is colorless and free of haze, obvious turbidity, and precipitate.

**Bacterial endotoxins** (85): not more than 0.25 USP Endotoxin Unit per mg of caffeine.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.2 and 5.2.

**Particulate matter** (788): not more than 150 particles are equal to or greater than 10  $\mu$ m, and not more than 25 particles are equal to or greater than 25  $\mu$ m.

**Related compounds**—

*Mobile phase* and *Theophylline solution*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*System sensitivity solution*—Transfer 2.5 mL of the *Standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Chromatographic system* (see *Chromatography* <621>)—Proceed as directed in the *Assay*. Chromatograph the *System sensitivity solution*, and record the peak responses as directed

for *Procedure*: the theophylline peak produces a discernible peak response at its retention time.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of any related compound in the portion of Injection taken by the formula:

$$100F(386.31/194.19)(C_s / C_w)(r_i / r_s)$$

in which *F* is the relative response factor and is equal to 0.878 for theobromine at a relative retention time of about 0.4, equal to 1.10 for paraxanthine at a relative retention time of about 0.6, equal to 0.905 for theophylline at a relative retention time of about 0.7, and equal to 1.0 for any other related compound; 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; *C<sub>s</sub>* is the concentration, in mg per mL, of USP Caffeine RS in the *Standard solution*; *C<sub>w</sub>* is the caffeine citrate concentration, in mg per mL, in the *Test solution*, as obtained in the *Assay*; *r<sub>i</sub>* is the individual peak response for each related compound obtained from the *Test solution*; and *r<sub>s</sub>* is the caffeine peak response obtained from the *Standard solution*: not more than 0.10% of any individual related compound is found; and not more than 0.1% of total impurities is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

*Mobile phase*—Prepare a mixture of 0.01 M sodium acetate, acetonitrile, and tetrahydrofuran (191:5:4). Adjust with glacial acetic acid to a pH of 4.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Theophylline solution*—Dissolve an accurately weighed quantity of theophylline in water, and dilute quantitatively, and stepwise if necessary, with water, to obtain a solution having a concentration of about 0.02 mg per mL.

*Standard preparation*—Transfer about 5 mg of USP Caffeine RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of *Theophylline solution*, dissolve in and dilute with water to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, mix, and pass through a polyvinylidene difluoride or equivalent membrane having a 0.45- $\mu$ m porosity.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm  $\times$  150-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for theophylline and 1.0 for caffeine; the resolution, *R<sub>s</sub>*, between theophylline and caffeine is not less than 6.0; the tailing factor, determined from the theophylline and caffeine peaks, is not more than 2.0; and the relative standard deviation for replicate injections, determined from the caffeine peaks, is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the caffeine peak responses. Calculate the quantity, in mg, of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ) in the volume of Injection taken by the formula:

$$250(386.31/194.19)C(r_u / r_s)$$

in which 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; *C* is the concentration, in mg per mL, of USP Caffeine RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Caffeine Citrate Oral Solution

» Caffeine Citrate Oral Solution is a sterile aqueous solution containing Caffeine and citric acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ). It contains no bacteriostat or other preservative.

**Packaging and storage**—Preserve in single-dose, tight containers, and store at a temperature between 15° and 30°.

**USP Reference standards** (11)—  
USP Caffeine RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** It meets the requirements of the test for *Citrate* (191).

**C:** Transfer about 4 g of potassium iodide to a 100-mL volumetric flask. Add 10 mL of water, and shake until the potassium iodide is dissolved. Transfer 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume, and mix. Transfer 5 drops of the solution so obtained to a 25-mL centrifuge tube containing 5.0 mL of the Oral Solution, and mix. Add 0.5 mL of 2.0 M hydrochloric acid solution, and mix: a brown precipitate is produced that dissolves on neutralization with 0.5 mL of sodium hydroxide TS.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.2 and 5.2.

### Related compounds—

*Mobile phase* and *Theophylline solution*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*System sensitivity solution*—Transfer 2.5 mL of the *Standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed in the *Assay*. Chromatograph the *System sensitivity solution*, and record the peak responses as directed for *Procedure*: the theophylline peak produces a discernible peak response at its retention time.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of any related compound in the portion of Oral Solution taken by the formula:

$$100F(386.31/194.19)(C_S / C_W)(r_i / r_S)$$

in which *F* is the relative response factor and is equal to 0.878 for theobromine at a relative retention time of about 0.4, equal to 1.10 for paraxanthine at a relative retention time of about 0.6, equal to 0.905 for theophylline at a relative retention time of about 0.7, and equal to 1.0 for any other related compound; 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Caffeine RS in the *Standard solution*; *C<sub>W</sub>* is the caffeine citrate concentration, in mg per mL, in the *Test solution*, as obtained in the *Assay*; *r<sub>i</sub>* is the individual peak response for each related

compound obtained from the *Test solution*; and *r<sub>S</sub>* is the caffeine peak response obtained from the *Standard solution*: not more than 0.10% of any individual related compound is found; and not more than 0.1% of total impurities is found.

### Assay—

*Mobile phase*—Prepare a mixture of 0.01 M sodium acetate, acetonitrile, and tetrahydrofuran (191:5:4). Adjust with glacial acetic acid to a pH of 4.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Theophylline solution*—Dissolve an accurately weighed quantity of theophylline in water, and dilute quantitatively, and stepwise if necessary, with water, to obtain a solution having a concentration of about 0.02 mg per mL.

*Standard preparation*—Transfer about 5 mg of USP Caffeine RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of the *Theophylline solution*, dissolve in and dilute with water to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, mix, and pass through a polyvinylidene difluoride or equivalent membrane having a 0.45- $\mu$ m porosity.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm  $\times$  150-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for theophylline and 1.0 for caffeine; the resolution, *R*, between theophylline and caffeine is not less than 6.0; the tailing factor, determined from the theophylline and caffeine peaks, is not more than 2.0; and the relative standard deviation for replicate injections, determined from the caffeine peaks, is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the caffeine peak responses. Calculate the quantity, in mg, of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ) in the volume of Oral Solution taken by the formula:

$$250(386.31/194.19)C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Caffeine RS in the *Standard preparation*; 386.31 and 194.19 are the molecular weights of caffeine citrate and caffeine, respectively; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Caffeine and Sodium Benzoate Injection

» Caffeine and Sodium Benzoate Injection is a sterile solution containing equal amounts of Caffeine and Sodium Benzoate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of anhydrous caffeine ( $C_8H_{10}N_4O_2$ ) and sodium benzoate ( $C_7H_5NaO_2$ ).

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Caffeine RS  
USP Endotoxin RS

**Identification—**

**A:** The caffeine obtained in the *Assay for caffeine* herein responds to *Identification* test A under *Caffeine*.

**B:** Dip the end of a platinum wire into a portion of Injection, and then introduce it into a nonluminous flame: the flame is colored intensely yellow.

**C:** To about 0.5 mL of Injection add a few drops of ferric chloride TS: a salmon-colored precipitate is formed. To another portion of Injection add 3 N hydrochloric acid: a white precipitate is formed.

**Bacterial endotoxins** (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of caffeine and sodium benzoate, based on the total, in mg, of the labeled amounts.

**pH** (791): between 6.5 and 8.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for caffeine**—Measure accurately a volume of Injection, equivalent to about 250 mg each of caffeine and sodium benzoate, transfer it completely with the aid of about 5 mL of water to a small separator, add 1 drop of phenolphthalein TS, and add 0.1 N sodium hydroxide, dropwise, until a permanent pink color is just produced. Shake the mixture with three or more 20-mL portions of chloroform to effect complete extraction of the caffeine, passing each chloroform extract through a small filter previously moistened with chloroform into a tared dish. (Retain the water layer for the *Assay for sodium benzoate*.) Wash the stem of the separator, the filter, and the funnel with 10 mL of hot chloroform, adding the washings to the dish. Evaporate the combined chloroform solutions on a steam bath, adding 2 mL of alcohol just before the last trace of chloroform is expelled. Complete the evaporation of the solvent, dry the residue, consisting of  $C_8H_{10}N_4O_2$ , at 80° for 4 hours, cool, and weigh.

**Assay for sodium benzoate**—To the water layer obtained in the *Assay for caffeine* add 75 mL of ether and 5 drops of methyl orange TS. Titrate with 0.1 N hydrochloric acid VS, mixing the liquids by vigorous shaking, until a permanent pink color is produced in the water layer. Each mL of 0.1 N hydrochloric acid is equivalent to 14.41 mg of  $C_7H_5NaO_2$ .

**Calamine**

Iron oxide ( $Fe_2O_3$ ), mixture with zinc oxide.  
Calamine (pharmaceutical preparation) [8011-96-9].

» Calamine is Zinc Oxide with a small proportion of ferric oxide, and contains, after ignition, not less than 98.0 percent and not more than 100.5 percent of zinc oxide ( $ZnO$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Identification—**

**A:** Treat 1 g with 10 mL of 3 N hydrochloric acid, and filter: the filtrate responds to the tests for *Zinc* (191).

**B:** Treat 1 g with 10 mL of 3 N hydrochloric acid, heat to boiling, and filter: the filtrate assumes a reddish color upon the addition of ammonium thiocyanate TS.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Loss on ignition** (733)—Weigh accurately about 2 g, and ignite at 500° to constant weight: it loses not more than 2.0% of its weight.

**Acid-insoluble substances**—Dissolve 2.0 g in 50 mL of 3 N hydrochloric acid. If an insoluble residue remains, collect it on a tared filter, wash with water, dry at 105° for 1 hour, cool, and weigh: the weight of the residue does not exceed 40 mg (2.0%).

**Alkaline substances**—Digest 1.0 g with 20 mL of water on a steam bath for 15 minutes, filter, and add 2 drops of phenolphthalein TS: if a red color is produced, not more than 0.20 mL of 0.10 N sulfuric acid is required to discharge it.

**Arsenic, Method I** (211): 8 ppm.

**Calcium**—Digest 1 g in 25 mL of 3 N hydrochloric acid for 30 minutes, filter to remove the insoluble ferric oxide, and add 6 N ammonium hydroxide to the filtrate until the precipitate first formed is redissolved, then add 5 mL more of 6 N ammonium hydroxide. To 10 mL of this solution add 2 mL of ammonium oxalate TS: not more than a slight turbidity is produced.

**Calcium or magnesium**—To another 10-mL portion of the solution prepared for the test for *Calcium* add 2 mL of dibasic sodium phosphate TS: not more than a slight turbidity is produced.

**Lead**—To 1 g add 15 mL of water, stir, then add 3 mL of glacial acetic acid, and warm on a steam bath until dissolved. Filter, and add 5 drops of potassium chromate TS: no turbidity is produced.

**Assay**—Digest about 1.5 g of freshly ignited Calamine, accurately weighed, with 50.0 mL of 1 N sulfuric acid VS, applying gentle heat until no further solution occurs. Filter the mixture, and wash the residue on the filter with hot water until the last washing is neutral to litmus paper. To the combined filtrate and washings add 2.5 g of ammonium chloride, cool, add methyl orange TS, and titrate with 1 N sodium hydroxide VS. Each mL of 1 N sulfuric acid is equivalent to 40.69 mg of  $ZnO$ .

**Calamine Topical Suspension**

» Prepare Calamine Topical Suspension as follows.

Calamine . . . . .	80 g
Zinc Oxide . . . . .	80 g
Glycerin . . . . .	20 mL
Bentonite Magma . . . . .	250 mL
Calcium Hydroxide Topical Solution, a sufficient quantity to make . . . .	1000 mL

Dilute the Bentonite Magma with an equal volume of Calcium Hydroxide Topical Solution. Mix the powders intimately with the Glycerin and about 100 mL of the diluted magma, triturating until a smooth, uniform paste is formed. Gradually incorporate the remainder of the diluted magma. Finally add enough Calcium Hydroxide Topical Solution to make 1000 mL, and shake.

If a more viscous consistency in the Calamine Topical Suspension is desired, the quantity of Bentonite Magma may be increased to not more than 400 mL.

NOTE—Shake the Calamine Topical Suspension before dispensing.

**Packaging and storage**—Preserve in tight containers.

**Microbial enumeration tests** (61) **and Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## Phenolated Calamine Topical Suspension

» Prepare Phenolated Calamine Topical Suspension as follows:

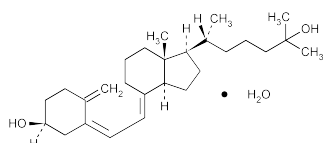
Liquefied Phenol . . . . .	10 mL
Calamine Topical Suspension . . . .	990 mL
To make . . . . .	1000 mL

Mix the ingredients.

NOTE—Shake Phenolated Calamine Topical Suspension before dispensing.

**Packaging and storage**—Preserve in tight containers.

## Calcifediol



$C_{27}H_{44}O_2 \cdot H_2O$  418.65

9,10-Secosterolesta-5,7,10(19)-triene-3,25-diol monohydrate, (3 $\beta$ ,5Z,7E)-.

25-Hydroxycholecalciferol monohydrate [63283-36-3].

» Calcifediol contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{44}O_2 \cdot H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers at controlled room temperature.

**USP Reference standards** (11)—

USP Calcifediol RS

**Identification, Infrared Absorption** (197M).

**Water, Method Ia** (921): between 3.8% and 5.0%, determined on a 0.2-g specimen.

**Assay**—

**Internal standard solution**—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 0.10 mg per mL.

**Mobile phase**—Prepare a suitable degassed solution of about 6 volumes of heptane, 6 volumes of water-saturated

heptane, 3 volumes of methylene chloride, and 5 volumes of ethyl acetate.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 20  $\mu$ g per mL.

**Assay preparation**—Transfer about 10 mg of Calcifediol, accurately weighed, to a 50-mL volumetric flask, dissolve in *Internal standard solution*, dilute with *Internal standard solution* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 4-mm  $\times$  30-cm column that contains packing L3, a detector that monitors absorption at 254 nm, and a pump capable of providing constant flow up to a minimum of 2000 psi.

**System suitability**—The relative standard deviation for peak response ratios for four replicate injections of *Standard preparation* is not more than 3.0%, and the resolution factor is not less than 3.0.

**Procedure**—Introduce equal volumes of the *Standard preparation* and the *Assay preparation* into the chromatograph (see *Chromatography* (621)), and measure the peak responses obtained. Calculate the quantity, in mg, of  $C_{27}H_{44}O_2 \cdot H_2O$  in the portion of Calcifediol taken by the formula:

$$0.5C(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Calcifediol RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of calcifediol to testosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Calcifediol Capsules

» Calcifediol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{27}H_{44}O_2 \cdot H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Calcifediol RS

**Identification**—Transfer the contents of a number of Capsules, equivalent to about 150  $\mu$ g of calcifediol, to a suitable container, add 1 mL of methanol, and shake vigorously for 1 minute. Separate the layers by centrifugation, and transfer as much of the top, methanol layer as possible to a second container. Evaporate this extract to dryness, and dissolve the residue in about 1 mL of chloroform. Proceed as directed under *Thin-layer Chromatographic Identification Test* (201), applying 20  $\mu$ L of this solution and 20  $\mu$ L of a solution containing about the same concentration of USP Calcifediol RS in chloroform, and using a solvent system consisting of 60 parts of cyclohexane and 40 parts of ethyl acetate.

**Dissolution** (711)—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

**Internal standard solution**—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 35 µg per mL.

**Mobile phase**—Prepare as directed in the Assay under Calcifediol.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 7 µg of USP Calcifediol RS per mL.

**Assay preparation**—Transfer a number of Calcifediol Capsules to a suitable container. Using a suitable implement, shear open a number of Capsules inside the container. Wash the implement with an accurately measured volume of *Internal standard solution* that will yield a solution having a concentration of about 7 µg of calcifediol per mL. Collect the rinsings in the container, and mix to obtain a homogeneous solution of the Capsule contents.

**Chromatographic system and System suitability**—Proceed as directed in the Assay under Calcifediol.

**Procedure**—Proceed as directed for *Procedure* in the Assay under Calcifediol. Calculate the quantity, in µg, of C<sub>27</sub>H<sub>44</sub>O<sub>2</sub> · H<sub>2</sub>O in the portion of Capsule contents taken by the formula:

$$CV_U(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Calcifediol RS in the *Standard preparation*; V<sub>U</sub> is the volume, in mL, of *Internal standard solution* taken for the Assay preparation; and R<sub>U</sub> and R<sub>S</sub> are the peak response ratios of calcifediol to testosterone obtained from the Assay preparation and the *Standard preparation*, respectively.

**Calcitonin Salmon**

CSNLSCTCVLG KLSQELHLKLO TYPRNTGSG TP —NH<sub>2</sub>

C<sub>145</sub>H<sub>240</sub>N<sub>44</sub>O<sub>48</sub>S<sub>2</sub> 3432 daltons  
[47931-85-1].

**DEFINITION**

Calcitonin Salmon is a polypeptide that has the same sequence as that of the hormone that regulates calcium metabolism and is secreted by the ultimobranchial gland of salmon. It is produced from either synthetic processes or microbial processes using recombinant DNA (rDNA) technology. The host cell-derived protein content and the host cell- or vector-derived DNA content of Calcitonin Salmon produced from an rDNA process are determined by validated methods. It contains NLT 90.0% and NMT 105.0% of calcitonin salmon, calculated on an acetic acid-free and anhydrous basis. [NOTE—1 mg of acetic acid-free, anhydrous Calcitonin Salmon is equivalent to 6000 USP Calcitonin Salmon Units. 1 USP Calcitonin Salmon Unit = 1 Calcitonin IU.]

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the Assay.

**ASSAY**

**Change to read:**

• **PROCEDURE**

**Solution A:** Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 100 mL of acetonitrile, and mix.

**Solution B:** Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 600 mL of acetonitrile, and mix.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**Standard solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**System suitability solution:** • Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS. • (RB 1-Apr-2012)

**Sample solution:** 1.0 mg/mL of Calcitonin Salmon in *Solution A*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A

**Tailing factor:** NMT 2.5 for calcitonin salmon

**Relative standard deviation:** NMT 3%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of calcitonin salmon (C<sub>145</sub>H<sub>240</sub>N<sub>44</sub>O<sub>48</sub>S<sub>2</sub>) in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r<sub>U</sub> = peak response of calcitonin salmon from the *Sample solution*

r<sub>S</sub> = peak response of calcitonin salmon from the *Standard solution*

C<sub>S</sub> = concentration of USP Calcitonin Salmon RS in the *Standard solution* (corrected for water and acetic acid content) (mg/mL)

C<sub>U</sub> = concentration of the *Sample solution* (corrected for water and acetic acid content) (mg/mL)



**Acceptance criteria:** 90.0%–105.0% on an acetic acid-free and anhydrous basis

## OTHER COMPONENTS

### • ACETIC ACID CONTENT (503)

**Sample solution:** 1 mg/mL of Calcitonin Salmon in *Diluent*, prepared as directed in the chapter

**Acceptance criteria:** 4%–15%

## IMPURITIES

### • HEAVY METALS, *Method II* (231): NMT 50 µg/g

### Change to read:

### • PROCEDURE: RELATED PEPTIDES AND OTHER RELATED SUBSTANCES

#### Test 1

[NOTE—This test is performed on material produced by both chemical synthesis processes and rDNA processes.]

**Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area response of each impurity from the *Sample solution*

$r_T$  = sum of the area responses of all the peaks from the *Sample solution*

#### Acceptance criteria

**Individual impurities:** NMT 3.0% of the total area of all peaks

**Total impurities:** NMT 5.0% of the sum of the areas of all the peaks including the principal peak

[NOTE—Disregard any peaks due to the solvent and any peaks whose area is less than 0.1% of the principal peak.]

#### Test 2

[NOTE—This test needs to be performed only on material produced using rDNA technology.]

**Buffer A:** Dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water.

**Buffer B:** Dissolve 2.72 g of monobasic potassium phosphate and 29.2 g of sodium chloride in 1000 mL of water.

**Buffer C (pH 3.0 citrate buffer):** Dissolve 4.8 g of citric acid in 80 mL of water. Adjust with 1 M sodium hydroxide to a pH of 3.0, and dilute with water to 100.0 mL.

**Solution A:** Acetonitrile and *Buffer A* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 5.0.

**Solution B:** Acetonitrile and *Buffer B* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 4.6.

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
15	0	100
15.1	100	0
22.1	100	0

• **Resolution solution:** Prepare a solution in water containing about 0.5 mg/mL each of USP Calcitonin

Salmon RS and USP Calcitonin Salmon Related Compound B RS. To 1 mL of this solution add 100 µL of pH 3.0 citrate buffer. • (RB 1-Apr-2012)

**Sample solution:** To 1 mL of a 0.5-mg/mL solution of Calcitonin Salmon add 100 mL of *Buffer C*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 20-cm; packing L9

**Flow rate:** 1.2 mL/min

**Injection volume:** 50 µL

### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon-glycine, [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon, and calcitonin salmon related compound B (calcitonin salmon-glycine) are 0.4, 0.6, and 0.9, respectively; and the retention time for calcitonin salmon is about 9 min.]

### Suitability requirements

**Resolution:** NLT 3.0 between calcitonin salmon and calcitonin salmon related compound B

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all the peaks

### Acceptance criteria

**Individual impurities:** See *Table 3*.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria NMT (%)
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon-glycine	0.4	0.2
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon	0.6	0.2
Calcitonin salmon related compound B	0.9	0.6

## SPECIFIC TESTS

### • AMINO ACID PROFILE

(See *Biotechnology-Derived Articles—Amino Acid Analysis* (1052).)

[NOTE—This test needs to be performed only on material of synthetic origin. The concentration of amino acids in the *Internal standard solution*, *Standard stock solution*, and *Standard solution* and the amount of material used to prepare the *Sample solution* can be adjusted depending on the method used for amino acid analysis. The concentrations given are based on analysis using *Method 1*.]

**Internal standard solution:** 1 mM solution of γ-aminobutyric acid

**Standard stock solution:** Prepare a mixture containing equimolar amounts of ammonia and the L form of lysine, histidine, arginine, aspartic acid, threonine, serine, proline, valine, glutamic acid, glycine, leucine, and tyrosine, together with half the equimolar amount of L-cysteine, in 0.1 M hydrochloric acid. The final concentration is about 2.5 mM for each amino acid.

**Standard solution:** Transfer 5 mL of the *Internal standard solution* and 2 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with 0.1 M hydrochloric acid to volume.

**Sample solution:** Place 1.5 mg of Calcitonin Salmon into a heavy-wall ignition tube. Add 1.0 mL of 6 N hydrochloric acid, and allow to cool. Immerse the lower half of the tube in a freezing mixture until the contents are frozen, then evacuate to approximately 10  $\mu$ m of Hg, purge with nitrogen (repeat the evacuation and nitrogen purge three times), and seal the tube while it is under a 10  $\mu$ m of Hg vacuum. Heat for 16 h at 110°–115° in an air oven. Cool, open the tube, dry in a vacuum desiccator, remove the contents, and allow to cool to room temperature. Dissolve in 0.1 M hydrochloric acid. Transfer to a 10-mL volumetric flask, add 1 mL of *Internal standard solution*, and dilute with 0.1 M hydrochloric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Standardize the amino acid analyzer using the *Standard solution*. Inject the *Sample solution* into the amino acid analyzer, and determine the relative proportion of amino acids.

Express the content of each amino acid in moles using an internal standard calibration technique. Calculate the relative proportions of the amino acids by taking as equivalent to one the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, valine, leucine, histidine, arginine, and lysine divided by 20.

**Acceptance criteria:** The requirements are met if the values fall within the limits in *Table 4*.

**Table 4**

Name	Acceptance Criteria
Aspartic acid	1.8–2.2
Glutamic acid	2.7–3.3
Proline	1.7–2.3
Glycine	2.7–3.3
Valine	0.9–1.1
Leucine	4.5–5.3
Histidine	0.9–1.1
Arginine	0.9–1.1
Lysine	1.8–2.2
Serine	3.2–4.2
Threonine	4.2–5.2
Tyrosine	0.7–1.1
Half cystine	1.4–2.1

#### • PEPTIDE MAPPING

(See *Biotechnology-Derived Articles—Peptide Mapping* (1055).)

[NOTE—This test needs to be performed only on material produced using rDNA technology.]

**Solution A:** Water and trifluoroacetic acid (1000:1)

**Solution B:** Acetonitrile, water, and trifluoroacetic acid (800:200:0.85)

**Mobile phase:** See *Table 5*.

**Table 5**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	65	35
60	40	60
60.1	0	100
65.1	0	100
65.2	100	0
80.2	100	0

**Trypsin solution:** Freshly prepare a solution containing 0.1 mg/mL of trypsin (previously treated with L-1-tosyl-amido-2-phenylethyl chloromethyl ketone [TPCK] to remove chymotrypsin activity) in water.

**Tris buffer:** 1 M tris(hydroxymethyl) aminomethane and 10 mM calcium chloride. Adjust with hydrochloric acid to a pH of 8.0.

**Stopping solution:** Water and trifluoroacetic acid (1:1)

**Standard solution:** Prepare a 1.0-mg/mL solution of USP Calcitonin Salmon RS. Transfer 1 mL of this solution to a clean vial. Add 100  $\mu$ L of *Tris buffer* and 50  $\mu$ L of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10  $\mu$ L of *Stopping solution*.

**Sample solution:** 1.0 mg/mL of Calcitonin Salmon. Transfer 1 mL of this solution to a clean vial. Add 100  $\mu$ L of *Tris buffer* and 50  $\mu$ L of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10  $\mu$ L of *Stopping solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

**Acceptance criteria:** The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

#### • BIOIDENTITY

**RPMI 1640 with L-glutamine:** Prepare a mixture of the ingredients in the quantities shown in *Table 6* in sufficient water to obtain 1 L of *RPMI 1640 with L-glutamine* solution, and sterilize by filtration.

**Table 6**

Calcium nitrate	100.00 mg
Potassium chloride	400.00 mg
Magnesium sulfate, anhydrous	48.84 mg
Potassium chloride	400 mg
Sodium chloride	6000 mg
Sodium phosphate, dibasic, anhydrous	800 mg
Sodium bicarbonate	2000 mg
Glucose	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic acid	20 mg
L-Cystine dihydrochloride	65 mg
L-Glutamic acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg

**Table 6** (Continued)

L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine disodium salt dihydrate	29 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline chloride	3 mg
D-Calcium pantothenate	0.25 mg
Folic acid	1 mg
<i>D</i> -Inositol	35 mg
Niacinamide	1 mg
<i>para</i> -Aminobenzoic acid	1 mg
Pyridoxine hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine hydrochloride	1 mg
Vitamin B <sub>12</sub>	0.005 mg

**Medium A** (growth medium): Using aseptic technique prepare the following tissue culture medium.

**Table 7**

RPMI 1640 with L-glutamine	500 mL
Fetal bovine serum	50 mL
1 M HEPES	5 mL
Penicillin/streptomycin solution (10,000 IU/mL per 10 mg/mL)	5 mL
Human insulin	10 IU
Hydrocortisone	0.5 mg

**Medium B** (stimulation medium): Dissolve 5 g of bovine serum albumin (BSA) in 500 mL of 2 mM RPMI 1640 with L-glutamine.

**Solution A** (0.2% BSA solution): Dissolve 50 mg of BSA in 25 mL of water. [NOTE—Use within one day.]

**Solution B** (formic acid/BSA solution): Add 25 mL of 0.1 M formic acid and 5 mL of Solution A to a 50-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Use within two days.]

**Solution C** (trypsin/EDTA solution): Prepare a sterile filtered solution containing 0.25% trypsin and 0.53 mM EDTA (tetrasodium ethylenediaminetetraacetate).

**Solution D** (Dulbecco's phosphate buffered saline): Dissolve 8 g of sodium chloride, 1.15 g of dibasic sodium phosphate, 0.2 g of monobasic potassium phosphate, 0.2 g of potassium chloride, 0.1 g of calcium chloride, and 0.1 g of magnesium chloride in 1 L of water.

**Standard stock solution:** 20 µg/mL of USP Calcitonin Salmon RS in Solution B

**Positive control solution:** Quantitatively dilute the Standard stock solution in Medium B to obtain a 1-ng/mL solution of USP Calcitonin Salmon RS.

**Negative control solution:** Medium B

[NOTE—Prior analysis should be performed to identify the linear portion of the dose-response curve. For example, the Standard solutions and Sample solutions given below.]

**Standard solution A:** 0.8 ng/mL of USP Calcitonin Salmon RS from the Standard stock solution in Medium B

**Standard solution B:** 0.4 ng/mL of USP Calcitonin

Salmon RS from Standard solution A in Medium B

**Standard solution C:** 0.2 ng/mL of USP Calcitonin

Salmon RS from Standard solution B in Medium B

**Standard solution D:** 0.1 ng/mL of USP Calcitonin

Salmon RS from Standard solution C in Medium B

**Sample stock solution:** 20 µg/mL of Calcitonin Salmon in Solution B

**Sample solution A:** 0.8 ng/mL of Calcitonin Salmon from the Sample stock solution in Medium B

**Sample solution B:** 0.4 ng/mL of Calcitonin Salmon from Sample solution A in Medium B

**Sample solution C:** 0.2 ng/mL of Calcitonin Salmon from Sample solution B in Medium B

**Sample solution D:** 0.1 ng/mL of Calcitonin Salmon from Sample solution C in Medium B

**Cell culture preparation:** Prepare a cell culture of the human mammary tumor cell line T-47D. Cells are propagated using Medium A at 37° and 5% carbon dioxide. The medium is changed every two days, and cells are passaged every 5–9 days using Solution C with a 1:4 subculture.

**Cell suspension:** For the test use a cell culture that is 5–9 days old. Remove the cell culture medium from the flask by aspiration, add 10 mL of Solution D, and rock the culture flask to rinse the entire monolayer. Remove the liquid by aspiration, add 2 mL of Solution C, spread over the entire monolayer, allow to stand for 3–5 min, and add 10 mL of Medium A. Homogenize the cell suspension using a pipet, transfer to a 15-mL polypropylene tube, centrifuge at about  $220 \times g$  for 5 min, pour off the supernatant, and resuspend the cell pellet in 10 mL of Medium A. Count the cells, and adjust the cell density through dilution using Medium A to  $2.5 \times 10^4$  cells/mL.

**Procedure:** Place 200 µL of the Cell suspension into each well of a 96-well culture plate (the tissue culture plate), and incubate for 18–24 h at 37° and 5% carbon dioxide. Fill each well of an empty, round-bottomed, 96-well culture plate (the prepared plate) with 150 µL of one of the following solutions: Positive control solution, Negative control solution, Standard solutions A–D, and Sample solutions A–D, so that each solution fills at least five wells on the prepared plate. After incubation remove the culture medium from the tissue culture plate. Using an 8-channel or 12-channel pipet, rapidly transfer 100 µL of solution from each well of the prepared plate to each well of the tissue culture plate. Incubate for 15 min at ambient temperature, remove the solution from each well, stop stimulation by immediately adding an appropriate cell-lysis buffer, and quantitate cAMP produced within the cells, using a validated kit. Perform the test three times, using three different 96-well culture plates. [NOTE—Some kits include a cell-lysis reagent and a sequestering agent for the cell-lysis reagent. The range of the test kit is between 0.05 and 10 ng/mL of cAMP. The number of cells used in the assay may vary, depending on the validated kit used to quantitate cAMP.]

**Analysis:** Potency is determined by a 3-dose, 6-point parallel-line assay, using standard statistical methods. The calculation is carried out using both the lower three concentrations and the upper three concentrations. For the assay to be valid, the requirements for regression, parallelism, and difference of quadratics must be met. If the requirements for validity are met to the same extent in both assessments (the lower and the higher assessments) the final result is determined from the concentration range that shows the higher value when the common slope is divided by the root mean square error.

**Acceptance criteria:** Combine the three potency values by using an unweighted mean on the log scale. Deter-

mine a 95% confidence interval in the log scale using standard statistical methods. Convert the average and confidence interval to the potency scale using antilogs to obtain a geometric mean and its confidence interval. The potency levels determined from all three performances of the test are valid if the data analysis indicates the three determinations to be homogeneous, and the confidence interval is fully contained within 64% and 156% of the geometric mean. If the confidence interval requirement is not met, additional assays may be performed to increase the number of assays and make the confidence interval narrower. The determination of whether it meets the identity requirement should be done only after the confidence interval requirement is met. The acceptance criterion for identity is that the geometric mean is within 80% and 125% of the Assay value.

#### Change to read:

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62)  
Sample: 25 mg  
Acceptance criteria: The total aerobic microbial count is NMT  $10^2$  cfu/g, and the total combined molds and yeasts count is NMT  $10^2$  cfu/g. (RB 1-Apr-2012)
  - **STERILITY TESTS** (71): Where the label states that Calcitonin Salmon is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
  - **WATER DETERMINATION, Method 1c** (921): NMT 10%
- ADDITIONAL REQUIREMENTS**
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a refrigerator or maintain in a frozen state, protected from light.
  - **LABELING:** The labeling states that the material is synthetic or of recombinant DNA origin.
  - **USP REFERENCE STANDARDS** (11)  
USP Calcitonin Salmon RS  
 $C_{145}H_{240}N_{44}O_{48}S_2$  3432 daltons  
USP Calcitonin Salmon Related Compound A RS  
N-Acetyl-cys<sup>1</sup>-calcitonin salmon.  
USP Calcitonin Salmon Related Compound B RS  
(calcitonin salmon-glycine)  
Calcitonin salmon-glycine.

## Calcitonin Salmon Injection

### DEFINITION

Calcitonin Salmon Injection is a sterile solution of Calcitonin Salmon in a suitable diluent. Each mL of Calcitonin Salmon Injection possesses an activity of NLT 80% and NMT 120% of that stated on the label.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, add 100 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.5, pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

**Solution B:** Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, add 600 mL of acetonitrile, and mix. Adjust with phosphoric

acid to a pH of 2.5, pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**Standard stock solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**Standard solution:** 0.1 mg/mL of USP Calcitonin Salmon RS from *Standard stock solution* diluted with *Solution A*

**System suitability solution:** Dissolve the contents of a vial of USP Calcitonin Salmon Related Compound A RS in 0.4 mL of *Solution A*, add 0.1 mL of the *Standard solution*, and mix. Take 0.1 mL of this solution, add 0.9 mL of *Solution A*, and mix.

**Sample solution:** Use the solution from an undiluted Injection vial.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection size:** 200  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

#### Suitability requirements

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 3%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the potency, in USP Calcitonin Salmon Units/mL, in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Calcitonin Salmon RS in the *Standard solution* (USP Calcitonin Salmon Units/mL)

**Acceptance criteria:** 80%–120%

### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.625 USP Endotoxin Unit/USP Calcitonin Salmon Unit
- **STERILITY TESTS** (71): Meets the requirements when tested as directed in the *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **pH** (791): 3.9–4.5
- **INJECTIONS** (1): Meets the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Avoid freezing. Store in a refrigerator.
- **LABELING:** Label it to indicate the activity in USP Calcitonin Salmon Units/mL. The labeling states that the material is synthetic. Label it to state that it is to be

stored in a refrigerator, and that freezing is to be avoided.

• **USP REFERENCE STANDARDS** <11>

USP Calcitonin Salmon RS  
USP Calcitonin Salmon Related Compound A RS  
N-Acetyl-cys<sup>1</sup>-calcitonin.  
C<sub>146</sub>H<sub>243</sub>N<sub>44</sub>O<sub>49</sub>S<sub>2</sub> 3463  
USP Endotoxin RS

## Calcitonin Salmon Nasal Solution

### DEFINITION

Calcitonin Salmon Nasal Solution is a solution of Calcitonin Salmon in a suitable diluent. It contains suitable preservatives, and is packaged in a form suitable for nasal administration so that the required dosage can be controlled as required. Each mL of Calcitonin Salmon Nasal Solution possesses an activity of NLT 80% and NMT 115% of that stated on the label.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

• **PROCEDURE**

**Solution A:** 3.26 mg/mL of tetramethylammonium hydroxide pentahydrate in water and acetonitrile (9:1). Adjust with phosphoric acid to a pH of 2.5.

**Solution B:** 1.45 mg/mL of tetramethylammonium hydroxide pentahydrate in acetonitrile and water (3:2). Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**Diluent:** 7.5 mg/mL of sodium chloride, 2 mg/mL of sodium acetate, and 2 mg/mL of glacial acetic acid in water

**Standard stock solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**Standard solution:** 0.1 mg/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Solution A*

**System suitability stock solution:** Dissolve the contents of a vial of USP Calcitonin Salmon Related Compound A RS in 0.4 mL of *Solution A*, and add 0.1 mL of the *Standard solution*.

**System suitability solution:** *System suitability stock solution* and *Solution A* (1:9)

**Sample solution:** Nasal Solution in *Diluent* (1:9)

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection size:** 200 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of Calcitonin Salmon in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of USP Calcitonin Salmon RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of calcitonin salmon in the *Sample solution* (mg/mL)

**Acceptance criteria:** 80%–115%

### SPECIFIC TESTS

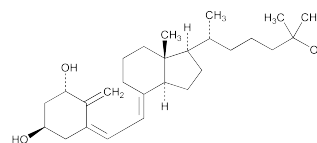
- MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 50 cfu/g. It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

- PH** <791>: 3.5–4.5

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in containers suitable for spraying the contents into the nasal cavities in a controlled individualized dosage. Store unopened containers in a refrigerator and opened containers at room temperature.
- LABELING:** Label it to indicate that it is for intranasal administration only. The labeling also states that it has been prepared either with Calcitonin Salmon of synthetic origin or Calcitonin Salmon of rDNA origin. Label it to state that it is to be stored in a refrigerator, and that freezing is to be avoided. Label it to indicate the activity in USP Calcitonin Salmon Units per mL.
- USP REFERENCE STANDARDS** <11>  
USP Calcitonin Salmon RS  
USP Calcitonin Salmon Related Compound A RS  
N-Acetyl-cys<sup>1</sup>-calcitonin.  
C<sub>146</sub>H<sub>243</sub>N<sub>44</sub>O<sub>49</sub>S<sub>2</sub> 3463

## Calcitriol



C<sub>27</sub>H<sub>44</sub>O<sub>3</sub> 416.64

9,10-Secocholesta-5,7,10(19)-triene-1,3,25-triol, (1 $\alpha$ ,3B,5Z,7E)-.

(5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-1 $\alpha$ ,3B,25-triol [32222-06-3].

Monohydrate 434.65 [77326-95-5].

» Calcitriol is anhydrous or contains one molecule of hydration. The anhydrous form contains not less than 97.0 percent and not more than 103.0 percent of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, calculated on the solvent-free basis. The monohydrate form contains not less than 97.0 percent and not more than

103.0 percent of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, calculated on the anhydrous basis.

**Caution**—Care should be taken to prevent inhaling particles of calcitriol and exposing the skin to it.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store as per labeling instructions.

**Labeling**—Where it is a monohydrate form, the label so indicates.

**USP Reference standards** (11)—  
USP Calcitriol RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method 1c* (921)(where it is labeled as a monohydrate): between 3.5% and 5.5%.

**Chromatographic purity**—[NOTE—Carry out the procedure as rapidly as possible, avoiding unnecessary exposure of solutions to light and air.]

*Tris buffer solution*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Prepare as directed for *Assay preparation*.

*Procedure*—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatograms for at least two times the retention time of the calcitriol peak, identify the impurities listed in *Table 1*, and measure the peak responses. Calculate the percentage of any individual impurity in the portion of Calcitriol taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the peak response of any individual peak other than the main calcitriol peak and the pre-calcitriol peak; and *r<sub>s</sub>* is the sum of the responses of all the peaks: in addition to not exceeding the limits in *Table 1*, not more than 1.0% of total impurities is found. Disregard any peak less than 0.1%.

Table 1

Name	Relative Retention Time	Limit (%)
Triazoline adduct of pre-calcitriol	0.43	0.1
trans-Calcitriol <sup>1</sup>	0.96	0.25
1β-Calcitriol <sup>2</sup>	1.15	0.1
Methylene calcitriol <sup>3</sup>	1.5	0.25
Any other individual unidentified impurity	—	0.1

<sup>1</sup>(5E,7E)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,25-triol

<sup>2</sup>(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1β,3β,25-triol

<sup>3</sup>(5Z,7E)-1α,3β-dihydroxy-17-((R)-7-hydroxy-7-methyloctan-2-yl)-9,10-secoandrosta-5,7,10(19)-triene

**Assay**—[NOTE—Carry out the procedure as rapidly as possible, avoiding unnecessary exposure of solutions to light and air.]

*Tris buffer solution*—Dissolve 1.0 g of tris(hydroxymethyl)aminomethane in 900 mL of water, adjust with phosphoric acid to a pH of 7.0 to 7.5, dilute with water to make 1000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and *Tris buffer solution* (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer an accurately weighed quantity of USP Calcitriol RS to a suitable volumetric flask, dissolve first in acetonitrile (without heating), using 55% of the final volume, then dilute with *Tris buffer solution* to volume, and mix to obtain a solution having a known concentration of about 100 µg of calcitriol per mL. [NOTE—Allow the solution to warm up to room temperature before diluting with *Tris buffer solution* to final volume.]

*System suitability solution*—Heat 2.0 mL of the *Standard preparation* at 80° for 30 minutes.

*Assay preparation*—Transfer an accurately weighed quantity of Calcitriol to a suitable volumetric flask, dissolve first in acetonitrile (without heating), using 55% of the final volume, then dilute with *Tris buffer solution* to volume, and mix to obtain a solution having a known concentration of about 100 µg of calcitriol per mL. [NOTE—Allow the solution to warm up to room temperature before diluting with *Tris buffer solution* to final volume.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for pre-calcitriol and 1.0 for calcitriol; and the resolution, *R*, between pre-calcitriol and calcitriol is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 10,000 theoretical plates; and the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the calcitriol and pre-calcitriol peaks. Calculate the percentage of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub> in the portion of Calcitriol taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which *C<sub>s</sub>* and *C<sub>u</sub>* are the concentrations, in µg per mL, of calcitriol in the *Standard preparation* and the *Assay preparation*, respectively; and *r<sub>u</sub>* and *r<sub>s</sub>* are the sums of the calcitriol and pre-calcitriol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Calcitriol Injection

» Calcitriol Injection is a sterile solution of Calcitriol. It contains an amount of Calcitriol equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of calcitriol (C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>). It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

**USP Reference standards** (11)—

USP Calcitriol Solution RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 100 USP Endotoxin Units per µg of calcitriol.

**pH** (791): between 5.9 and 8.0, determined on a portion to which, if necessary, 0.30 mL of saturated potassium chloride solution has been added for each 100 mL of Injection.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—[NOTE—Avoid unnecessary exposure of solutions to light or air.]

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (74:26). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)) so that the retention time for calcitriol is not less than 20 minutes.

**Standard preparation**—Transfer 3.0 mL of USP Calcitriol Solution RS, equilibrated to room temperature, to a container; add 3.0 mL of water; and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 3 µg of calcitriol, to a container; add a sufficient amount of water to dilute to a total volume of 3.0 mL; add 3.0 mL of methanol; and mix.

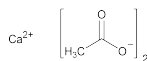
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 264-nm detector, a 4.6-mm × 4.5-cm guard column that contains 5-µm packing L1, and a 4.6-mm × 7.5-cm analytical column that contains 3-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of calcitriol (C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>) in each mL of the Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of calcitriol in the USP Calcitriol Solution RS; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Calcium Acetate



C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub> 158.17  
Acetic acid, calcium salt;  
Calcium acetate [62-54-4].

### DEFINITION

Calcium Acetate contains NLT 99.0% and NMT 100.5% of C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub>, calculated on the anhydrous basis.

### IDENTIFICATION

- A. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191) and *Acetate* (191)  
**Sample solution**: 50 mg/mL  
**Acceptance criteria**: Meets the requirements

### ASSAY

#### PROCEDURE

**Sample**: 300 mg

**Analysis**: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add

15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate (C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub>).  
**Acceptance criteria**: 99.0%–100.5% on the anhydrous basis

### IMPURITIES

- ARSENIC**, *Method I* (211): NMT 3 ppm
- CHLORIDE AND SULFATE**, *Chloride* (221): A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).
- CHLORIDE AND SULFATE**, *Sulfate* (221): A 0.25-g portion shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.06%).
- HEAVY METALS**, *Method I* (231)  
**Test preparation**: Dissolve 0.8 g of Calcium Acetate in 20 mL of water. Add 3.0 mL of glacial acetic acid, dilute with water to 25 mL, and adjust with glacial acetic acid to a pH of 3.8–4.0, measured with a pH meter.  
**Monitor preparation**: Prepare as directed for the *Test preparation*, 2.0 mL of *Standard Lead Solution* being added.  
**Acceptance criteria**: NMT 25 ppm

- LEAD** (251): NMT 10 ppm

#### LIMIT OF ALUMINUM

[NOTE—Use where it is labeled as intended for parenteral use or for use in hemodialysis or peritoneal dialysis.]

**Buffer**: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.

**Aluminum standard solution**: 1.0 µg/mL of aluminum. Prepare as directed for *Standard Preparations* under *Aluminum* (206).

**Standard solution**: Prepare a solution containing 2.0 mL of *Aluminum standard solution*, 5 mL of *Buffer*, and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

**Sample solution**: Dissolve 1.0 g of Calcium Acetate in 50 mL of water, and add 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

**Blank solution**: Prepare a solution containing 50 mL of water and 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode**: Fluorescence

**Excitation wavelength**: 392 nm

**Emission wavelength**: 518 nm

#### Analysis

**Samples**: *Standard solution*, *Sample solution*, and *Blank solution*. Use the *Blank solution* to zero the instrument.

**Acceptance criteria**: The fluorescence of the *Sample solution* is NMT that of the *Standard solution* (2 ppm).

#### LIMIT OF BARIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis.]

**Barium chloride solution**: 500 µg/mL of barium in water, from anhydrous barium chloride

**Buffer**: Ammonium sulfate solution (1 in 10)

**Standard solution**: To a tube add 1 g of ammonium acetate, 2 mL of 1 N hydrochloric acid, 3.0 mL of *Barium chloride solution*, and sufficient water to bring the volume to 40 mL.

**Sample stock solution**: 250 mg/mL of Calcium Acetate and 25 mg/mL of ammonium acetate in 1 N hydrochloric

ric acid. The pH of this solution is 4.5–5.5. Filter and cover the solution.

**Sample solutions:** To four separate tubes add 1.0, 1.5, 2.0, and 2.5 mL of *Barium chloride solution*. To each tube add a sufficient volume of the *Sample stock solution* to bring the volume to 40 mL.

**Analysis:** To the *Sample solutions* and the *Standard solution* add, with brisk stirring, 3.0 mL of *Buffer*, and allow to stand for 20 min.

**Acceptance criteria:** The *Sample solutions* containing 1.0 and 1.5 mL of *Barium chloride solution* remain clear or are only faintly turbid. The *Sample solution* containing 2.0 mL of *Barium chloride solution* is not more turbid than the *Standard solution*.

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer:** 294 mg/mL of sodium citrate dihydrate in water  
**Standard stock solution:** 1.11 mg/mL of USP Sodium Fluoride RS, in water

**Standard solution:** Combine 20.0 mL of *Standard stock solution* with 50.0 mL of *Buffer*, and dilute with water to 100.0 mL. Equivalent to 100 µg/mL of fluoride.

**Sample solution:** Transfer 2.0 g of Calcium Acetate to a beaker containing a plastic-coated stirring bar. Add 20.0 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH <791>).

#### Analysis

**Samples:** *Standard response line* and *Sample solution*  
Transfer 50.0 mL of *Buffer* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*.

Calculate the amount of fluoride (ppm) in the sample taken by multiplying *C* by 50.

**Acceptance criteria:** 50 ppm

#### • LIMIT OF MAGNESIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 1000 µg/mL of magnesium in 1 N nitric acid, from magnesium oxide

**Standard solution:** 5.0 µg/mL of magnesium, from *Standard stock solution*

**Sample solution:** 2 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of *Sample solution* with water to 25.0 mL (0 µg/mL of magnesium).

**Linearity solution B:** Dilute 2.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (0.4 µg/mL of magnesium).

**Linearity solution C:** Dilute 4.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (0.8 µg/mL of magnesium).

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectroscopy

**Analytical wavelength:** 285.2 nm

**Flame:** Air–acetylene

**Lamp:** Magnesium hollow-cathode

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content (0, 0.4, and 0.8 µg/mL) of magnesium, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of magnesium in the *Sample solution*.

Calculate the percentage of magnesium in the sample by multiplying this value by 0.0625.

**Acceptance criteria:** NMT 0.05%

#### • LIMIT OF NITRATE

**Sample solution:** 100 mg/mL of Calcium Acetate in water

**Analysis:** To 10 mL of the *Sample solution* add 5 mg of sodium chloride, 0.05 mL of indigo carmine TS, and, with stirring, 10 mL of nitrogen-free sulfuric acid.

**Acceptance criteria:** The blue color persists for NLT 10 min.

#### • LIMIT OF POTASSIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 23.84 mg/mL of potassium chloride, using potassium chloride previously dried at 105° for 2 h, equivalent to 12.5 mg/mL of potassium

**Standard solution:** 31.25 µg/mL of potassium, from *Standard stock solution*

**Sample solution:** 12.5 mg/mL of calcium acetate

**Linearity solution A:** Dilute 20.0 mL of *Sample solution* with water to 25.0 mL (0 µg/mL of potassium).

**Linearity solution B:** Dilute 2.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (2.5 µg/mL of potassium).

**Linearity solution C:** Dilute 4.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (5.0 µg/mL of potassium).

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectroscopy

**Analytical wavelength:** 766.7 nm

**Lamp:** Potassium hollow-cathode

**Flame:** Air–acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content (0, 2.5, and 5.0 µg/mL) of potassium, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of potassium in the *Sample solution*.

Calculate the percentage of potassium in the sample by multiplying this value by 0.01.

**Acceptance criteria:** NMT 0.05%

#### • LIMIT OF SODIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 25.42 mg/mL of sodium chloride, using sodium chloride previously dried at 105° for 2 h, equivalent to 10.0 mg/mL of sodium



**Standard solution:** 250 µg/mL of sodium, from *Standard stock solution*

**Sample solution:** 10 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of *Sample solution* with water to 25.0 mL (0 µg/mL of sodium).

**Linearity solution B:** Dilute 2.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (20 µg/mL of sodium).

**Linearity solution C:** Dilute 4.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (40 µg/mL of sodium).

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectroscopy

**Analytical wavelength:** 589.0 nm

**Lamp:** Sodium hollow-cathode

**Flame:** Air-acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content (0, 20, and 40 µg/mL) of sodium, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the sample by multiplying this value by 0.0125.

**Acceptance criteria:** NMT 0.5%

#### • LIMIT OF STRONTIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 2.45 mg/mL of strontium acetate in water, equivalent to 1000 µg/mL of strontium

**Standard solution:** 50.0 µg/mL of strontium, from *Standard stock solution*

**Sample solution:** 20 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of *Sample solution* with water to 25.0 mL (0 µg/mL of strontium).

**Linearity solution B:** Dilute 2.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (4 µg/mL of strontium).

**Linearity solution C:** Dilute 4.0 mL of *Standard solution* and 20.0 mL of *Sample solution* with water to 25.0 mL (8 µg/mL of strontium).

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectroscopy

**Analytical wavelength:** 460.7 nm

**Lamp:** Strontium hollow-cathode

**Flame:** Nitrous oxide-acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content (0, 4, and 8 µg/mL) of strontium, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of strontium in the *Sample solution*.

Calculate the percentage of strontium in the sample by multiplying this value by 0.00625.

**Acceptance criteria:** NMT 0.05%

#### • READILY OXIDIZABLE SUBSTANCES

**Sample solution:** 20 mg/mL of Calcium Acetate in boiling water

**Analysis:** Add a few glass beads to 100 mL of the *Sample solution*, 6 mL of 10 N sulfuric acid, and 0.3 mL of 1 N potassium permanganate. Mix, boil gently for 5 min, and allow the precipitate to settle.

**Acceptance criteria:** The pink color in the supernatant is not completely discharged.

#### SPECIFIC TESTS

##### • pH (791)

**Sample solution:** 50 mg/mL

**Acceptance criteria:** 6.3–9.6

##### • WATER DETERMINATION, Method I (921)

**Sample:** 0.7 g

**Analysis:** Proceed as directed in the chapter, adding 20.0 mL of glacial acetic acid to the titration vessel in addition to the methanol.

**Acceptance criteria:** NMT 7.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where Calcium Acetate is intended for use in hemodialysis or peritoneal dialysis, it is so labeled.

• **USP REFERENCE STANDARDS (11)**

USP Sodium Fluoride RS

## Calcium Acetate Tablets

#### DEFINITION

Calcium Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium acetate ( $\text{C}_4\text{H}_6\text{CaO}_4$ ).

#### IDENTIFICATION

• **IDENTIFICATION TESTS—GENERAL,** *Calcium* (191) and *Acetate* (191)

**Sample solution:** 100 mg/mL of calcium acetate from powdered Tablets

**Acceptance criteria:** Meet the requirements

#### ASSAY

##### • PROCEDURE

**Sample:** Amount equivalent to 300 mg of calcium acetate from NLT 20 powdered Tablets

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret, and add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration with the 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate ( $\text{C}_4\text{H}_6\text{CaO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

##### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrometry

**Analytical wavelength:** 422.8 nm

**Standard solution:** Calcium at a known concentration in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

**Acceptance criteria:** NLT 80% (Q) of the labeled amount of calcium acetate ( $\text{C}_4\text{H}_6\text{CaO}_4$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****• LIMIT OF ALUMINUM**

**Buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.

**Aluminum standard solution** 1.0 µg/mL of aluminum. Prepare as directed for *Standard Preparation* under *Aluminum* <206>.

**Standard solution:** Prepare a solution containing 2.0 mL of *Aluminum standard solution*, 5 mL of *Buffer*, and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

**Sample solution:** Dissolve a portion of powdered Tablets (NLT 10) equivalent to 1.0 g of calcium acetate in 50 mL of water, and add 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

**Blank solution:** Prepare a solution containing 50 mL of water and 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Fluorescence

**Excitation wavelength:** 392 nm

**Emission wavelength:** 518 nm

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*. Use the *Blank solution* to zero the instrument.

**Acceptance criteria:** The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (NMT 2 ppm).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Calcium Ascorbate**

426.34

**DEFINITION**

Calcium Ascorbate contains NLT 98.0% and NMT 101.0% of calcium ascorbate dihydrate ( $\text{C}_{12}\text{H}_{14}\text{CaO}_{12} \cdot 2\text{H}_2\text{O}$ ), calculated on the as-is basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* <191>: A 100 mg/mL solution meets the requirements.
- **B.** A 100 mg/mL solution decolorizes a 100 mg/mL solution of dichlorophenol-indophenol.
- **C. INFRARED ABSORPTION** <197M>

**ASSAY****• PROCEDURE**

**Sample:** 300 mg of Calcium Ascorbate

**Blank:** 50 mL of water

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N iodine VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* into a 250-mL conical flask, add 50 mL of water, and mix to dissolve. Immediately titrate with the *Titrant*, adding 3 mL of starch TS

as the endpoint is approached. Perform a *Blank* determination.

Calculate the percentage of calcium ascorbate dihydrate ( $\text{C}_{12}\text{H}_{14}\text{CaO}_{12} \cdot 2\text{H}_2\text{O}$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 106.6 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.0%–101.0% on the as-is basis

**IMPURITIES**

- **ARSENIC, Method I** <211>: NMT 3 µg/g

- **HEAVY METALS, Method II** <231>: NMT 10 ppm

**• LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer solution:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1052 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of *Standard solution* contains 100 µg of fluoride ion.

**Sample solution:** Transfer 2.0 g of Calcium Ascorbate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH <791>).

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration,  $C$  (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in the portion of Calcium Ascorbate taken:

$$\text{Result} = (C \times V) / W$$

$C$  = concentration of fluoride ion in the *Sample solution* (µg/mL), obtained from the *Standard response line*

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Calcium Ascorbate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 10 ppm

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** <781S>

**Sample solution:** 50 mg/mL in carbon dioxide-free water [NOTE—Perform measurements immediately after preparation.]

- Acceptance criteria: +95° to +97°
- **PH** <791>  
Sample solution: 100 mg/mL  
Acceptance criteria: 6.8–7.4
- **LOSS ON DRYING** <731>: Dry 3 g at 105° for 2 h: it loses NMT 0.1% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** <11>  
USP Calcium Ascorbate RS  
USP Sodium Fluoride RS

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**Calcium Carbonate**


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CaCO<sub>3</sub> 100.09  
Carbonic acid, calcium salt (1:1);  
Calcium carbonate (1:1) [471-34-1].

**DEFINITION**

Calcium Carbonate, dried at 200° for 4 h, contains calcium equivalent to NLT 98.0% and NMT 100.5% of calcium carbonate (CaCO<sub>3</sub>).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium** <191>: The addition of acetic acid to it produces effervescence (presence of carbonate), and the resulting solution, after boiling, meets the requirements of the tests.

**ASSAY**

- **TITRIMETRY** <541>  
Sample: 200 mg of Calcium Carbonate, previously dried at 200° for 4 h  
Blank: 100 mL of water and 15 mL of 1 N sodium hydroxide  
Titrimetric system  
(See *Titrimetry* <541>.)  
Mode: Direct titration  
Titrant: 0.05 M edetate disodium VS  
Indicator: 300 mg of hydroxy naphthol blue  
Endpoint detection: Visual, change to distinct blue  
Analysis: Transfer the *Sample* to a 250-mL beaker. Moisten thoroughly with a few mL of water, and add, dropwise, sufficient 3 N hydrochloric acid to dissolve. Add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with the *Titrant*. Calculate the percentage of calcium carbonate (CaCO<sub>3</sub>) in the *Sample* taken:

$$\text{Result} = [(V - B) \times M \times F \times 100] / W$$

*V* = *Sample* titrant volume (mL)  
*B* = *Blank* titrant volume (mL)  
*M* = titrant molarity (mmol/mL)  
*F* = equivalency factor, 100.09 mg/mmol  
*W* = weight of the *Sample* (mg)

Acceptance criteria: 98.0%–100.5% on the dried basis

**IMPURITIES**• **ACID-INSOLUBLE SUBSTANCES**

Sample: 5.0 g  
Analysis: Mix the *Sample* with 10 mL of water, and add hydrochloric acid, dropwise, with agitation, until it ceases to cause effervescence, then add water to make the mixture measure 200 mL, and filter. Wash the insoluble residue with water until the last washing shows no chloride, and ignite and weigh the residue.  
Acceptance criteria: NMT 0.2%; the weight of the residue does not exceed 10 mg.

• **ARSENIC, Method I** <211>

Sample solution: Slowly dissolve 1.0 g in 15 mL of hydrochloric acid, and dilute with water to 55 mL.

Analysis: Omit the addition of 20 mL of 7 N sulfuric acid specified in *Arsenic* <211>, *Method I, Procedure*.

Acceptance criteria: NMT 3 ppm

- **BARIUM:** A platinum wire, dipped in the filtrate obtained in the test for *Acid-Insoluble Substances* and held in a nonluminous flame, does not impart a green color.

• **HEAVY METALS** <231>

Test preparation: Mix 1.0 g with 5 mL of water, slowly add 8 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water, filter, and add water to the filtrate to make 25 mL.

Acceptance criteria: NMT 20 ppm

• **IRON** <241>

Sample solution: 40 mg in 5 mL of 2 N hydrochloric acid. Transfer to a beaker with the aid of water, and dilute with water to 10 mL.

Standard solution: Transfer 4.0 mL of the *Standard Iron Solution*, prepared as directed in *Iron* <241>, to a beaker, and dilute with water to 10 mL.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

Analytical wavelength: 530 nm

Blank: Water

Analysis: Separately to the *Sample solution* and *Standard solution* add 2 mL of citric acid solution (1 in 5) and 2 drops of thioglycolic acid, adjust with ammonia TS to a pH of 9.5 ± 0.1, dilute with water to 20 mL, and allow to stand for 5 min. Dilute with water to 50 mL. Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution*.

Acceptance criteria: NMT 0.1%; the absorbance of the solution from the *Sample solution* does not exceed that of the *Standard solution*.

• **LEAD** <251>

Sample solution: 1.0 g in 5 mL of water

Analysis: To the *Sample solution* slowly add 8 mL of 3 N hydrochloric acid, evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.

Acceptance criteria: NMT 3 ppm

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Solution A: 294 mg/mL of sodium citrate dihydrate in water

Sample: 2.0 g

Standard stock solution: 1.11 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Combine 20.0 mL of the *Standard stock solution* with 50.0 mL of *Solution A*, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100 µg of fluoride ion]

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* <791>).

Standard response line: Transfer 50.0 mL of *Solution A* and 4.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Analysis: Transfer the *Sample* to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 4.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make

100 mL of test solution. Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of fluoride ion in the *Sample solution*. Calculate the content of fluoride in the specimen taken:

$$\text{Result} = (V \times C)/W$$

$V$  = volume of the *Sample solution* (mL)  
 $C$  = concentration of fluoride in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $W$  = weight of *Sample* (g)

Acceptance criteria: 50 ppm

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**

**Sample solution:** 1.0 g

**Analysis:** Mix the *Sample* with 35 mL of water. Carefully add 3 mL of hydrochloric acid, heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well-established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

**Acceptance criteria:** NMT 1.0%; the weight of the residue is NMT 5 mg.

• **MERCURY, Method IIa (261)**

**Mercury stock solution and Standard mercury solution:** Proceed as directed in *Mercury* (261).

**Standard solution:** Proceed as directed in *Mercury* (261), except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.

**Sample stock solution:** 4.0 g in a 100-mL beaker, and cautiously dissolve in 14 mL of 6 N hydrochloric acid

**Sample solution:** Proceed as directed in *Mercury* (261) using the *Sample stock solution*, except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Proceed as directed in *Mercury* (261).

**Acceptance criteria:** 0.5 ppm

**SPECIFIC TESTS**

• **LOSS ON DRYING (731):** Dry a sample at  $200^\circ$  for 4 h: it loses NMT 2.0% of its weight.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**  
 USP Sodium Fluoride RS

## Calcium Carbonate Lozenges

**DEFINITION**

Calcium Carbonate Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ).

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 6 N hydrochloric acid to a Lozenge produces effervescence, and the resulting solution, after being

boiled to expel carbon dioxide and then neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.

**ASSAY**

• **PROCEDURE**

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

**Lanthanum chloride solution:** Transfer 10 g of potassium chloride and 20 g of lanthanum chloride to a 2000-mL volumetric flask. Add 1000 mL of water and 40 mL of hydrochloric acid, mix, and allow to cool. Dilute with water to volume.

**Standard stock solution:** Transfer 250 mg of chelometric standard calcium carbonate, previously dried at  $110^\circ$  for 2 h and then cooled in a desiccator, to a 500-mL volumetric flask. Add 100 mL of water and 12 mL of 1 N hydrochloric acid, swirl to dissolve the calcium carbonate, and allow to cool. Dilute with water to volume. This stock solution contains about 500  $\mu\text{g/mL}$  of calcium carbonate.

**Standard solutions:** To three separate 100-mL volumetric flasks add 2.0, 3.0, and 4.0 mL of the *Standard stock solution*, and dilute each with *Lanthanum chloride solution* to volume. These *Standard solutions* contain 10, 15, and 20  $\mu\text{g/mL}$  of calcium carbonate, respectively.

**Sample stock solution:** Transfer the equivalent to 3000 mg of calcium carbonate, from powdered Lozenges, to a 1000-mL volumetric flask. Add 100 mL of 1 N hydrochloric acid and 300 mL of water, and sonicate to dissolve the powder. Dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of *Sample stock solution* to a 1000-mL volumetric flask, and dilute with *Lanthanum chloride solution* to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Calcium hollow-cathode

**Flame:** Nitrous oxide-acetylene

**Analytical wavelength:** Calcium emission line at 422.7 nm

**Blank:** *Lanthanum chloride solution*

**Analysis**

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*  
 Plot the absorbances of the *Standard solutions* versus their concentrations of calcium carbonate, in  $\mu\text{g/mL}$ , by drawing a straight line best fitting the three plotted points. From the graph determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of calcium carbonate in the *Sample solution*. Calculate the percentage of label claim of calcium carbonate ( $\text{CaCO}_3$ ) in the portion of Lozenges taken:

$$\text{Result} = (C/C_U) \times 100$$

$C$  = measured concentration of calcium carbonate in the *Sample solution* ( $\mu\text{g/mL}$ ), as calculated above

$C_U$  = nominal concentration of calcium carbonate in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

**OTHER COMPONENTS**

• **SODIUM CONTENT** (if so labeled)

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

**Standard stock solution:** Transfer 2.542 g of sodium chloride, previously dried at  $105^\circ$  for 2 h, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** To three separate 100-mL volumetric flasks, add 1.0, 3.0, and 5.0 mL of the *Standard stock solution*, and dilute each with water to volume. These *Standard solutions* contain 1.0, 3.0, and 5.0 µg/mL of sodium, respectively.

**Sample stock solution:** Prepare as directed in the Assay. Pass a portion of it, if necessary, through a filter of 0.5-µm or finer pore size, and use the clear solution.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Sodium hollow-cathode

**Flame:** Air-acetylene

**Analytical wavelength:** Sodium emission line at 589.6 nm

**Blank:** Water

#### Analysis

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*  
Plot the absorbances of the *Standard solutions* versus their contents of sodium, in µg/mL, by drawing a straight line best fitting the three plotted points. From the graph determine the quantity, *C*, in µg, of sodium in each mL of the *Sample solution*.

Calculate the percentage of label claim of sodium in the portion of Lozenges taken:

$$\text{Result} = (C/C_U) \times 100$$

*C* = measured concentration of sodium in the *Sample solution* (µg/mL), as calculated above

*C<sub>U</sub>* = nominal concentration of sodium in the *Sample solution* (µg/mL)

**Acceptance criteria:** NMT 115.0% of the labeled amount

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY** (301)

**Analysis:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq of acid and NLT the number of mEq calculated by:

$$\text{Result} = (F_C \times C) \times 0.9$$

*F<sub>C</sub>* = theoretical acid-neutralizing capacity of CaCO<sub>3</sub>, 0.02 mEq

*C* = quantity of CaCO<sub>3</sub> in the sample tested (mg), based on the labeled quantity

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Calcium Carbonate Oral Suspension

#### DEFINITION

Calcium Carbonate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate (CaCO<sub>3</sub>).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191): The addition of acetic acid to it produces effervescence (presence of carbonate). The resulting solution, after boiling, meets the requirements.

#### ASSAY

##### • PROCEDURE

**Sample solution:** Transfer a portion of Oral Suspension, equivalent to 1 g of calcium carbonate, previously well shaken in its original container, to a beaker with the aid of 25 mL of water. Add 20 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume. Mix, and filter.

**Blank:** 100 mL of water, 15 mL of 1 N sodium hydroxide, and 5 mL of triethanolamine

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** 100 mg of hydroxy naphthol blue

**Endpoint detection:** Visual, change to distinct blue

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container. Dilute with water to 100 mL. Add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with the *Titrant*.

Calculate the percentage of the labeled amount of calcium carbonate (CaCO<sub>3</sub>) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

*V<sub>S</sub>* = volume of the *Titrant* consumed by the *Sample solution* (mL)

*V<sub>B</sub>* = volume of the *Titrant* consumed by the *Blank* (mL)

*M* = *Titrant* molarity (mmol/mL)

*F* = equivalency factor, 100.09 mg/mmol

*W* = nominal amount of calcium carbonate taken for the *Analysis* (mg)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Solution A:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Combine 20.0 mL of the *Standard stock solution* with 50.0 mL of *Solution A*, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100 µg of fluoride ion.]

**Sample solution:** Transfer a portion of Oral Suspension, equivalent to 2.0 g of calcium carbonate, to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 4.0 mL of hydrochloric acid. Stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make 100.0 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* (791)).

**Standard response line:** Transfer 50.0 mL of *Solution A* and 4.0 mL of hydrochloric acid to a beaker. Add water to make 100.0 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, and stir for 15 min. Read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

**Analysis:** Rinse and dry the electrodes, and insert them into the *Sample solution*. Stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in the sample taken:

$$\text{Result} = (V \times C)/W$$

- $V$  = volume of the *Sample solution* (mL)  
 $C$  = determined concentration of fluoride in the *Sample solution* (μg/mL)  
 $W$  = nominal weight of calcium carbonate taken (g)

**Acceptance criteria:** 50 μg/g, with respect to the labeled amount of calcium carbonate

• **ARSENIC, Method I (211)**

**Test preparation:** Slowly dissolve a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 15 mL of hydrochloric acid. Dilute with water to 55 mL.

**Analysis:** Proceed as directed in the chapter, except omit the addition of 20 mL of 7 N sulfuric acid specified under *Procedure*.

**Acceptance criteria:** NMT 3 μg/g, with respect to the labeled amount of calcium carbonate

• **LEAD (251)**

**Test preparation:** Mix a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 5 mL of water.

**Analysis:** To the *Test preparation* slowly add 8 mL of 3 N hydrochloric acid. Evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.

**Acceptance criteria:** NMT 3 μg/g, with respect to the labeled amount of calcium carbonate

• **HEAVY METALS (231)**

**Test preparation:** Mix a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate with 5 mL of water. Slowly add 8 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water. Filter, and add water to the filtrate to make 25 mL.

**Acceptance criteria:** NMT 20 μg/g, with respect to the labeled amount of calcium carbonate

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $10^2$  cfu/mL. It meets the requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **PH (791):** 7.5–8.7

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **USP REFERENCE STANDARDS (11)**  
USP Sodium Fluoride RS

## Calcium Carbonate Tablets

**DEFINITION**

Calcium Carbonate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ). For Tablets labeled for any indication other than, or in addition to, antacid use, the Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of calcium carbonate.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 6 N acetic acid to the Tablets produces effervescence, and the resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements.

**ASSAY**

• **PROCEDURE**

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 200 mg of calcium carbonate, to a suitable crucible. Ignite to constant weight. Cool the crucible, add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution.

**Blank:** 150 mL of water and 15 mL of 1 N sodium hydroxide

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** 300 mg of hydroxy naphthol blue

**Endpoint detection:** Visual, change to distinct blue

**Analysis:** Transfer the *Sample solution* completely to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Titrate with the *Titrant*.

Calculate the percentage of calcium carbonate ( $\text{CaCO}_3$ ) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

$V_S$  = volume of the *Titrant* consumed by the *Sample solution* (mL)

$V_B$  = volume of the *Titrant* consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mmol/mL)

$F$  = equivalency factor, 100.09 mg/mmol

$W$  = weight of calcium carbonate taken (mg)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of  $\text{CaCO}_3$ . For Tablets labeled for any indication other than, or in addition to, antacid use, 90.0%–115.0% of the labeled amount of  $\text{CaCO}_3$

**PERFORMANCE TESTS**

• **DISSOLUTION (711)**

[NOTE—For Tablets labeled for any indication other than, or in addition to, antacid use.]

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Lanthanum chloride solution:** 50 mg/mL of lanthanum chloride in 0.1 N hydrochloric acid

**Standard stock solution:** 100 μg/mL of calcium in 0.1 N hydrochloric acid

**Standard solutions:** Into separate 100-mL volumetric flasks containing 10.0 mL of *Lanthanum chloride solution* pipet 3-, 4-, 5-, and 6-mL portions of *Standard stock solution* and dilute each with 0.1 N hydrochloric acid to volume to obtain solutions with calcium concentrations of 3, 4, 5, and 6 μg/mL, respectively.

**Sample solution:** Filter a portion of the solution under test. Pipet a volume of the filtrate, estimated to contain 1 mg of calcium, into a 250-mL volumetric flask. Add 25.0 mL of *Lanthanum chloride solution*, and dilute with 0.1 N hydrochloric acid to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 422.8 nm

**Lamp:** Calcium hollow-cathode

**Flame:** Air-acetylene

**Blank:** *Lanthanum chloride solution* and 0.1 N hydrochloric acid (1:9)

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*  
Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* against the *Blank*. Construct a standard curve by plotting absorbances versus calcium concentrations of the *Standard solutions*, then from it obtain the concentration,  $C$ , in μg/mL of calcium, of the *Sample solution*.

Calculate the percentage of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

- $M_r$  = molecular weight of calcium carbonate, 100.09  
 $A_r$  = atomic weight of calcium, 40.08  
 $C$  = measured concentration of calcium in the *Sample solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY (301):** For Tablets labeled for antacid use  
**Analysis:** Proceed as directed in the chapter.  
**Acceptance criteria:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated as follows:

$$\text{Result} = (C \times A_{NC}) \times F$$

- $C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled amount  
 $A_{NC}$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq/mg  
 $F$  = acceptance factor for the lower limit of the required acid-neutralizing capacity, 0.9

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is for use as an antacid, or as a dietary supplement, or both.

### Calcium Carbonate and Magnesia Tablets

#### DEFINITION

Calcium Carbonate and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 90.0% and NMT 115.0% of the labeled amount of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ].

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 3 N hydrochloric acid to the Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**  
**Sample solution:** Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.  
**Acceptance criteria:** The solution meets the requirements.

#### ASSAY

##### • CALCIUM CARBONATE

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to

volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of it for the test for *Magnesium Hydroxide*.]

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

##### • MAGNESIUM HYDROXIDE

**Sample solution:** Use the *Sample solution* from the test for *Calcium Carbonate*.

**Analysis:** Transfer a portion of the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

**Acceptance criteria:** 90.0%–115.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesia

#### SPECIFIC TESTS

##### • ACID-NEUTRALIZING CAPACITY (301)

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

- $F_M$  = theoretical acid-neutralizing capacity of  $\text{Mg}(\text{OH})_2$ , 0.0343 mEq  
 $M$  = quantity of  $\text{Mg}(\text{OH})_2$  in the sample tested (mg), based on the labeled quantity  
 $F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq  
 $C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled quantity

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

### Calcium Carbonate and Magnesia Chewable Tablets

#### DEFINITION

Calcium Carbonate and Magnesia Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 90.0% and NMT 115.0% of the labeled amount of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ].

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 3 N hydrochloric acid to the Chewable Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized

with 6 N ammonium hydroxide, meets the requirements of the tests.

• **B. IDENTIFICATION TESTS—GENERAL, *Magnesium* <191>**

**Sample solution:** Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.

**Acceptance criteria:** The solution meets the requirements.

**ASSAY**

• **CALCIUM CARBONATE**

**Sample solution:** Finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of it for the test for *Magnesium Hydroxide*.]

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**

**Sample solution:** Use the *Sample solution* from the test for *Calcium Carbonate*.

**Analysis:** Transfer a portion of the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium

**SPECIFIC TESTS**

• **ACID-NEUTRALIZING CAPACITY <301>**

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{Mg}(\text{OH})_2$ , 0.0343 mEq

$M$  = quantity of  $\text{Mg}(\text{OH})_2$  in the sample tested (mg), based on the labeled quantity

$F_C$  = theoretical acid-neutralizing capacities of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled quantity

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label the Chewable Tablets to indicate that they must be chewed before being swallowed.

## Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets

**Former Title:** *Calcium Carbonate, Magnesia, and Simethicone Tablets*

» Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of calcium carbonate ( $\text{CaCO}_3$ ) and magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ], and an amount of polydimethylsiloxane [ $-(\text{CH}_3)_2\text{SiO}-$ ] $_n$  that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label it to indicate that the Chewable Tablets are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, in mg per Chewable Tablet, if it is greater than 5 mg per Chewable Tablet.

**USP Reference standards <11>—**

USP Polydimethylsiloxane RS

**Identification—**

**A: Infrared Absorption <197S>—**

**Solution—**Using Chewable Tablets, proceed to obtain IR absorption spectra as directed in the *Assay for polydimethylsiloxane under Alumina, Magnesia, and Simethicone Chewable Tablets*.

**B:** The addition of 1 N hydrochloric acid to a Chewable Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests for *Calcium* <191>.

**C:** Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 minutes. Filter this solution, and to the filtrate add 2 mL of 1 N hydrochloric acid: this solution meets the requirements of the tests for *Magnesium* <191>.

**Uniformity of dosage units (905):** meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium hydroxide.

**Acid-neutralizing capacity <301>—**Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**Content of sodium (if so labeled)—**

**Lanthanum chloride solution—**Prepare as directed in the *Assay for calcium carbonate and magnesium hydroxide*.

**Dilute hydrochloric acid—**Prepare as directed in the *Assay for polydimethylsiloxane*.

**Standard solution—**Transfer 2.542 g of sodium chloride, previously dried at 105° for 2 hours, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask containing 6.0 mL of *Dilute hydrochloric acid* and 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. This solution contains 2.0 µg of sodium (Na) per mL.

**Test solution—**Transfer 3.0 mL of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* to a 50-mL volumetric flask containing 1.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

**Blank solution—**Transfer 15.0 mL of *Dilute hydrochloric acid* and 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.



**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Calculate the mg of sodium (Na) in each Chewable Tablet taken by the formula:

$$(5C/6)(A/W)(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of sodium in the *Standard solution*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances of the *Test solution* and the *Standard solution*, respectively. Each Chewable Tablet contains not more than the number of mg of sodium stated on the label.

#### **Assay for polydimethylsiloxane—**

**Saccharin solution**—Prepare a solution of saccharin in 4-methyl-2-pentanone containing 12.5 mg per mL.

**Dilute hydrochloric acid**—Mix 200 mL of hydrochloric acid with sufficient water to make 1000 mL.

**Standard preparation**—Dissolve a suitable quantity of USP Polydimethylsiloxane RS in 4-methyl-2-pentanone to obtain a stock solution having a known concentration of about 1 mg per mL. On the day of use, transfer 20.0 mL of this solution and 5.0 mL of *Saccharin solution* to a 250-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix. This solution contains about 0.08 mg of USP Polydimethylsiloxane RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of polydimethylsiloxane, to a 125-mL separator. Cautiously add 50.0 mL of *Dilute hydrochloric acid*, and swirl until the reaction subsides. Insert the stopper, and mix. Carefully release the pressure, add 50.0 mL of 4-methyl-2-pentanone, and mix for 10 minutes. Allow the layers to separate, and drain the aqueous layer into a suitable stoppered container. [NOTE—Retain this aqueous layer for use in preparing the *Assay preparation* in the *Assay for calcium carbonate and magnesium hydroxide* and for the preparation of the *Test solution* in the test for *Content of sodium*.] Filter the organic layer through a filter containing 50 g of anhydrous sodium sulfate. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, add 1.0 mL of *Saccharin solution*, dilute with methyl isobutyl ketone to volume, and mix.

**Blank solution**—Transfer 1.0 mL of *Saccharin solution* to a 50-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the silicon emission line at 251.6 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a silicon hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of polydimethylsiloxane in each Chewable Tablet taken by the formula:

$$250C(A/W)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the *Standard preparation*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

#### **Assay for calcium carbonate and magnesium hydroxide—**

**Lanthanum chloride solution**—Transfer 26.8 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

**Dilute hydrochloric acid**—Prepare as directed in the *Assay for polydimethylsiloxane*.

**Calcium stock standard solution**—Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Dilute hydrochloric acid*, and swirl to dissolve the calcium carbonate. Dilute with water to volume, and mix. This solution contains 1000  $\mu\text{g}$  of calcium (Ca) per mL.

**Magnesium stock standard solution**—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume, and mix. This solution contains 1000  $\mu\text{g}$  of magnesium (Mg) per mL.

**Calcium and magnesium standard preparation**—To a 250-mL volumetric flask add 10.0 mL of *Calcium stock standard solution* and 5.0 mL of *Magnesium stock standard solution*, dilute with water to volume, and mix. This solution contains 40  $\mu\text{g}$  of calcium (Ca) and 20  $\mu\text{g}$  of magnesium (Mg) per mL. On the day of use, transfer 4.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. This solution contains 1.6  $\mu\text{g}$  of calcium (Ca) and 0.8  $\mu\text{g}$  of magnesium (Mg) per mL.

**Assay preparation**—Transfer an accurately measured volume of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane*, equivalent to about 28 mg of calcium carbonate, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

**Blank solution**—Transfer 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.

**Procedure for calcium carbonate**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the calcium emission line at 422.7 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a calcium hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of calcium carbonate ( $\text{CaCO}_3$ ) in each Chewable Tablet taken by the formula:

$$(100.09/40.08)(1000C/3V)(A/W)(A_U / A_S)$$

in which 100.09 is the molecular weight of calcium carbonate; 40.08 is the atomic weight of calcium; C is the concentration, in  $\mu\text{g}$  per mL, of calcium in the *Standard preparation*; V is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

**Procedure for magnesium hydroxide**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a magnesium hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank.

Calculate the quantity, in mg, of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ] in each Chewable Tablet taken by the formula:

$$(58.34/24.305)(1000C/3V)(A/W)(A_U/A_S)$$

in which 58.34 is the molecular weight of magnesium hydroxide; 24.305 is the atomic weight of magnesium; C is the concentration, in  $\mu\text{g}$  per mL, of magnesium in the *Standard preparation*; V is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation*; A is the average weight, in mg, of each Chewable Tablet taken; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Calcium and Magnesium Carbonates Oral Suspension

### DEFINITION

Calcium and Magnesium Carbonates Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ( $\text{MgCO}_3$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium <191>:** The addition of 3 N hydrochloric acid to a quantity of Oral Suspension, equivalent to 500 mg of calcium carbonate, produces effervescence, and the resulting solution, after having been filtered, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium <191>:**  
**Sample solution:** Heat a quantity of Oral Suspension, equivalent to 800 mg of magnesium carbonate, with 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.  
**Acceptance criteria:** Meets the requirements

### ASSAY

#### • CALCIUM CARBONATE

**Sample solution:** Transfer a portion of Oral Suspension equivalent to 400 mg of calcium carbonate, previously well shaken in its original container and free of air bubbles, to a beaker, with the aid of 20 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, transfer with the aid of water to a 100-mL volumetric flask, dilute with water to volume, filter and use the filtrate. [NOTE—Reserve a portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

**Analysis:** Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM CARBONATE

**Sample solution:** Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

**Analysis:** Transfer the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium carbonate combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of

0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of  $\text{MgCO}_3$ .

**Acceptance criteria:** 85.0%–115.0%

### PERFORMANCE TESTS

- **DELIVERABLE VOLUME <698>:** Meets the requirements

### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>:** The total aerobic microbial count is NMT 100 cfu/mL, and it meets the requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **PH <791>:** 7.0–8.6
- **ACID-NEUTRALIZING CAPACITY <301>:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{MgCO}_3$ , 0.024 mEq

$M$  = quantity of  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantity (mg)

$F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantity (mg)

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

## Calcium and Magnesium Carbonates Tablets

### DEFINITION

Calcium and Magnesium Carbonates Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ( $\text{MgCO}_3$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium <191>:** The addition of 1 N hydrochloric acid to 1 Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium <191>:**  
**Sample solution:** Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.  
**Acceptance criteria:** Meet the requirements

### ASSAY

#### • CALCIUM CARBONATE

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with the aid of 25 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

**Analysis:** Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add

15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

Acceptance criteria: 90.0%–110.0%

#### • MAGNESIUM CARBONATE

**Sample solution:** Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

**Analysis:** Transfer the *Sample solution* equivalent to 120 mg of calcium carbonate and magnesium carbonate combined to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of  $\text{MgCO}_3$ .

Acceptance criteria: 85.0%–115.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION <701>

**Time:** NMT 10 min, except that where Tablets are labeled as gelatin-coated, the time is NMT 30 min, simulated gastric fluid TS being substituted for water in the test

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium carbonate

#### SPECIFIC TESTS

##### • ACID-NEUTRALIZING CAPACITY <301>

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{MgCO}_3$ , 0.024 mEq

$M$  = quantity of  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantity (mg)

$F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantity (mg)

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Tablets that are gelatin-coated are so labeled.

## Calcium Chloride

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  147.01  
Calcium chloride dihydrate [10035-04-8].

$\text{CaCl}_2$  110.98  
Anhydrous [10043-52-4].

#### DEFINITION

Calcium Chloride contains an amount of  $\text{CaCl}_2$  equivalent to NLT 99.0% and NMT 107.0% of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium <191>**  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Chloride <191>**  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Sample solution:** Dissolve 1 g of Calcium Chloride in a mixture of water and 3 N hydrochloric acid (100:5). Transfer the solution to a 250-mL volumetric flask, and dilute with water to volume.

**Analysis:** Pipet 50 mL of the *Sample solution* into a suitable container, and add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ).

Acceptance criteria: 99.0%–107.0% of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

#### IMPURITIES

##### • ALUMINUM <206>

[NOTE—Perform if labeled as intended for use in hemodialysis.]

**Test preparation:** Use a 2.0-g sample.

Acceptance criteria: NMT 1 ppm

##### • HEAVY METALS <231>

**Sample solution:** 80 mg/mL

Acceptance criteria: NMT 10 ppm

##### • IRON, ALUMINUM, AND PHOSPHATE

**Sample solution:** 50 mg/mL

**Analysis:** Add 2 drops of 3 N hydrochloric acid and 1 drop of phenolphthalein TS to the *Sample solution*.

Then add ammonium chloride–ammonium hydroxide TS, dropwise, until the solution is faintly pink. Add 2 drops in excess, and heat the liquid to boiling.

Acceptance criteria: No turbidity or precipitate is produced.

##### • LIMIT OF MAGNESIUM AND ALKALI SALTS

**Sample solution:** 20 mg/mL

**Analysis:** To 50 mL of *Sample solution* add 500 mg of ammonium chloride. Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature. Transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish, add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: The weight of the residue is NMT 5 mg (1.0%).

#### SPECIFIC TESTS

##### • PH <791>

**Sample solution:** 50 mg/mL

Acceptance criteria: 4.5–9.2

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where Calcium Chloride is intended for use in hemodialysis, it is so labeled.

## Calcium Chloride Injection

» Calcium Chloride Injection is a sterile solution of Calcium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

**Identification**—It responds to the tests for *Calcium* and *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of calcium chloride.

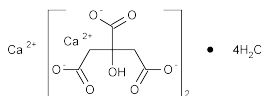
**pH** (791): between 5.5 and 7.5 in the undiluted Injection, except where the concentration is greater than 1 in 20, in which case this range applies to the Injection diluted with water to yield a concentration of 1 in 20.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 1 g of calcium chloride, to a 250-mL volumetric flask, add 5 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Pipet 50 mL of the resulting solution into a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

## Calcium Citrate



$\text{C}_{12}\text{H}_{10}\text{Ca}_3\text{O}_{14} \cdot 4\text{H}_2\text{O}$  570.49  
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, calcium salt (2:3), tetrahydrate;  
Calcium citrate (3:2), tetrahydrate [5785-44-4].

### DEFINITION

Calcium Citrate contains four molecules of water of hydration. When dried at 150° to constant weight, it contains NLT 97.5% and NMT 100.5% of  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ .

### IDENTIFICATION

#### • A.

**Analysis:** Dissolve 0.5 g in a mixture of 10 mL of water and 2.5 mL of 2 N nitric acid. Add 1 mL of mercuric sulfate TS, heat to boiling, and add 1 mL of potassium permanganate TS.

**Acceptance criteria:** A white precipitate is formed.

#### • B.

**Sample:** 0.5 g of Calcium Citrate

**Analysis:** Ignite completely the *Sample* at as low a temperature as possible, cool, and dissolve the residue in dilute glacial acetic acid (1:10). Filter, and add 10 mL of ammonium oxalate TS to the filtrate.

**Acceptance criteria:** A voluminous white precipitate that is soluble in hydrochloric acid is formed.

### ASSAY

#### • PROCEDURE

**Sample solution:** Dissolve 350 mg of Calcium Citrate, previously dried at 150° to constant weight, in 12 mL of 0.5 M hydrochloric acid, and dilute with water to about 100 mL.

**Analysis:** While stirring the *Sample solution*, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.307 mg of calcium citrate ( $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ ).

**Acceptance criteria:** 97.5%–100.5% on the dried basis

### IMPURITIES

#### • ARSENIC, Method I (211)

**Test preparation:** Dissolve 1 g of Calcium Citrate in 5 mL of 3 N hydrochloric acid, and dilute with water to 35 mL.

**Acceptance criteria:** NMT 3 ppm

#### • HEAVY METALS, Method I (231)

**Test preparation:** Dissolve 1 g of Calcium Citrate in a mixture of hydrochloric acid and water (2:20). Add 1.5 mL of ammonium hydroxide, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 20 ppm

#### • LEAD (251)

**Test preparation:** Dissolve 0.5 g of Calcium Citrate in 20 mL of 3 N hydrochloric acid. Evaporate this solution on a steam bath to 10 mL, dilute with water to 20 mL, and cool. Use 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb) for the test.

**Acceptance criteria:** NMT 10 ppm

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Standard stock solution:** 1000 µg/mL of fluoride ion from USP Sodium Fluoride RS in water

**Standard solution:** 5 µg/mL of fluoride ion from *Standard stock solution*. [NOTE—Prepare on the day of use.]

**Linearity solution A:** Transfer 1.0 mL of the *Standard solution* to a 250-mL plastic beaker. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1.0 M sodium citrate, and 10 mL of 0.2 M edetate disodium. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer to a 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.05 µg/mL of fluoride.

**Linearity solution B:** Transfer 5.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water,". This solution contains 0.25 µg/mL of fluoride.

**Linearity solution C:** Transfer 10.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water,". This solution contains 0.50 µg/mL of fluoride.

**Sample solution:** Transfer 1.0 g of Calcium Citrate to a 100-mL beaker. Add 10 mL of water and, while stirring, 10 mL of 1 N hydrochloric acid. When dissolved, boil rapidly for 1 min, transfer the solution to a 250-mL plastic beaker, and cool in ice water. Add 15 mL of 1.0 M sodium citrate and 10 mL of 0.2 M edetate disodium.

dium, and adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer this solution to a 100-mL volumetric flask, and dilute with water to volume.

**Electrode system:** Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH <791>).

#### Analysis

**Samples:** *Linearity solution A*, *Linearity solution B*, *Linearity solution C*, and *Sample solution*

Transfer 50 mL of each *Linearity solution A*, *Linearity solution B*, and *Linearity solution C* to separate 250-mL plastic beakers, and measure the potential of each solution with the *Electrode system*. Between each reading wash the electrodes with water, and absorb any residual water by blotting the electrodes dry. Plot the logarithms of the fluoride concentrations (0.05, 0.25, and 0.50  $\mu\text{g/mL}$ , respectively) versus potential to obtain a Standard response line.

Transfer 50 mL of the *Sample solution* to a 250-mL plastic beaker, and measure the potential with the *Electrode system*. From the measured potential and the Standard response line determine the concentration, *C*, in  $\mu\text{g/mL}$ , of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride in the specimen taken by multiplying *C* by 0.01.

**Acceptance criteria:** NMT 0.003%

#### • LIMIT OF ACID-INSOLUBLE SUBSTANCES

**Sample solution:** Dissolve 5 g of Calcium Citrate by heating with a mixture of hydrochloric acid and water (10:50) for 30 min.

**Analysis:** Filter, wash, and dry at 105° for 2 h the residue so obtained.

**Acceptance criteria:** The weight of the residue is NMT 10 mg (0.2%).

#### SPECIFIC TESTS

- **LOSS ON DRYING** <731>: Dry a sample at 150° for 4 h: it loses from 10.0% to 13.3% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Sodium Fluoride RS

## Calcium Glubionate Syrup

#### DEFINITION

Calcium Glubionate Syrup is a solution containing equimolar amounts of Calcium Gluconate and Calcium Lactobionate or with Calcium Lactobionate predominating. It contains NLT 95.0% and NMT 105.0% of the labeled amount of calcium (Ca).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, *Calcium* <191>  
**Sample solution:** Syrup in water (1 in 10)  
**Acceptance criteria:** Meets the requirements
- **B. THIN-LAYER CHROMATOGRAPHY**  
**Standard solution:** 2 mg/mL of calcium gluconate and 4 mg/mL of calcium lactobionate in water  
**Sample solution:** Equivalent to 0.4 mg/mL of calcium, from Syrup in water  
**Chromatographic system**  
(See *Chromatography* <621>, *Thin-Layer Chromatography*.)  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel  
**Application volume:** 5  $\mu\text{L}$   
**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber and dry at 100° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

**Acceptance criteria:** The two principal spots of the *Sample solution* correspond in color, size, and  $R_f$  value to those obtained from the *Standard solution*. [NOTE—Sucrose, if present in the *Sample solution*, appears in the chromatogram as a spot between the two principal spots.]

#### ASSAY

##### • PROCEDURE

**Sample solution:** Transfer a portion of Syrup equivalent to 70 mg of Ca into a suitable beaker. Add 2 mL of 3 N hydrochloric acid, and dilute with water to 150 mL.

**Analysis:** While stirring the *Sample solution* with a magnetic stirrer, add 20 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

**Acceptance criteria:** 95.0%–105.0%

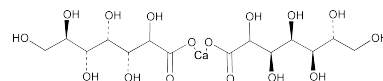
#### SPECIFIC TESTS

- **pH** <791>: 3.4–4.5

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, at a temperature not exceeding 30°, and avoid freezing.

## Calcium Gluceptate



$\text{C}_{14}\text{H}_{26}\text{CaO}_{16}$  (anhydrous) 490.42  
Glucoheptonic acid, calcium salt (2:1);  
Calcium glucoheptonate (1:2) [29039-00-7].

#### DEFINITION

Calcium Gluceptate is anhydrous or contains varying amounts of water of hydration. It consists of the calcium salt of the alpha epimer of glucoheptonic acid or of a mixture of the alpha and beta epimers of glucoheptonic acid. It contains NLT 95.0% and NMT 102.0% of calcium gluceptate ( $\text{C}_{14}\text{H}_{26}\text{CaO}_{16}$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B. IDENTIFICATION TESTS—GENERAL**, *Calcium* <191>: A 20-mg/mL solution meets the requirements.

#### ASSAY

##### • PROCEDURE

**Sample:** 800 mg of Calcium Gluceptate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* with 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add 25 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium gluceptate ( $C_{14}H_{26}CaO_{16}$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 490.4 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 95.0%–102.0% on the dried basis

#### IMPURITIES

##### • CHLORIDE AND SULFATE, *Chloride* <221>

**Standard:** 1.0 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g

**Acceptance criteria:** NMT 0.07%

##### • CHLORIDE AND SULFATE, *Sulfate* <221>

**Standard:** 1.0 mL of 0.020 N sulfuric acid

**Sample:** 2.0 g

**Acceptance criteria:** NMT 0.05%

##### • HEAVY METALS <231>

**Test preparation:** Dissolve 1 g in 25 mL of water.

**Acceptance criteria:** NMT 20 ppm

##### • REDUCING SUGARS

**Sample:** 0.50 g

**Analysis:** Dissolve the *Sample* in 10 mL of hot water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

**Acceptance criteria:** No red precipitate is formed immediately.

#### SPECIFIC TESTS

##### • PH <791>

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 6.0–8.0

##### • LOSS ON DRYING <731>

(See *Thermal Analysis* <891>.)

[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on Drying*.]

**Sample:** 10–25 mg

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the specimen under test at a rate of 5°/min in an atmosphere of nitrogen, at a flow rate of 40 mL/min. Record the thermogram to 150°.

**Acceptance criteria:** See *Table 1*.

Table 1

Form	Loss on Drying (%)
Anhydrous	NMT 1.0
2H <sub>2</sub> O (dihydrate)	NMT 6.9
3 1/2 H <sub>2</sub> O	NMT 11.4

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **LABELING:** Label to indicate whether hydrous or anhydrous; if hydrous, label to indicate also the degree of hydration.

##### • USP REFERENCE STANDARDS <11>

USP Calcium Gluceptate RS

## Calcium Gluceptate Injection

» Calcium Gluceptate Injection is a sterile solution of Calcium Gluceptate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of calcium (Ca).

**Packaging and storage—**Preserve in tight, single-dose containers, preferably of Type I or Type II glass.

**Labeling—**The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

##### USP Reference standards <11>—

USP Calcium Gluceptate RS

USP Endotoxin RS

##### Identification—

**A: Infrared Absorption <197K>—**Prepare the test specimen as follows. Transfer 5 mL of Injection to a separator, add 10 mL of chloroform, shake, and allow the layers to separate. Draw off and discard the chloroform layer, and repeat the extraction with a second 10-mL portion of chloroform. Drain the water layer into a small beaker, evaporate to dryness, and dry in vacuum at 60° for 16 hours.

**B:** A dilution of the Injection with water (1 in 5) responds to the tests for *Calcium* <191>.

**Bacterial endotoxins <85>—**It contains not more than 0.32 USP Endotoxin Unit per mg of calcium gluceptate.

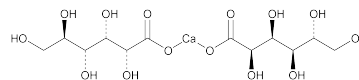
**pH <791>:** between 5.6 and 7.0.

**Particulate matter <788>:** meets the requirements for small-volume injections.

**Other requirements—**It meets the requirements under *Injections* <1>.

**Assay—**To an accurately measured volume of Injection, equivalent to about 45 mg of calcium, add 2 mL of 3 N hydrochloric acid and 148 mL of water. While stirring, preferably with a magnetic stirrer, add about 15 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

## Calcium Gluconate



$C_{12}H_{22}CaO_{14}$

430.37

$C_{12}H_{22}CaO_{14} \cdot H_2O$

448.39

D-Gluconic acid, calcium salt (2:1);  
Calcium D-gluconate (1:2) [299-28-5].  
Monohydrate [18016-24-5].

**DEFINITION**

Calcium Gluconate is anhydrous or contains one molecule of water of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ), calculated on the dried basis. The monohydrate form contains NLT 99.0% and NMT 101.0% of calcium gluconate monohydrate ( $C_{12}H_{22}CaO_{14} \cdot H_2O$ ) where labeled as intended for use in preparing injectable dosage forms, and NLT 98.5% and NMT 102.0% of calcium gluconate monohydrate ( $C_{12}H_{22}CaO_{14} \cdot H_2O$ ) where labeled as not intended for use in preparing injectable dosage forms.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL**, *Calcium* <191>: A 20 mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHY**  
**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS  
**Sample solution:** 10 mg/mL of Calcium Gluconate, heating in a water bath at 60°, if necessary, to dissolve  
**Chromatographic system**  
 (See *Chromatography* <621>, *Thin-Layer Chromatography*).  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel  
**Application volume:** 5  $\mu$ L  
**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)  
**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
 Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.  
**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

**ASSAY**

- **PROCEDURE**  
**Sample:** 800 mg of Calcium Gluconate  
**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid  
**Titrimetric system**  
 (See *Titrimetry* <541>).  
**Mode:** Direct titration  
**Titrant:** 0.05 M edetate disodium VS  
**Endpoint detection:** Visual  
**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination. Calculate the percentage of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $M$  = *Titrant* molarity (mM/mL)  
 $F$  = equivalency factor, 430.4 mg/mM  
 $W$  = *Sample* weight (mg)

**Acceptance criteria:** Anhydrous form, 98.0%–102.0% on the dried basis; monohydrate form, 99.0%–101.0% where labeled as intended for use in preparing injectable dosage forms; and monohydrate form, 98.5%–102.0% where labeled as not intended for use in preparing injectable dosage forms

**IMPURITIES**

- **ARSENIC**, *Method I* <211>  
**Test preparation:** Dissolve 1.0 g in a mixture of 10 mL of hydrochloric acid and 20 mL of water, and dilute with water to 55 mL.  
**Analysis:** Proceed as directed in the chapter, except to omit the addition of 20 mL of 7 N sulfuric acid.  
**Acceptance criteria:** NMT 3 ppm
- **CHLORIDE AND SULFATE**, *Chloride* <221>: A 1.0-g portion shows no more chloride than corresponds to 0.07 mL of 0.020 N hydrochloric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 1.0-g portion shows no more chloride than corresponds to 1 mL of 0.020 N hydrochloric acid (0.07%).
- **CHLORIDE AND SULFATE**, *Sulfate* <221>: A 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 0.1 mL of 0.020 N sulfuric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 1 mL of 0.020 N sulfuric acid (0.05%).
- **HEAVY METALS**, *Method II* <231>: NMT 10 ppm; NMT 20 ppm where Calcium Gluconate is labeled as not intended for use in the preparation of injectable dosage forms
- **LIMIT OF IRON**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

**Standard solutions:** 0.2, 0.4, and 1.0  $\mu$ g/mL of iron, prepared as follows. Separately transfer 2.0, 4.0, and 10.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* <241>, to individual 100-mL volumetric flasks, each containing 1.37 g of calcium chloride, previously tested and shown to contain less than 5 ppm of iron, and dilute with 2 N hydrochloric acid to volume.

**Sample solution:** Transfer 1.0 g of Calcium Gluconate to a 100-mL quartz glass flask. Add 20 mL of 12 N nitric acid, and heat to boiling until fumes are evolved. Add 0.5 mL of 30% hydrogen peroxide, and heat again until fumes are evolved. Repeat this process until the volume is reduced to about 5 mL. Cool, add 1.0 mL of perchloric acid, and heat to boiling. [CAUTION—Do not heat above 190° or evaporate to dryness because of danger of explosion.] Transfer this solution to a 25-mL volumetric flask, and dilute with 2 N hydrochloric acid to volume.

**Blank solution:** Use 0.34 g of calcium chloride, previously tested and shown to contain less than 5 ppm of iron, instead of Calcium Gluconate, and prepare as directed under *Sample solution*.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 248.3 nm

**Lamp:** Iron hollow-cathode

**Flame:** Air-acetylene

**Analysis**

**Samples:** *Standard solutions*, *Sample solution*, and *Blank solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*, using the *Blank solution* as the blank and making deuterium background corrections. Plot the absorbances of the *Standard solutions* versus concentration, in  $\mu$ g/mL, of iron, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu$ g/mL, of iron in the *Sample solution*.

Calculate the concentration of iron, in ppm, in the portion of Calcium Gluconate taken:

$$\text{Result} = (C \times V)/W$$

- C = concentration of iron in the *Sample solution* obtained from the regression equation ( $\mu\text{g/mL}$ )  
 V = volume of *Sample solution* (mL)  
 W = weight of Calcium Gluconate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 5 ppm

• **LIMIT OF MAGNESIUM AND ALKALI METALS**

[NOTE—Calcium Gluconate labeled as not intended for use in preparing injectable dosage forms is exempt from this requirement.]

**Sample:** 1.0 g

**Analysis:** Dissolve completely the *Sample* in 100 mL of boiling water. Add 10 mL of ammonium chloride TS, 1 mL of ammonium hydroxide, and 50 mL of hot (maintained at 70°–80°) ammonium oxalate TS. Allow to stand for 4 h, dilute with water to 200 mL, and filter. Evaporate 100 mL of the filtrate to dryness, and ignite to constant weight.

**Acceptance criteria:** NMT 0.4%: The weight of the residue does not exceed 2 mg.

• **LIMIT OF PHOSPHATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

**Standard stock solution 1:** 0.716 mg/mL of monobasic potassium phosphate

**Standard stock solution 2:** Dilute 1.0 mL of *Standard stock solution 1* with water to 100 mL.

**Standard solution:** Dilute 2.0 mL of *Standard stock solution 2* with water to 100 mL.

**Sample stock solution:** To 10.0 g of Calcium Gluconate add 90 mL of hot water (70°–80°), and heat to boiling, with swirling, for 10 s to obtain a clear solution.

**Sample solution:** Dilute 1 mL of the hot *Sample stock solution* with water to 100 mL.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 To the *Standard solution* and *Sample solution* add 4 mL of sulfomolybdic acid TS, and mix. To both solutions add 0.1 mL of a freshly prepared mixture of 3 N hydrochloric acid and stronger acid stannous chloride TS (10:1), and mix.

**Acceptance criteria:** NMT 0.01%: After 10 min any color in the *Sample solution* is not more intense than that in the *Standard solution*.

• **LIMIT OF OXALATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

[NOTE—Use deionized water where water is indicated.]

**Mobile phase:** 0.0017 M sodium bicarbonate and 0.0018 M sodium carbonate in water

**Solution A:** 0.0125 M sulfuric acid in water

**Solution B:** Dilute 1 mL of hydrochloric acid with water to 1200 mL.

**Standard solution:** 1.5  $\mu\text{g/mL}$  of sodium oxalate in *Solution B*

**Sample solution:** 20 mg/mL of Calcium Gluconate in *Solution B*. Sonicate if necessary.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** Ion chromatography

**Detector:** Conductance

**Columns**

**Analytical:** 4-mm  $\times$  25-cm; 15- $\mu\text{m}$  packing L12

**Guard:** 4-mm  $\times$  5-cm; 15- $\mu\text{m}$  packing L12

**Anion suppressor:** Micromembrane anion suppressor column connected in series with the guard and analytical columns. The anion suppressor column is equipped with a micromembrane that separates the *Mobile phase* from the *Solution A* flowing counter-current to the *Mobile phase* at a rate of about 7 mL/min.

[NOTE—Condition the system for about 15 min with *Mobile phase* at a flow rate of 2 mL/min.]

**Flow rate:** 2 mL/min

**Injection size:** 50  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2500 theoretical plates

**Tailing factor:** NMT 1.2

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of oxalate in the portion of Calcium Gluconate taken:

$$\text{Result: } (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

$r_U$  = peak response for oxalate from the *Sample solution*

$r_S$  = peak response for oxalate from the *Standard solution*

$C_S$  = concentration of sodium oxalate in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = concentration of Calcium Gluconate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of oxalate, 88.03

$M_{r2}$  = molecular weight of sodium oxalate, 134.00

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** NMT 0.01%

• **REDUCING SUBSTANCES**

**Sample:** 1.0 g of Calcium Gluconate

**Blank:** 20 mL of water

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 250-mL conical flask, dissolve in 20 mL of hot water, cool, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with the *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{(V_B - V_S) \times N \times F\} / W \times 100$$

$V_B$  = *Back titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back titrant* volume consumed by the *Sample* (mL)

$N$  = *Back titrant* normality (mEq/mL)

$F$  = equivalency factor, 27 mg/mEq

$W$  = *Sample weight* (mg)

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**

- **LOSS ON DRYING** <731>: Dry a sample at 105° for 16 h: the anhydrous form loses NMT 3.0% of its weight; the monohydrate form, where labeled as intended for use in preparing injectable dosage forms, loses NMT 1.0% of its weight, and where labeled as not intended for use in preparing injectable dosage forms, loses NMT 2.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or monohydrate. Where the quantity of calcium gluconate is indicated in the labeling of any solution containing Calcium Gluconate, this shall be understood to be in terms of anhydrous calcium gluconate ( $\text{C}_{12}\text{H}_{22}\text{CaO}_{14}$ ).



Calcium Gluconate intended for use in preparing injectable dosage forms is so labeled. Calcium Gluconate not intended for use in preparing injectable dosage forms is so labeled; in addition, it may be labeled also as intended for use in preparing oral dosage forms.

- **USP REFERENCE STANDARDS** (11)  
USP Potassium Gluconate RS

## Calcium Gluconate Injection

» Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of total Ca. The calcium is in the form of calcium gluconate, except that a small amount may be replaced with an equal amount of calcium in the form of Calcium Saccharate, or other suitable calcium salts, for the purpose of stabilization. It may contain sodium hydroxide added for adjustment of the pH.

Injection intended for veterinary use only may be prepared from Calcium Gluconate solubilized with Boric Acid, or from Glyconolactone, Boric Acid, and Calcium Carbonate.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**Labeling**—Label the Injection to indicate its content, if any, of added calcium salts, calculated as percentage of calcium in the Injection. The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL. The labeling indicates that the Injection must be clear at the time of use, and that if crystallization has occurred, warming may redissolve the precipitate. Injection intended for veterinary use only is so labeled. If Injection contains Boric Acid, it is so labeled.

**USP Reference standards** (11)—

USP Endotoxin RS  
USP Potassium Gluconate RS

**Identification**—

**A:** A volume of Injection diluted, if necessary, with water to obtain a test solution of calcium gluconate (1 in 100) responds to Identification test *B* under *Calcium Gluconate*.

**B:** A dilution of the Injection with water (1 in 5) responds to the tests for *Calcium* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.17 USP Endotoxin Unit per mg of calcium gluconate.

**pH** (791): between 6.0 and 8.2, except that in the case where it is labeled as intended for veterinary use only and as containing boric acid, it is between 2.5 and 4.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—To an accurately measured volume of Injection, equivalent to about 500 mg of calcium gluconate, add 2 mL of 3 N hydrochloric acid, and dilute with water to 150 mL. While stirring, preferably with a magnetic stirrer, add about 20 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue

endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

## Calcium Gluconate Tablets

### DEFINITION

Calcium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191)

**Sample stock solution:** A warm, filtered solution in water, equivalent to 100 mg/mL of calcium gluconate from powdered Tablets

**Sample solution:** Equivalent to 20 mg/mL of calcium gluconate from a dilution of the *Sample stock solution*

**Acceptance criteria:** Meet the requirements

- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Equivalent to 10 mg/mL of calcium gluconate from a dilution of the *Sample stock solution* obtained from *Identification* test A

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

### ASSAY

- **PROCEDURE**

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium gluconate

**Blank:** Proceed as directed in the *Analysis*, without the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue, 300 mg

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution. Transfer the solution to a suitable container, and add about 150 mL of water. While stirring, preferably with a magnetic stirrer, add 20 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) in the portion of Tablets taken:

$$\text{Result} = \{(V_s - V_b) \times M \times F / W\} \times 100$$

$V_s$  = Titrant volume consumed by the Sample (mL)  
 $V_b$  = Titrant volume consumed by the Blank (mL)  
 $M$  = actual molarity of the Titrant (mM/mL)  
 $F$  = equivalency factor, 430.4 (mg/mM)  
 $W$  = nominal weight of calcium gluconate in the Sample (mg)

Acceptance criteria: 95.0%–105.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Solution having a known concentration of calcium in Medium

Sample solution: Filtered portion of the solution under test, suitably diluted with Medium if necessary

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 422.8 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) dissolved:

$$\text{Result} = (A_u/A_s) \times (C_s \times D \times V/L) \times (M_r/A_r) \times 100$$

$A_u$  = absorbance of the Sample solution

$A_s$  = absorbance of the Standard solution

$C_s$  = concentration of calcium in the Standard solution (mg/mL)

$D$  = dilution factor for the Sample solution

$V$  = volume of Medium, 900 mL

$L$  = label claim (mg/Tablet)

$M_r$  = molecular weight of calcium gluconate, 430.4

$A_r$  = atomic weight of calcium, 40.078

Tolerances: NLT 75% (Q) of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** <11>  
 USP Potassium Gluconate RS

## Calcium Hydroxide

$Ca(OH)_2$  74.09

Calcium hydroxide.

Calcium hydroxide [1305-62-0].

» Calcium Hydroxide contains not less than 95.0 percent and not more than 100.5 percent of  $Ca(OH)_2$ .

**Packaging and storage**—Preserve in tight containers.

### Identification—

A: When mixed with from three to four times its weight of water, it forms a smooth magma. The clear, supernatant from the magma is alkaline to litmus.

B: Mix 1 g with 20 mL of water, and add sufficient 6 N acetic acid to effect solution: the resulting solution responds to the tests for Calcium <191>.

**Limit of acid-insoluble substances**—Dissolve 2.0 g in 30 mL of 4 N hydrochloric acid, and heat to boiling. Filter the mixture, wash the residue with hot water, and ignite: the weight of the residue does not exceed 10 mg (0.5%).

**Carbonate**—Mix 2 g with 50 mL of water: the addition of an excess of 3 N hydrochloric acid to the mixture does not cause more than a slight effervescence.

**Heavy metals** <231>—Dissolve 2.0 g in 20 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water, and filter. Dilute the filtrate with water to 40 mL, and to 20 mL of the resulting solution add 1 mL of 0.1 N hydrochloric acid, then add water to make 25 mL: the limit is 20 µg per g.

**Limit of magnesium and alkali salts**—Dissolve 0.50 g in a mixture of 30 mL of water and 10 mL of 3 N hydrochloric acid, and proceed as directed in the test for *Magnesium and alkali salts* under *Calcium Carbonate*, beginning with "heat the solution, and boil for 1 minute." The weight of the residue does not exceed 12 mg (4.8%).

**Assay**—Transfer about 1.5 g of Calcium Hydroxide, accurately weighed, to a beaker, and gradually add 30 mL of 3 N hydrochloric acid. When the solution is dissolved, transfer it to a 500-mL volumetric flask, rinse the beaker thoroughly, adding the rinsings to the flask, dilute with water to volume, and mix. Pipet 50 mL of the solution into a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.705 mg of  $Ca(OH)_2$ .

## Calcium Hydroxide Topical Solution

» Calcium Hydroxide Topical Solution is a solution containing, in each 100 mL, not less than 140 mg of calcium hydroxide [ $Ca(OH)_2$ ].

Prepare Calcium Hydroxide Topical Solution as follows (see *Pharmaceutical Compounding—Non-sterile Preparations* <795>):

Calcium Hydroxide .....	3 g
Purified Water .....	1000 mL

Add the Calcium Hydroxide to 1000 mL of cool Purified Water, and agitate the mixture vigorously and repeatedly during 1 hour. Allow the excess calcium hydroxide to settle. Dispense only the clear supernatant.

NOTE—The solubility of calcium hydroxide, which varies with the temperature at which the solution is stored, is about 170 mg per 100 mL at 15° and less at a higher temperature. The official concentration is based upon a temperature of 25°.

The undissolved portion of the mixture is not suitable for preparing additional quantities of Calcium Hydroxide Topical Solution.

**Packaging and storage**—Preserve in well-filled, tight containers, at a temperature not exceeding 25°.

**Identification—**

**A:** It absorbs carbon dioxide from the air, a film of calcium carbonate forming on the surface of the liquid.

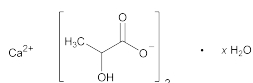
**B:** When heated, it becomes turbid, owing to the separation of calcium hydroxide.

**C:** It responds to the tests for *Calcium* <191>.

**Alkalies and their carbonates—**A portion of it, saturated with carbon dioxide and subsequently boiled, is neutral in reaction.

**Assay—**Pipet 100 mL of Topical Solution into a suitable container, add 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.705 mg of calcium hydroxide  $[\text{Ca}(\text{OH})_2]$ .

## Calcium Lactate



$\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$  308.30

$\text{C}_6\text{H}_{10}\text{CaO}_6$  218.22

Propanoic acid, 2-hydroxy-, calcium salt (2:1), hydrate;

Calcium lactate (1:2) hydrate [41372-22-9].

Calcium lactate (1:2) pentahydrate [5743-47-5].

Anhydrous [814-80-2].

**DEFINITION**

Calcium Lactate contains NLT 98.0% and NMT 101.0% of calcium lactate ( $\text{C}_6\text{H}_{10}\text{CaO}_6$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* <191>: Meets the requirements

• **B. INFRARED ABSORPTION** <197K>

**ASSAY****• PROCEDURE**

**Sample:** Weighed portion of Calcium Lactate equivalent to 350 mg of  $\text{C}_6\text{H}_{10}\text{CaO}_6$

**Blank:** 150 mL of water and 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium lactate ( $\text{C}_6\text{H}_{10}\text{CaO}_6$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mM/mL)

$F$  = equivalency factor, 218.2 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES****• HEAVY METALS** <231>

**Test preparation:** Dissolve 1 g in 2.5 mL of 1 N acetic acid, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 20 ppm

**• LIMIT OF MAGNESIUM AND ALKALI SALTS**

**Sample:** 1.0 g Calcium Lactate

**Analysis:** Mix the *Sample* with 40 mL of water, carefully add 1 mL of hydrochloric acid, and heat the solution to boiling. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid. Evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

**Acceptance criteria:** NMT 1.0%: The weight of the residue does not exceed 5.0 mg.

**• VOLATILE FATTY ACID**

**Sample solution:** Stir 500 mg of Calcium Lactate with 1 mL of sulfuric acid, and warm.

**Acceptance criteria:** The mixture does not emit an odor of volatile fatty acid.

**SPECIFIC TESTS****• ACIDITY**

**Sample solution:** 50 mg/mL

**Analysis:** Titrate 20 mL of *Sample solution* with 0.10 N sodium hydroxide, using phenolphthalein TS as the indicator.

**Acceptance criteria:** NMT 0.50 mL is required for neutralization, equivalent to NMT 0.45% as lactic acid.

**• LOSS ON DRYING** <731>

**Sample:** 1–2 g

**Analysis:** Distribute the *Sample* evenly in a suitable weighing dish to a depth of NMT 3 mm, and dry at 120° for 4 h.

**Acceptance criteria:** See *Table 1*.

**Table 1**

Form	Loss on Drying (%)
Pentahydrate	22.0–27.0
Trihydrate	15.0–20.0
Monohydrate	5.0–8.0
Anhydrous	NMT 3.0

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The label indicates whether it is the dried form or is hydrous; if the latter, the label indicates the degree of hydration. Where the quantity of Calcium Lactate is indicated in the labeling of any solution containing Calcium Lactate, this shall be understood to be in terms of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ).

• **USP REFERENCE STANDARDS** <11>

USP Calcium Lactate RS

## Calcium Lactate Tablets

### DEFINITION

Calcium Lactate Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ ).

[NOTE—An equivalent amount of Calcium Lactate with less water of hydration may be used in place of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ ) in preparing Calcium Lactate Tablets.]

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**  
**Sample solution:** A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets  
**Acceptance criteria:** Meet the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Lactate (191)**  
**Sample solution:** A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets  
**Acceptance criteria:** Meet the requirements

### ASSAY

#### • PROCEDURE

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 350 mg of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ )

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue, 300 mg

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable container, and add 150 mL of water and 2 mL of 3 N hydrochloric acid. Stir, using a magnetic stirrer, for 3–5 min. While stirring, add 30 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ ) in the portion of Tablets taken:

$$\text{Result} = \left\{ \frac{(V_S - V_B) \times M \times F}{W} \right\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 308.4 (mg/mM)

$W$  = nominal weight of calcium lactate pentahydrate in the *Sample* taken (mg)

**Acceptance criteria:** 94.0%–106.0%

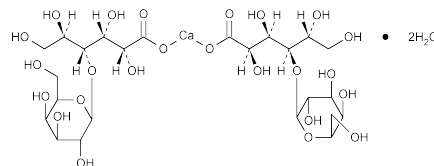
### PERFORMANCE TESTS

- **DISSOLUTION, Procedure for a Pooled Sample (711)**  
**Medium:** Water; 500 mL  
**Apparatus 1:** 100 rpm  
**Time:** 45 min  
**Analysis:** Determine the amount of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ ) dissolved, as directed in the *Assay*, making any necessary modifications.  
**Tolerances:** NLT 75% (Q) of the labeled amount of calcium lactate pentahydrate ( $C_6H_{10}CaO_6 \cdot 5H_2O$ ) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The quantity of Calcium Lactate stated in the labeling is in terms of calcium lactate pentahydrate.

## Calcium Lactobionate



$C_{24}H_{42}CaO_{24} \cdot 2H_2O$  790.68  
D-Gluconic acid, 4-O-β-D-galactopyranosyl-, calcium salt (2:1), dihydrate;  
Lactobionic acid, calcium salt (2:1), dihydrate;  
Calcium lactobionate (1:2), dihydrate [110638-68-1].

### DEFINITION

Calcium Lactobionate contains NLT 96.0% and NMT 102.0% of calcium lactobionate ( $C_{24}H_{42}CaO_{24} \cdot 2H_2O$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 20 mg/mL solution meets the requirements.
- **B. INFRARED ABSORPTION (197K)**
- **C. THIN LAYER CHROMATOGRAPHY**  
**Standard solution:** 10 mg/mL of USP Calcium Lactobionate RS in water  
**Sample solution:** 10 mg/mL of Calcium Lactobionate in water  
**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel  
**Application volume:** 5 μL  
**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)  
**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.

#### Analysis:

**Samples:** *Standard solution* and *Sample solution*  
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 100° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

### ASSAY

#### • PROCEDURE

**Sample:** 800 mg of Calcium Lactobionate

**Blank:** 150 mL of water and 2 mL of 3 N hydrochloric acid

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 15 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the

titration to a blue endpoint. Perform the *Blank* determination.  
Calculate the percentage of calcium lactobionate ( $C_{24}H_{42}CaO_{24} \cdot 2H_2O$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

- $V_S$  = Titrant volume consumed by the *Sample* (mL)  
 $V_B$  = Titrant volume consumed by the *Blank* (mL)  
 $M$  = Titrant molarity (mM/mL)  
 $F$  = equivalency factor, 790.7 mg/mmol  
 $W$  = *Sample* weight (mg)  
 Acceptance criteria: 96.0%–102.0%

## IMPURITIES

### • HALIDES

**Standard solution:** 0.7 mL of 0.020 N hydrochloric acid  
**Sample:** 1.2 g  
**Analysis:** Proceed as directed for *Chloride and Sulfate* <221>, *Chloride*.

**Acceptance criteria:** NMT 0.04%

### • CHLORIDE AND SULFATE, *Sulfate* <221>

**Standard solution:** 1 mL of 0.020 N sulfuric acid  
**Sample:** 2.0 g

**Analysis:** Dissolve the *Sample* in boiling water.

**Acceptance criteria:** NMT 0.05%

### • HEAVY METALS <231>

**Test preparation:** Mix 1 g in 4 mL of 1.2 N hydrochloric acid. Add water to make 25 mL, warm gently until dissolved, and cool to room temperature.

**Acceptance criteria:** NMT 20 ppm

### • REDUCING SUBSTANCES

**Sample:** 1.0 g of Calcium Lactobionate

**Blank:** 20 mL of water

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back-titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 250-mL conical flask, dissolve in the *Blank*, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

- $V_B$  = Back-titrant volume consumed by the *Blank* (mL)  
 $V_S$  = Back-titrant volume consumed by the *Sample* (mL)  
 $N$  = Back-titrant normality (mEq/mL)  
 $F$  = equivalency factor, 27 mg/mEq  
 $W$  = *Sample* weight (mg)  
 Acceptance criteria: NMT 1.0%

## SPECIFIC TESTS

### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 100 mg/mL

**Acceptance criteria:** +22.0° to +26.5°

### • pH <791>

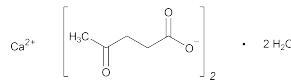
**Sample solution:** 50 mg/mL

**Acceptance criteria:** 5.4–7.4

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS <11>**  
USP Calcium Lactobionate RS

## Calcium Levulinate



$C_{10}H_{14}CaO_6 \cdot 2H_2O$  306.32

$C_{10}H_{14}CaO_6$  270.30

Pentanoic acid, 4-oxo-, calcium salt (2:1), dihydrate;  
 Calcium levulinate (1:2) dihydrate [5743-49-7].

Anhydrous [591-64-0].

## DEFINITION

Calcium Levulinate contains NLT 97.5% and NMT 100.5% of calcium levulinate ( $C_{10}H_{14}CaO_6$ ), calculated on the dried basis.

## IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, *Calcium* <191>:** A 100 mg/mL solution meets the requirements.

### • B.

**Sample solution:** 0.5 g in 5 mL of water

**Analysis:** To the *Sample solution* add 5 mL of 1 N sodium hydroxide, and filter. To the filtrate add 5 mL of iodine TS.

**Acceptance criteria:** A precipitate of iodoform is produced.

## ASSAY

### • PROCEDURE

**Sample:** 600 mg of Calcium Levulinate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium levulinate ( $C_{10}H_{14}CaO_6$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

- $V_S$  = Titrant volume consumed by the *Sample* (mL)  
 $V_B$  = Titrant volume consumed by the *Blank* (mL)  
 $M$  = Titrant molarity (mM/mL)  
 $F$  = equivalency factor, 270.3 mg/mmol  
 $W$  = *Sample* weight (mg)  
 Acceptance criteria: 97.5%–100.5% on the dried basis

## IMPURITIES

### • CHLORIDE AND SULFATE, *Chloride* <221>

**Standard:** 1.0 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g

**Acceptance criteria:** NMT 0.07%

### • CHLORIDE AND SULFATE, *Sulfate* <221>

**Standard:** 1.0 mL of 0.020 N sulfuric acid

**Sample:** 2.0 g

**Acceptance criteria:** NMT 0.05%

### • HEAVY METALS <231>: NMT 20 ppm

### • REDUCING SUGARS

**Sample:** 0.50 g

**Analysis:** Dissolve the *Sample* in 10 mL of water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to

stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

**Acceptance criteria:** No red precipitate is formed immediately.

#### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* (741): 119°–125°
- **PH** (791)  
Sample solution: 100 mg/mL  
Acceptance criteria: 7.0–8.5
- **LOSS ON DRYING** (731): Dry a sample at a pressure not exceeding 5 mm of mercury at 60° for 5 h: it loses 10.5%–12.0% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

### Calcium Levulinate Injection

» Calcium Levulinate Injection is a sterile solution of Calcium Levulinate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{10}H_{14}CaO_6 \cdot 2H_2O$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

#### USP Reference standards (11)—

USP Endotoxin RS

**Identification**—It responds to the *Identification* tests under *Calcium Levulinate*.

**Bacterial endotoxins** (85)—It contains not more than 35.70 USP Endotoxin Units per mg of calcium levulinate.

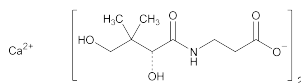
**pH** (791): between 6.0 and 8.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 600 mg of calcium levulinate, to a 400-mL beaker, add 2 mL of hydrochloric acid, and proceed as directed in the *Assay* under *Calcium Levulinate*, beginning with “While stirring with a magnetic stirrer.” Each mL of 0.05 M edetate disodium is equivalent to 15.32 mg of  $C_{10}H_{14}CaO_6 \cdot 2H_2O$ .

### Calcium Pantothenate



$C_{18}H_{32}CaN_2O_{10}$  476.53  
β-Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (*R*)-;

Calcium D-pantothenate (1:2) [137-08-6].

#### DEFINITION

Calcium Pantothenate is the calcium salt of the dextrorotatory isomer of pantothenic acid. It contains NLT 5.7% and NMT 6.0% of nitrogen (N), and NLT 8.2% and NMT 8.6% of calcium (Ca), both calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191): A 50-mg/mL solution meets the requirements.

#### COMPOSITION

- **NITROGEN DETERMINATION**, *Method I* (461)  
Sample: 500 mg  
Analysis: Proceed as directed in the chapter.  
Acceptance criteria: 5.7%–6.0% on the dried basis
- **CONTENT OF CALCIUM**  
Sample: 800 mg of Calcium Pantothenate  
Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid  
Titrimetric system  
(See *Titrimetry* (541).)  
Mode: Direct titration  
Titrant: 0.05 M edetate disodium VS  
Endpoint detection: Visual  
Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with *Titrant* to a distinct blue endpoint. Perform the *Blank* determination.  
Calculate the percentage of calcium (Ca) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F] / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $M$  = actual molarity of the *Titrant* (mM/mL)  
 $F$  = equivalency factor, 40.08 mg/mM  
 $W$  = *Sample* weight (mg)

**Acceptance criteria:** 8.2%–8.6% on the dried basis

#### IMPURITIES

- **HEAVY METALS** (231)  
Test preparation: 1.0 g in 25 mL of water  
Acceptance criteria: NMT 20 ppm
- **ORDINARY IMPURITIES** (466)  
Standard solution: Use water as the solvent. Use USP Beta Alanine RS, in place of USP Calcium Pantothenate RS, as the Standard.  
Test solution: Use water as the solvent.  
Eluant: Alcohol and water (65:35)  
Visualization: 4  
Acceptance criteria: NMT 1.0%

#### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* (781S)  
Sample solution: 50 mg/mL in water  
Acceptance criteria: +25.0° to +27.5°
- **ALKALINITY**  
Sample: 1.0 g  
Analysis: Dissolve the *Sample* in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution is complete, add 1.0 mL of 0.10 N hydrochloric acid, then add 0.05 mL of phenolphthalein TS.  
Acceptance criteria: No pink color is produced within 5 s.
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 5.0% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** <11>  
USP Beta Alanine RS  
3-Aminopropionic acid.  
USP Calcium Pantothenate RS

## Calcium Pantothenate Tablets

### DEFINITION

Calcium Pantothenate Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of the dextrorotatory isomer of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium** <191>  
**Sample solution:** Digest a quantity of powdered Tablets, equivalent to 150 mg of calcium pantothenate, with 15 mL of 1 N sodium hydroxide, and filter.  
**Acceptance criteria:** Meet the requirements
- **B.**  
**Sample solution:** 5 mL of the filtrate obtained in *Identification test A*  
**Analysis:** Add 5 mL of 1 N hydrochloric acid and 2 drops of ferric chloride TS to the *Sample solution*.  
**Acceptance criteria:** A strong yellow color is produced.

### ASSAY

#### Change to read:

- **▲CALCIUM PANTOTHENATE**  
**Buffer solution:** Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.  
**Mobile phase:** Methanol and *Buffer solution* (1:9)  
**System suitability solution:** 0.5 mg/mL of USP Calcium Pantothenate RS and 0.1 mg/mL of USP Racemic Panthenol RS in water  
**Standard solution:** 0.5 mg/mL of USP Calcium Pantothenate RS in water  
**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of calcium pantothenate, to a 100-mL volumetric flask. Add 5 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.  
**Chromatographic system**  
(See *Chromatography* <621>, *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 205 nm  
**Column:** 4.6-mm × 25-cm; 5-μm packing L1  
**Column temperature:** 50°  
**Flow rate:** 2 mL/min  
**Injection volume:** 25 μL  
**System suitability**  
**Sample:** *System suitability solution*  
[NOTE—The relative retention times for pantothenate and panthenol are 1.0 and 1.1, respectively.]  
**Suitability requirements**  
**Resolution:** NLT 1.5 between pantothenate and panthenol  
**Relative standard deviation:** NMT 2.0%  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
Measure the peak areas for calcium pantothenate. Calculate the percentage of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of calcium pantothenate in the *Sample solution* (mg/mL)▲<sup>USP36</sup>

**Acceptance criteria:** 95.0%–115.0%

### OTHER COMPONENTS

#### • CONTENT OF CALCIUM

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium pantothenate

**Blank:** Proceed as directed in the *Analysis*, without the *Sample*.

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue, 300 mg

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to completely dissolve. Transfer the solution to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Titrate with *Titrant* to a distinct blue endpoint. Perform a blank determination.

Calculate the percentage of calcium in the content of calcium pantothenate, as determined by the *Assay*, in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 40.08 mg/mM

$W$  = weight of calcium pantothenate in the *Sample* taken, as determined by the *Assay* (mg)

**Acceptance criteria:** 7.9%–9.7% of the weight of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) in the Tablets, as determined by the *Assay*

### PERFORMANCE TESTS

#### • DISSOLUTION, Procedure for a Pooled Sample <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Mobile phase:** Phosphoric acid and water (1:1000)

**Standard solution:** A known concentration of USP Calcium Pantothenate RS in *Medium*

**Sample solution:** A filtered portion of the solution under test, suitably diluted with *Medium* if necessary, having a concentration of  $C_{18}H_{32}CaN_2O_{10}$  similar to that of the *Standard solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times [C_S \times D \times (V/L)] \times 100$$

- $r_U$  = peak area of calcium pantothenate from the *Sample solution*  
 $r_S$  = peak area of calcium pantothenate from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)  
**Tolerances:** NLT 75% ( $Q$ ) of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to indicate the content of dextrorotatory calcium pantothenate.

**Change to read:**

- **USP REFERENCE STANDARDS** (11)  
 USP Calcium Pantothenate RS  
 ▲USP Racemic Panthenol RS▲<sup>USP36</sup>

**Racemic Calcium Pantothenate**

$C_{18}H_{32}CaN_2O_{10}$  476.53  
 $\beta$ -Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), ( $\pm$ );  
 Calcium DL-pantothenate (1:2) [6381-63-1].

**DEFINITION**

Racemic Calcium Pantothenate is a mixture of the calcium salts of the dextrorotatory and levorotatory isomers of pantothenic acid. It contains NLT 5.7% and NMT 6.0% of nitrogen (N), and NLT 8.2% and NMT 8.6% of calcium (Ca), both calculated on the dried basis.

[NOTE—The physiological activity of Racemic Calcium Pantothenate is approximately one-half that of Calcium Pantothenate.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191): A 50-mg/mL solution meets the requirements.

**COMPOSITION**

- **NITROGEN DETERMINATION**, *Method I* (461)  
 Sample: 500 mg  
 Analysis: Proceed as directed in the chapter.  
 Acceptance criteria: 5.7%–6.0% on the dried basis
- **CONTENT OF CALCIUM**  
 Sample: 800 mg  
 Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid  
**Titrimetric system**  
 (See *Titrimetry* (541).)  
 Mode: Direct titration  
 Titrant: 0.05 M edetate disodium VS  
 Endpoint detection: Visual  
 Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with the *Titrant* to a distinct blue endpoint. Perform the *Blank* determination. Calculate the percentage of calcium (Ca) in the *Sample* taken:

$$\text{Result} = \left\{ \left[ (V_S - V_B) \times M \times F \right] / W \right\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

- $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $M$  = actual molarity of the *Titrant* (mM/mL)  
 $F$  = equivalency factor, 40.08 mg/mM  
 $W$  = *Sample* weight (mg)  
**Acceptance criteria:** 8.2%–8.6% on the dried basis

**IMPURITIES**

- **HEAVY METALS** (231)  
 Test preparation: 1.0 g in 25 mL of water  
 Acceptance criteria: NMT 20 ppm

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S)  
 Test solution: 50 mg/mL in water  
 Acceptance criteria:  $-0.05^\circ$  to  $+0.05^\circ$
- **ALKALINITY**  
 Sample: 1.0 g  
 Analysis: Dissolve the *Sample* in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution is complete, add 1.6 mL of 0.10 N hydrochloric acid, and then add 0.05 mL of phenolphthalein TS.  
 Acceptance criteria: No pink color is produced within 5 s.
- **LOSS ON DRYING** (731): Dry a sample at  $105^\circ$  for 3 h: it loses NMT 5.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label preparations containing it in terms of the equivalent amount of dextrorotatory calcium pantothenate.
- **USP REFERENCE STANDARDS** (11)  
 USP Calcium Pantothenate RS

**Dibasic Calcium Phosphate Dihydrate****Pharmacopeial Discussion Group Sign-Off Document**

Attribute	JP	EP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Acid-Insoluble Substances	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Carbonate	+	+	+
Barium	+	+	+
Loss on Ignition	+	+	+
Assay	+	+	+

**Legend:** +, will adopt and implement; –, will not stipulate.

**Nonharmonized attributes:** *Packaging and Storage*, *Heavy Metals*, *Limit of Fluoride*, *Iron*

**Specific local attributes:** *Identification C* (EP), *Lead* (USP), *Description* (JP)

$CaHPO_4 \cdot 2H_2O$  172.09  
 Phosphoric acid, calcium salt (1:1);  
 Calcium phosphate, dihydrate (1:1) [7789-77-7].

**DEFINITION**

Dibasic Calcium Phosphate Dihydrate contains two molecules of water of hydration. It contains NLT 98.0% and NMT 105.0% of dibasic calcium phosphate dihydrate ( $CaHPO_4 \cdot 2H_2O$ ).

**IDENTIFICATION**

- **A.**  
 Sample: 0.1 g of Dibasic Calcium Phosphate Dihydrate



**Analysis:** Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.

**Acceptance criteria:** A white precipitate is formed.

• **B.**

**Sample:** 0.1 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to 70°, and add 2 mL of 10% ammonium molybdate solution (freshly prepared).

**Acceptance criteria:** A yellow precipitate of ammonium phosphomolybdate is formed.

## ASSAY

### • PROCEDURE

**Buffer:** Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.

**Sample solution:** Transfer 400 mg of Dibasic Calcium Phosphate Dihydrate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.

**Blank:** 20 mL of water containing 1.2 mL of diluted hydrochloric acid

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.02 M edetate disodium VS

**Back-titrant:** 0.02 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T–sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner.

Calculate the percentage of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F] / W\} \times 100$$

$V_B$  = Back-titrant volume consumed by the *Blank* (mL)

$V_S$  = Back-titrant volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 172.1 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.0%–105.0%

## IMPURITIES

### • CARBONATE

**Sample:** 1.0 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.

**Acceptance criteria:** No effervescence occurs.

### • CHLORIDE AND SULFATE, Chloride (221)

**Standard:** 0.70 mL of 0.010 N hydrochloric acid

**Sample:** 0.2 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** To the *Sample* add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 50 mL of the filtrate add 1 mL of silver nitrate TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.25%).

### • CHLORIDE AND SULFATE, Sulfate (221)

**Standard:** 1.0 mL of 0.010 N sulfuric acid

**Sample:** 0.5 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** To the *Sample* add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to

100 mL, and filter if necessary. To 20 mL of the filtrate add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL. Add 1 mL of barium chloride TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.5%).

### • ARSENIC, Method I (211)

**Test preparation:** 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.

**Acceptance criteria:** NMT 3 µg/g

### • BARIUM

**Sample:** 0.5 g Dibasic Calcium Phosphate Dihydrate

**Analysis:** Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter, if necessary. To the filtrate add 2 mL of potassium sulfate TS.

**Acceptance criteria:** No turbidity is produced within 10 min.

### • HEAVY METALS, Method I (231)

**Test preparation:** Warm 1.3 g with 3 mL of 3 N hydrochloric acid to completely dissolve. Cool, dilute with water to 50 mL, and filter.

**Acceptance criteria:** NMT 30 ppm

### • LIMIT OF ACID-INSOLUBLE SUBSTANCES

**Sample solution:** Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.

**Analysis:** After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at  $600 \pm 50^\circ$ .

**Acceptance criteria:** The weight of the residue does not exceed 10 mg (NMT 0.2%).

### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer solution:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1052 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

**Sample solution:** Transfer 2.0 g of Dibasic Calcium Phosphate dihydrate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH (791)).

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential in mV. From the measured potential and the *Standard response line* determine the concentration,  $C$  (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride (ppm) in the portion of Dibasic Calcium Phosphate Dihydrate taken:

$$\text{Result} = (V \times C)/W$$

- $V$  = Sample solution volume (mL)  
 $C$  = concentration of fluoride ion, determined from the *Standard response line*, in the *Sample solution* (µg/mL)  
 $W$  = weight of Dibasic Calcium Phosphate Dihydrate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 50 ppm

### SPECIFIC TESTS

#### • LOSS ON IGNITION (733)

**Sample:** 1 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** Ignite the *Sample* at 800°–825° to constant weight.

**Acceptance criteria:** 24.5%–26.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.
- **USP REFERENCE STANDARDS (11)**  
USP Sodium Fluoride RS

## Anhydrous Dibasic Calcium Phosphate

### Pharmacopeial Discussion Group Sign-Off Document

Attribute	JP	EP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Acid-insoluble substances	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Carbonate	+	+	+
Barium	+	+	+
Loss on ignition	+	+	+
Assay	+	+	+

**Legend:** + will adopt and implement; - will not stipulate.

**Nonharmonized attributes:** *Packaging and storage, Heavy metals, Limit of fluoride, Iron*

**Specific local attributes:** Identification C (EP), Lead (USP), Description (JP)

CaHPO<sub>4</sub> 136.06  
 Phosphoric acid, calcium salt (1:1);  
 Calcium phosphate (1:1) [7757-93-9].

### DEFINITION

Anhydrous Dibasic Calcium Phosphate contains NLT 98.0% and NMT 103.0% of anhydrous dibasic calcium phosphate (CaHPO<sub>4</sub>).

### IDENTIFICATION

- **A.**  
**Sample:** 0.1 g of Anhydrous Dibasic Calcium Phosphate  
**Analysis:** Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.  
**Acceptance criteria:** A white precipitate is formed.
- **B.**  
**Sample:** 0.1 g of Anhydrous Dibasic Calcium Phosphate  
**Analysis:** Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to 70°, and add 2 mL of 10% ammonium molybdate solution (freshly prepared).

**Acceptance criteria:** A yellow precipitate of ammonium phosphomolybdate is formed.

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.

**Sample solution:** Transfer 400 mg of Anhydrous Dibasic Calcium Phosphate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.

**Blank:** 20 mL of water containing 1.2 mL of diluted hydrochloric acid

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.02 M edetate disodium VS

**Back-titrant:** 0.02 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T–sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner.

Calculate the percentage of anhydrous dibasic calcium phosphate (CaHPO<sub>4</sub>) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

$V_B$  = Back-titrant volume consumed by the *Blank* (mL)

$V_S$  = Back-titrant volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 136.06 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.0%–103.0%

### IMPURITIES

#### • CARBONATE

**Sample:** 1.0 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.

**Acceptance criteria:** No effervescence occurs.

#### • CHLORIDE AND SULFATE, Chloride (221)

**Standard:** 0.70 mL of 0.010 N hydrochloric acid

**Sample:** 0.2 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** To the *Sample* add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 50 mL of the filtrate add 1 mL of silver nitrate TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.25%).

#### • CHLORIDE AND SULFATE, Sulfate (221)

**Standard:** 1.0 mL of 0.010 N sulfuric acid

**Sample:** 0.5 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** To the *Sample* add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 20 mL of the filtrate add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL. Add 1 mL of barium chloride TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.5%).

#### • ARSENIC, Method I (211)

**Test preparation:** 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.

**Acceptance criteria:** NMT 3 µg/g

• **BARIUM**

**Sample:** 0.5 g Anhydrous Dibasic Calcium Phosphate

**Analysis:** Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter if necessary. To the filtrate add 2 mL of potassium sulfate TS. **Acceptance criteria:** No turbidity is produced within 10 min.

• **HEAVY METALS, Method I (231)**

**Test preparation:** Warm 1.3 g with 3 mL of 3 N hydrochloric acid to completely dissolve. Cool, dilute with water to 50 mL, and filter.

**Acceptance criteria:** NMT 30 ppm

• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**

**Sample solution:** Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.

**Analysis:** After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at  $600 \pm 50^\circ$ .

**Acceptance criteria:** The weight of the residue does not exceed 10 mg (NMT 0.2%).

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer solution:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1052 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

**Sample solution:** Transfer 2.0 g of Anhydrous Dibasic Calcium Phosphate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH (791)).

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential in mV. From the measured potential and the *Standard response line* determine the concentration, *C* (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride (ppm) in the portion of Anhydrous Dibasic Calcium Phosphate taken:

$$\text{Result} = (V \times C)/W$$

*V* = *Sample solution* volume (mL)

*C* = concentration of fluoride ion, determined from the *Standard response line*, in the *Sample solution* (µg/mL)

*W* = weight of Anhydrous Dibasic Calcium Phosphate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 50 ppm

**SPECIFIC TESTS**

• **LOSS ON IGNITION (733)**

**Sample:** 1 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** Ignite the *Sample* at  $800^\circ$ – $825^\circ$  to constant weight.

**Acceptance criteria:** 6.6%–8.5%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.

• **USP REFERENCE STANDARDS (11)**

USP Sodium Fluoride RS

## Dibasic Calcium Phosphate Tablets

**DEFINITION**

Dibasic Calcium Phosphate Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

[NOTE—An equivalent amount of Dibasic Calcium Phosphate with less water of hydration may be used in place of  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  in preparing Dibasic Calcium Phosphate Tablets.]

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**

**Sample solution:** A filtered portion of the *Sample solution* from the *Assay*

**Acceptance criteria:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Phosphate (191)**

**Sample solution:** A filtered portion of the *Sample solution* from the *Assay*, neutralized with ammonium hydroxide

**Acceptance criteria:** Meets the requirements

**ASSAY**

• **PROCEDURE**

**Sample solution:** Transfer a portion of powder, equivalent to 1 g of dibasic calcium phosphate dihydrate, from NLT 20 powdered Tablets, to a 100-mL volumetric flask containing 15 mL of hydrochloric acid and 10 mL of water. Heat on a steam bath, with occasional mixing, to dissolve the dibasic calcium phosphate, but not longer than 30 min. Cool, add water to volume, and mix. If the solution is not clear, filter, discarding the first 10 mL of the filtrate.

**Blank:** Water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue

**Endpoint detection:** Visual

**Analysis:** Transfer 25.0 mL of the *Sample solution* to a 250-mL beaker equipped with a magnetic stirrer. With constant stirring, add, in the order named, 0.5 mL of triethanolamine, 300 mg of *Indicator*, and from a 50-mL buret, about 23 mL of *Titrant*. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear blue. Continue to add it dropwise until the color changes to violet, and add an additional 0.5 mL. The pH is 12.3–12.5. Continue the titration dropwise with the *Titrant* to the appearance of a clear blue endpoint that persists for NLT 60 s. Perform a blank determination.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) in the portion of Tablets taken:

$$\text{Result} = \{[(V_s - V_b) \times N \times F]/W\} \times 100$$

- $V_s$  = Titrant volume consumed by the *Sample solution* (mL)  
 $V_b$  = Titrant volume consumed by the *Blank* (mL)  
 $M$  = actual molarity of the *Titrant* (mM/mL)  
 $F$  = equivalency factor, 172.08 mg/mM  
 $W$  = nominal weight of dibasic calcium phosphate dihydrate in the *Sample solution* taken for Analysis (mg)

Acceptance criteria: 92.5%–107.5%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of calcium in *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 422.7 nm

**Lamp:** Calcium hollow-cathode

**Flame:** Air-acetylene

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the concentration of calcium (Ca) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) dissolved:

$$\text{Result} = (A_u/A_s) \times (C_s \times D \times V/L) \times (M_r/A_r) \times 100$$

- $A_u$  = absorbance of the *Sample solution*  
 $A_s$  = absorbance of the *Standard solution*  
 $C_s$  = concentration of calcium in the *Standard solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)  
 $M_r$  = molecular weight of dibasic calcium phosphate, 172.08  
 $A_r$  = atomic weight of calcium, 40.08

**Tolerances:** NLT 75% (Q) of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The quantity of dibasic calcium phosphate stated in the labeling is in terms of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

## Calcium Polycarbophil

Calcium polycarbophil [9003-97-8].

» Calcium Polycarbophil is the calcium salt of polyacrylic acid cross-linked with divinyl glycol.

**Packaging and storage**—Preserve in tight containers.

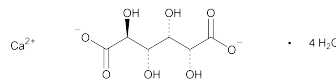
**Identification**—When tested as directed in the test for *Absorbing power*, it absorbs about 35 times its original weight.

**Loss on drying** <731>—Dry it in vacuum at 130° for 4 hours: it loses not more than 10.0% of its weight.

**Absorbing power**—Transfer about 250 mg, accurately weighed, to a tared 50-mL centrifuge tube fitted with a tight closure. Add 35 mL of 0.1 N hydrochloric acid to the tube, seal the tube, and shake by mechanical means for 30 minutes. Centrifuge at 2000 rpm for 20 minutes, and decant and discard the supernatant. [NOTE—Exercise care to avoid any loss of particles.] Add 35 mL of 0.1 N hydrochloric acid, and shake for 30 minutes. Centrifuge, decanting and discarding the supernatant. Repeat the foregoing steps, using water instead of acid. Add 35 mL of a sodium bicarbonate solution (15 in 1000), and shake, venting as necessary to release any carbon dioxide liberated. Shake for 1 hour, centrifuge, and decant the supernatant. Add 35 mL of sodium bicarbonate solution (15 in 1000), and shake for 1 hour. Allow the tube and contents to stand overnight or until the contents have settled, and centrifuge. Withdraw the supernatant, and weigh the tube and contents. Calculate the weight of sodium bicarbonate solution absorbed: not less than 35.0 g of the sodium bicarbonate solution is absorbed by 1.0 g of Calcium Polycarbophil, calculated on the dried basis.

**Content of calcium**—Transfer about 2 g of Calcium Polycarbophil, accurately weighed, to a tared crucible. Cover, leaving the lid slightly ajar, and place in a muffle furnace. Heat to 600° over 2 hours, increase the temperature to 1000° over 1 hour, and maintain at 1000° for 1 hour. Allow to cool slowly. Dissolve the residue in dilute hydrochloric acid (1 in 5), quantitatively transfer with the aid of dilute hydrochloric acid (1 in 5) to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (1 in 5) to volume. Pipet 15 mL of this solution into a 250-mL beaker, and add, while stirring with a magnetic stirrer, 100 mL of water, 20.0 mL of 0.05 M edetate disodium VS, and 300 mg of hydroxy naphthol blue. Adjust with 1 N sodium hydroxide solution to a pH of 9.0 to 9.5. Adjust with about 10 mL of 2 N sodium hydroxide to a pH of 12.4. Titrate with 0.05 M edetate disodium VS to a persistent blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca). The content of Ca found is not less than 18.0% and not more than 22.0%, calculated on the dried basis.

## Calcium Saccharate



$\text{C}_6\text{H}_8\text{CaO}_8 \cdot 4\text{H}_2\text{O}$

320.26

D-Glucaric acid, calcium salt (1:1) tetrahydrate;

Calcium D-glucarate (1:1), tetrahydrate [5793-89-5].

## DEFINITION

Calcium Saccharate is the calcium salt of D-saccharic acid. It contains NLT 98.5% and NMT 102.0% of  $\text{C}_6\text{H}_8\text{CaO}_8 \cdot 4\text{H}_2\text{O}$ .

## IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium** <191>

**Sample:** 0.2 g

**Analysis:** Dissolve the *Sample* in 10 mL of water by the addition of 2 mL of hydrochloric acid.

Acceptance criteria: Meets the requirements

- **B. INFRARED ABSORPTION** (197M): Meets the requirements

## ASSAY

### PROCEDURE

Sample: 600 mg

Blank: 150.0 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water with the aid of a sufficient volume of hydrochloric acid.

While stirring, preferably with a magnetic stirrer, add 30 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration to a blue endpoint. Perform a *Blank* determination and make any necessary corrections.

Calculate the percentage of calcium saccharate ( $\text{C}_6\text{H}_8\text{CaO}_8 \cdot 4\text{H}_2\text{O}$ ) in the portion of Calcium Saccharate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 320.2 mg/mEq

$W$  = *Sample* weight (mg)

Acceptance criteria: 98.5%–102.0%

## IMPURITIES

- **CHLORIDE AND SULFATE, Chloride** (221)

Standard: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of nitric acid

Acceptance criteria: NMT 0.07%

- **CHLORIDE AND SULFATE, Sulfate** (221)

Standard: 0.6 mL of 0.020 N sulfuric acid

Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of hydrochloric acid

Acceptance criteria: NMT 0.12%

- **HEAVY METALS, Method II** (231): NMT 20 ppm

- **SUCROSE AND REDUCING SUGARS**

Sample: 0.5 g

Analysis: Dissolve the *Sample* in 10 mL of water with the addition of 2 mL of hydrochloric acid, and boil the solution for about 2 min. Cool, add 15 mL of sodium carbonate TS, allow to stand for 5 min, and filter. Add 5 mL of the clear filtrate to about 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

Acceptance criteria: No red precipitate is formed immediately.

## SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)

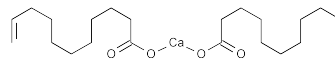
Sample solution: 60 mg/mL in 4.8 N hydrochloric acid that has been allowed to stand for 1 h

Acceptance criteria: +18.5° to +22.5°

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Calcium Saccharate RS

## Calcium Undecylenate



$\text{C}_{22}\text{H}_{38}\text{O}_4\text{Ca}$  406.61

10-Undecenoic acid, calcium (2+) salt.

Calcium 10-undecenoate.

» Calcium Undecylenate contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{22}\text{H}_{38}\text{O}_4\text{Ca}$  (calcium undecylenate), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### Identification—

**A:** A filtered solution (1 in 20) in 3 N hydrochloric acid responds to the tests for *Calcium* (191).

**B:** Transfer 40 mL of water and 10 g of calcium undecylenate to a 250-mL separator. Cautiously and slowly add 10 mL of hydrochloric acid, while swirling. Insert the stopper, and shake. [NOTE—The separator will become quite warm, and pressure must be carefully and frequently relieved through the stopcock. If a curdy, white material remains after 5 minutes of shaking, add additional hydrochloric acid, 1 mL at a time, and shake until a clear oily phase is formed.] Allow the phases to separate, drain, and discard the bottom aqueous layer. Drain and discard the middle oily layer, if present. Filter the top layer of undecylenic acid through a pledget of cotton into a 10-mL graduated cylinder, noting the volume obtained. Transfer the filtrate to a 250-mL flask, and add an equal volume of aniline. Reflux for 1 hour, swirling the flask occasionally. Allow to cool, and pour 60 mL of alcohol through the condenser into the flask. Remove the flask from the condenser, add 1 g of charcoal, and stir. Filter the slurry into a 250-mL beaker. Add water dropwise until a few crystals form or the solution becomes slightly cloudy. [NOTE—If too much water is added, an oil will form. Add alcohol dropwise until the oil dissolves.] Allow the mixture to stand or refrigerate until crystals are formed. Collect the crystals on a filter paper inserted in a 45-mm porous glass filter funnel. Wash the crystals with 75 mL of 25% alcohol: the crystals have a clean, white, glossy appearance. If not, recrystallize by dissolving the crystals in about 50 mL of alcohol. Add about 1 g of charcoal, stir, filter into a 150-mL beaker, and continue as directed above, beginning with "Add water dropwise." Dry the crystals in vacuum at 50° for 2 hours: the crystals so obtained melt between 66° and 67.5°, the procedure for *Class Ia* being used (see *Melting Range or Temperature* (741)). [NOTE—If the melting point is low, additional drying or recrystallization may be necessary.]

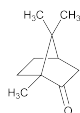
**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses between 2.0% and 5.7% of its weight.

**Particle size** (786)—Test in accordance with this procedure, except to use not more than 25 g, and except that a single No. 100 sieve is used and is to be shaken for not less than 30 minutes or until sifting is practically complete: not less than 99.0% of it passes through a No. 100 sieve.

**Limit of free undecylenic acid**—Transfer 10 g of Calcium Undecylenate, accurately weighed, to a 400-mL beaker, add 250 mL of solvent hexane, and mix for 2 hours using a magnetic stirrer. Filter into a 500-mL flask, evaporate with the aid of a current of air to about 20 mL, and add 100 mL of neutralized alcohol. Add 3 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 18.43 mg of  $\text{C}_{11}\text{H}_{20}\text{O}_2$ : not more than 0.1% is found.

**Assay**—Boil 40.0 mL of 0.1 N hydrochloric acid VS with about 600 mg of Calcium Undecylenate, accurately weighed, for 10 minutes, or until the undecylenic acid layer is clear, adding water, as necessary, to maintain the original volume. Transfer the mixture, with the aid of water, to a 500-mL separator. Dilute with water to about 75 mL, and extract with two 100-mL portions of solvent hexane. Wash the combined extracts with water until the last washing is neutral to litmus, add the washings to the original water layer. Cool, add 3 drops of methyl orange TS, and titrate the excess hydrochloric acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N sodium hydroxide is equivalent to 20.33 mg of  $C_{22}H_{38}O_4Ca$ .

## Camphor



$C_{10}H_{16}O$  152.23  
Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-;  
Camphor;  
2-Bornanone [76-22-2].

### DEFINITION

Camphor is a ketone of *Cinnamomum camphora* (L.) Nees et Ebermaier (Fam. Lauraceae) (natural Camphor), or is produced synthetically (synthetic Camphor).

### IMPURITIES

#### • LIMIT OF NONVOLATILE RESIDUE

**Sample:** 2.0 g of Camphor

**Analysis:** Heat the *Sample* in a tared dish on a steam bath until sublimation is complete. Dry the residue at 120° for 3 h, cool, and weigh.

**Acceptance criteria:** 0.05%; the weight of the residue does not exceed 1.0 mg.

#### • HALOGENS

**Sample:** Mix 100 mg of finely divided Camphor with 200 mg of sodium peroxide in a clean, dry, hard glass test tube about 25 mm in internal diameter and 20 cm in length. Suspend the tube at an angle of about 45°, using a clamp placed at the upper end. Gently heat the tube, starting near the upper end, but not heating the clamp. Gradually bring the heat toward the lower part of the tube until incineration is complete.

**Analysis:** Dissolve the residue in 25 mL of warm water, acidify with nitric acid, and filter the solution into a comparison tube. Wash the test tube and the filter with two 10-mL portions of hot water, adding the washings to the filtered solution. To the filtrate add 0.50 mL of 0.10 N silver nitrate, dilute with water to 50 mL, and mix.

**Acceptance criteria:** 0.035%; the turbidity does not exceed that produced in a blank test with the same quantities of the same reagents and 0.050 mL of 0.020 N hydrochloric acid.

### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 174°–179°

• **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 100 mg/mL in alcohol  
Synthetic Camphor is optically inactive.

**Acceptance criteria:** +41° to +43° for natural Camphor

• **APPEARANCE OF SOLUTION:** A 100-mg/mL solution in solvent hexane is clear.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.
- **LABELING:** Label it to indicate whether it is of natural sources or is prepared synthetically.

## Camphor Spirit

» Camphor Spirit is an alcohol solution containing, in each 100 mL, not less than 9.0 g and not more than 11.0 g of camphor ( $C_{10}H_{16}O$ ).

Camphor .....	100 g
Alcohol, a sufficient quantity,	
to make .....	1000 mL

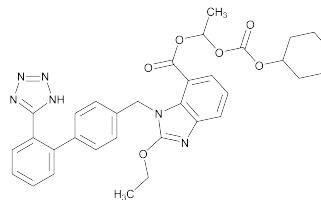
Dissolve the camphor in about 800 mL of the alcohol, and add alcohol to make 1000 mL. Filter, if necessary.

**Packaging and storage**—Preserve in tight containers.

**Alcohol content, Method II** (611): between 80.0% and 87.0% of  $C_2H_5OH$ , the dilution to approximately 2% alcohol being made with methanol instead of with water.

**Assay**—Transfer 2.0 mL of Camphor Spirit to a suitable pressure bottle containing 50 mL of freshly prepared dinitrophenylhydrazine TS. Close the pressure bottle, immerse it in a water bath, and maintain at about 75° for 16 hours. Cool to room temperature, and transfer the contents to a beaker with the aid of 100 mL of 3 N sulfuric acid. Allow to stand at room temperature for not less than 12 hours, transfer the precipitate to a tared filter crucible, and wash with 100 mL of 3 N sulfuric acid followed by 75 mL of cold water in divided portions. Continue the suction until the excess water is removed, dry the crucible and precipitate at 80° for 2 hours, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of  $C_{10}H_{16}O$  in the specimen taken.

## Candesartan Cilexetil



$C_{33}H_{34}N_6O_6$

1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[[[(cyclohexyloxy)carbonyl]oxy]ethyl ester, (±);

(±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). 610.66  
[145040-37-5].

### DEFINITION

Candesartan Cilexetil contains NLT 98.7% and NMT 101.0% of  $C_{33}H_{34}N_6O_6$ , calculated on anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K): If the spectra obtained show differences, proceed with the samples prepared as follows. Separately dissolve a quantity of USP Candesartan Cilexetil RS and Candesartan Cilexetil in alcohol. [NOTE—Heating the solution may be necessary for complete dissolution.] Cool the solution in an ice bath, filter the crystals, and dry at 105°.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the procedure for *Organic Impurities*.

**ASSAY**• **PROCEDURE**

**Sample solution:** 8.33 mg/mL of Candesartan Cilexetil in glacial acetic acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Potentiometric

**Titrant:** 0.1 N perchloric acid

**Analysis:** Titrate with 8 mL of 0.1 N perchloric acid VS using a blank determination under the same conditions. Each mL of the *Titrant* is equivalent to 61.07 mg of C<sub>33</sub>H<sub>34</sub>N<sub>6</sub>O<sub>6</sub>.

**Acceptance criteria:** 98.7%–101.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%, determined from a 1-g sample

**Organic Impurities**• **PROCEDURE**

**Diluent:** Acetonitrile and water (3:2)

**Solution A:** Acetonitrile, glacial acetic acid, and water (57:1:43)

**Solution B:** Acetonitrile, glacial acetic acid, and water (90:1:10)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	0	100

[NOTE—Equilibration for about 10 min may be necessary between injections.]

**System suitability solution:** 0.04 mg/mL of USP Candesartan Cilexetil RS and 0.125 mg/mL of acenaphthene in *Diluent*

**Standard solution:** 4 µg/mL of USP Candesartan Cilexetil RS in *Diluent*

**Sample solution:** 0.4 mg/mL of Candesartan Cilexetil in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 0.8 mL/min

**Injection size:** 10 µL

**System suitability**

[NOTE—The *Mobile phase* used for testing system suitability is 100% *Solution A* in an isocratic mode.]

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 5.0 between candesartan cilexetil and acenaphthene

**Tailing factor:** NMT 1.5 for candesartan cilexetil

**Relative standard deviation:** NMT 3.0% for the candesartan cilexetil peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Candesartan Cilexetil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of candesartan cilexetil from the *Standard solution*

$C_S$  = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Candesartan Cilexetil in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See *Table 1*.

**Total impurities:** NMT 0.6%. [NOTE— Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethyl candesartan <sup>a</sup>	0.4	0.2
Desethyl candesartan cilexetil <sup>b</sup>	0.5	0.3
Candesartan cilexetil	1.0	—
N <sup>2</sup> -Ethyl candesartan cilexetil <sup>c</sup>	2.0	0.2
Any other unknown impurity	—	0.10

<sup>a</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>b</sup> ±1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxobenzimidazole-7-carboxylate.

<sup>c</sup> ±1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(*N*-ethyl-tetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method 1** (921): NMT 0.3%

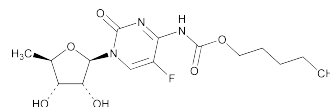
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Candesartan Cilexetil RS

1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[[cyclohexyloxy]carbonyloxy]ethyl ester, (±); (±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester).  
610.66

**Capecitabine**

C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub>

359.35

Carbamic acid, [1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]-, pentyl ester;  
Pentyl 1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate [154361-50-9].

**DEFINITION**

Capecitabine contains NLT 98.0% and NMT 102.0% of  $C_{15}H_{22}FN_3O_6$ , calculated on the anhydrous and solvent-free basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)

**Sample:** 2 mg of sample in 300 mg of potassium bromide

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol, acetonitrile, and water (7:1:12)

**Solution A:** 0.1% mixture of glacial acetic acid in water

**Solution B:** Methanol, acetonitrile, and *Solution A* (7:1:12)

**Solution C:** Methanol, acetonitrile, and *Solution A* (16:1:3)

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

[NOTE—The following solutions may be sonicated if necessary.]

**System suitability solution:** 0.6 µg/mL each of USP Capecitabine RS, USP Capecitabine Related Compound A RS, USP Capecitabine Related Compound B RS, and USP Capecitabine Related Compound C RS in *Diluent*  
**Standard solution:** 0.6 mg/mL of USP Capecitabine RS in *Diluent*

**Sample solution:** 0.6 mg/mL of Capecitabine in *Diluent*  
**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Autosampler temperature:** 5°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impu-*

*Table 1*. The relative retention times are measured with respect to capecitabine.]

**Suitability requirements**

**Resolution:** NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{15}H_{22}FN_3O_6$  in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Capecitabine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm

**Organic Impurities**• **PROCEDURE**

**Diluent, Solution B, Solution C, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for capecitabine from the *Standard solution*

$C_S$  = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Capecitabine in the *Sample solution* (mg/mL)

$F$  = relative response factor for an impurity, from *Impurity Table 1*

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.5%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	0.3
Capecitabine related compound B	0.19	0.81	0.3
2',3'-Di-O-acetyl-5'-deoxy-5-fluorocytidine	0.36	0.89	0.1
5'-Deoxy-5-fluoro-N4-(2-methyl-1-butyloxy-carbonyl)cytidine + 5'-Deoxy-5-fluoro-N4-(3-methyl-1-butyloxy-carbonyl)cytidine	0.95	1.01	0.5
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.06	1.00	0.3



Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
[1-[5-Deoxy-2-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.09	1.00	0.2
Capecitabine related compound C	1.11	0.91	0.3
[1-[5-Deoxy-3-O-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.20	1.00	0.3
2',3'-Di-O-acetyl-5'-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine	1.37	0.85	0.1
Individual unspecified impurity	—	1.00	0.1

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S): +96.0° to +100.0°  
**Sample solution:** 10 mg/mL, on the anhydrous and solvent-free basis, in methanol, at 20°
- **WATER DETERMINATION**, *Method 1c* (921): NMT 0.3%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Capecitabine RS
  - USP Capecitabine Related Compound A RS  
 5'-Deoxy-5-fluorocytidine.  
 $C_9H_{12}FN_3O_4$  245.21
  - USP Capecitabine Related Compound B RS  
 5'-Deoxy-5-fluorouridine.  
 $C_9H_{11}FN_2O_5$  246.19
  - USP Capecitabine Related Compound C RS  
 2',3'-O-Carbonyl-5'-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine.  
 $C_{16}H_{20}FN_3O_7$  385.34

Time (min)	Solution B (%)	Solution C (%)
20	49	51
30	49	51
31	100	0
40	100	0

[NOTE—The following solutions may be sonicated as necessary.]

**System suitability solution:** Includes 0.6 μg/mL of USP Capecitabine RS, 0.6 μg/mL of USP Capecitabine Related Compound A RS, 0.6 μg/mL of USP Capecitabine Related Compound B RS, and 0.6 μg/mL of USP Capecitabine Related Compound C RS in *Diluent*

**Standard solution:** 0.6 mg/mL of USP Capecitabine RS in *Diluent*

**Sample solution:** Equivalent to 0.6 mg/mL of capecitabine, from powdered Tablets (NLT 20), in *Diluent*. [NOTE—Pass through a PVDF membrane filter of 0.45-μm pore size, and use the filtrate.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 40°

**Autosampler temperature:** 5°

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1*. The relative retention times are measured with respect to capecitabine.]

**Suitability requirements**

**Resolution:** NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{15}H_{22}FN_3O_6$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of capecitabine in the *Sample solution* (mg/mL)

## Capecitabine Tablets

**DEFINITION**

Capecitabine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of capecitabine ( $C_{15}H_{22}FN_3O_6$ ).

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)

**Analytical wave number:** 1500–1760  $\text{cm}^{-1}$

**Sample:** Grind 1 Tablet to a fine powder with a mortar and pestle. Mix 1 mg of this sample with 300 mg of potassium bromide.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol, acetonitrile, and water (7:1:12)

**Solution A:** 0.1% mixture of glacial acetic acid in water

**Solution B:** Methanol, acetonitrile, and *Solution A* (7:1:12)

**Solution C:** Methanol, acetonitrile, and *Solution A* (16:1:3)

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0

Acceptance criteria: 93.0%–105.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 900 mL, degassed

Apparatus 2: 50 rpm

Time: 30 min

#### Standard solutions

For Tablets labeled to contain 150 mg: 17 mg of USP Capecitabine RS in 100 mL of Medium

For Tablets labeled to contain 500 mg: 28 mg of USP Capecitabine RS in 50 mL of Medium

Sample solution: Pass a portion of the solution under test through a fiberglass filter of 0.45-μm pore size.

Analysis: Determine the amount of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> dissolved by selecting a wavelength with appropriate sensitivity between 300 and 330 nm on portions of the Sample solution, suitably diluted with Medium, if necessary, in comparison with the appropriate Standard solution, using a 1-mm quartz cell. Calculate the percentage of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> dissolved in each Tablet:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

A<sub>U</sub> = absorbance of the Sample solution

A<sub>S</sub> = absorbance of the Standard solution

C<sub>S</sub> = concentration of capecitabine in the Standard solution (mg/mL)

V = volume of medium, 900 mL

L = Tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

Diluent, Solution A, Solution B, Solution C, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

## Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r<sub>U</sub> = peak response for each impurity from the Sample solution

r<sub>S</sub> = peak response for capecitabine from the Standard solution

C<sub>S</sub> = concentration of USP Capecitabine RS in the Standard solution (mg/mL)

C<sub>U</sub> = nominal concentration of capecitabine in the Sample solution (mg/mL)

F = relative response factor for each impurity, from Impurity Table 1

## Acceptance criteria

Individual impurities: See Impurity Table 1.

Total degradation products: NMT 2.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Capecitabine RS

USP Capecitabine Related Compound A RS

5'-Deoxy-5-fluorocytidine.

C<sub>9</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>4</sub> 245.21

USP Capecitabine Related Compound B RS

5'-Deoxy-5-fluorouridine.

C<sub>9</sub>H<sub>11</sub>FN<sub>2</sub>O<sub>5</sub> 246.19

USP Capecitabine Related Compound C RS

2',3'-O-Carbonyl-5'-deoxy-5-fluoro-N<sup>4</sup>-(pentyloxycarbonyl)cytidine.

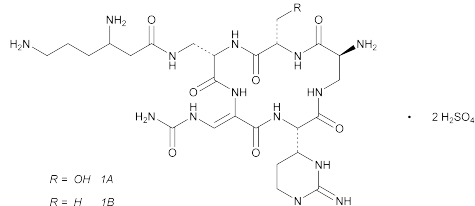
C<sub>16</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>7</sub> 385.34

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	1.0
Capecitabine related compound B	0.19	0.81	1.0
2',3'-Di-O-acetyl-5'-deoxy-5-fluorocytidine*	0.36	0.89	—
5'-Deoxy-5-fluoro-N <sup>4</sup> -(2-methyl-1-butyloxycarbonyl)cytidine + 5'-Deoxy-5-fluoro-N <sup>4</sup> -(3-methyl-1-butyloxycarbonyl)cytidine*	0.95	1.01	—
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.06	1.00	—
[1-[5-Deoxy-2-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.09	1.00	—
Capecitabine related compound C	1.11	0.91	0.5
[1-[5-Deoxy-3-O-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.20	1.00	—
2',3'-Di-O-acetyl-5'-deoxy-5-fluoro-N <sup>4</sup> -(pentyloxycarbonyl)cytidine*	1.37	0.85	—
Individual unspecified degradation product	—	1.00	0.1

The impurities marked with an "\*" are process impurities and are not included in the total degradation products.

## Capreomycin Sulfate



Capreomycin sulfate [1405-37-4].

$\text{C}_{25}\text{H}_{44}\text{N}_{14}\text{O}_8$  668.71

Capreomycin 1A (free base);

3,6-Diamino-*N*-[[(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)-iminohexahydropyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl]methyl]hexanamide [37290-35-6].

$\text{C}_{25}\text{H}_{44}\text{N}_{14}\text{O}_7$  652.71

Capreomycin 1B (free base);

3,6-Diamino-*N*-[[(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-11-[(*R*)-iminohexahydropyrimidin-4-yl]-2-methyl-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl]methyl]hexanamide [33490-33-4].

### DEFINITION

Capreomycin Sulfate is the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*, suitable for parenteral use. It has a potency equivalent to NLT 700 µg/mg and NMT 1050 µg/mg of capreomycin.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.

### ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* <81>.
- Acceptance criteria: 700–1050 µg/mg

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** <281>: NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid
- **HEAVY METALS, Method II** <231>: NMT 30 ppm

### SPECIFIC TESTS

#### CAPREOMYCIN 1 CONTENT

**Solution A:** 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5-µm or less pore size.

**Mobile phase:** Methanol and *Solution A* (2:3)

**System suitability solution:** 0.25 mg/mL of USP Capreomycin Sulfate RS in water

**Sample solution:** 0.25 mg/mL of Capreomycin Sulfate in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 268 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L10

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 1.5 between capreomycin 1A and capreomycin 1B

**Tailing factor:** NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)

### Analysis

[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]

**Sample:** *Sample solution*

Calculate the percentage of capreomycin 1 in the portion of Capreomycin Sulfate taken:

$$\text{Result} = [(r_{1A} + r_{1B})/r_T] \times 100$$

$r_{1A}$  = peak area response of capreomycin 1A

$r_{1B}$  = peak area response of capreomycin 1B

$r_T$  = total response for all peaks

**Acceptance criteria:** NLT 90.0%

#### • pH <791>: 4.5–7.5

**Sample solution:** 30 mg/mL

- **LOSS ON DRYING** <731>: Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h; it loses NMT 10.0% of its weight.

- **BACTERIAL ENDOTOXINS TEST** <85>: Where it is intended for use in preparing injectable dosage forms: NMT 0.35 USP Endotoxin Unit/mg of capreomycin

- **OTHER REQUIREMENTS:** Where the label states that Capreomycin Sulfate is sterile, it meets the requirements under *Injections* <1>.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** <11>
  - USP Capreomycin Sulfate RS
  - USP Endotoxin RS

## Capreomycin for Injection

### DEFINITION

Capreomycin for Injection contains an amount of Capreomycin Sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of capreomycin.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.

### ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* <81>.

Acceptance criteria: 90.0%–115.0%

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** <281>: NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid
- **HEAVY METALS, Method II** <231>: NMT 30 ppm

### SPECIFIC TESTS

#### • CAPREOMYCIN 1 CONTENT

**Solution A:** 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5-µm or less pore size.

**Mobile phase:** Methanol and *Solution A* (2:3)

**System suitability solution:** 0.25 mg/mL of USP Capreomycin Sulfate RS in water

**Sample solution:** 0.25 mg/mL of Capreomycin for Injection in water

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 268 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L10

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between capreomycin 1A and capreomycin 1B

**Tailing factor:** NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)

**Analysis**

[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]

**Sample:** *Sample solution*

Calculate the percentage of capreomycin 1 in the portion of Capreomycin for Injection taken:

$$\text{Result} = (r_{1A} + r_{1B})/r_T \times 100$$

$r_{1A}$  = peak area response for capreomycin 1A

$r_{1B}$  = peak area response for capreomycin 1B

$r_T$  = total response for all peaks

**Acceptance criteria:** The capreomycin 1 content is NLT 90.0%.

• **PH <791>:** 4.5–7.5

**Sample solution:** 30 mg/mL

• **Loss on Drying <731>:** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h: it loses NMT 10.0% of its weight.

• **BACTERIAL ENDOTOXINS TEST <85>:** NMT 0.35 USP Endotoxin Unit/mg of capreomycin

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* <1>, *Constituted Solutions*.

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* <1>.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

• **USP REFERENCE STANDARDS <11>**

USP Capreomycin Sulfate RS

USP Endotoxin RS

noids is not more than 15 percent, all calculated on the dried basis.

**Caution—Handle Capsaicin with care. Prevent inhalation of particles of it and prevent its contact with any part of the body.**

**Packaging and storage—**Preserve in tight containers, protected from light, and store in a cool place.

**Labeling—**Label it to state the percentage content of total capsaicinoids.

**USP Reference standards <11>—**

USP Capsaicin RS

USP Dihydrocapsaicin RS

**Identification—**Prepare a test solution of Capsaicin in methanol containing 1 mg per mL. Prepare a Standard solution of USP Capsaicin RS in methanol containing 1 mg per mL. Separately apply 10-μL portions of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatograms in a solvent system consisting of a mixture of ether and methanol (19:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to air-dry. Spray the plate with a 0.5% solution of 2,6-dibromoquinone-chlorimide in methanol, allow to stand in a chamber containing ammonia fumes, and examine the chromatograms: the blue color and the  $R_f$  value of the principal spot obtained from the test solution correspond to those properties of the principal spot obtained from the Standard solution.

**Melting range <741>:** between 57° and 66°, but the range between beginning and end of melting does not exceed 5°.

**Loss on drying <731>:** Dry it in vacuum over phosphorus pentoxide at 40° for 5 hours: it loses not more than 1.0% of its weight.

**Residue on ignition <281>:** not more than 1.0%.

**Content of capsaicin, dihydrocapsaicin, and other capsaicinoids—**

**Mobile phase—**Prepare a mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (600:400). Pass through a filter having a porosity of 0.5 μm or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard capsaicin solution—**Quantitatively dissolve an accurately weighed quantity of USP Capsaicin RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL.

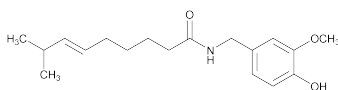
**Standard dihydrocapsaicin solution—**Quantitatively dissolve an accurately weighed quantity of USP Dihydrocapsaicin RS in methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

**Test solution—**Transfer about 25 mg of Capsaicin, accurately weighed, to a 250-mL volumetric flask, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 281-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L11 and is maintained at a constant temperature of about 30°. Adjust the flow rate to obtain a retention time of about 20 minutes for the main capsaicin peak. Chromatograph the *Standard capsaicin solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2%.

**Procedure—**Separately inject equal volumes (about 20 μL) of the *Standard capsaicin solution*, the *Standard dihydrocapsaicin solution*, and the *Test solution* into the chromatograph, record the chromatogram for a period of time that is twice that of the retention time of capsaicin, and measure the areas of the responses for all of the peaks. Calculate the

## Capsaicin



$C_{18}H_{27}NO_3$  305.41

6-Nonenamide, (*E*)-*N*-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methyl.

(*E*)-8-Methyl-*N*-vanillyl-6-nonenamide [404-86-4].

» Capsaicin contains not less than 90.0 percent and not more than 110.0 percent of the labeled percentage of total capsaicinoids. The content of capsaicin ( $C_{18}H_{27}NO_3$ ) is not less than 55 percent, and the sum of the contents of capsaicin and dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ) is not less than 75 percent, and the content of other capsaici-

percentage of capsaicin ( $C_{18}H_{27}NO_3$ ) in the portion of Capsaicin taken by the formula:

$$25,000(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the capsaicin peak responses obtained from the *Test solution* and the *Standard capsaicin solution*, respectively. Not less than 55% is found. Calculate the percentage of dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ) in the portion of Capsaicin taken by the formula:

$$25,000(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dihydrocapsaicin RS in the *Standard dihydrocapsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the dihydrocapsaicin peak responses obtained from the *Test solution* and the *Standard dihydrocapsaicin solution*, respectively. The sum of the percentage of capsaicin found and of the percentage of dihydrocapsaicin found is not less than 75%. Using the chromatograms obtained from the *Standard capsaicin solution* and the *Test solution*, calculate the percentage of other capsaicinoids in the portion of Capsaicin taken by the formula:

$$25,000(C / W)(r_T / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*;  $r_T$  is the sum of the peak responses of the capsaicinoids other than capsaicin and dihydrocapsaicin in the chromatogram obtained from the *Test solution*; and  $r_S$  is the capsaicin peak response obtained from the *Standard capsaicin solution*. Not more than 15% of other capsaicinoids is found.

## Capsicum

### DEFINITION

Capsicum is the dried ripe fruit of *Capsicum frutescens* L., known in commerce as African chillies or as Tabasco pepper; or of *Capsicum annuum* L. var. *conoides* Irish; or of *Capsicum annuum* var. *longum* Sendt, known in commerce as Louisiana Long Pepper, or of a hybrid between the Honka variety of Japanese Capsicum and the Old Louisiana Sport Capsicum known in commerce as Louisiana Sport Pepper (Fam. Solanaceae).

### SPECIFIC TESTS

#### • BOTANIC CHARACTERISTICS

##### Macroscopic

**Unground Capsicum:** Occurs as oblong-conical fruits, often curved (Louisiana Long Pepper), usually laterally compressed, from 10 to 25 mm in length and from 4 to 8 mm in diameter (African Chillies), or up to 15 cm in length and 2.5 cm in diameter (Louisiana Long Pepper), or up to 5.5 cm in length and up to 13 mm in diameter (Louisiana Sport Pepper), or up to 4 cm in length and up to 9 mm in diameter (Tabasco Pepper). The fruit is two to three locular, the dissepiments being united at the base to a conical, central placenta. The pericarp is thin and membranous, its outer surface dark reddish brown to dusky yellowish orange, glabrous, shriveled, its inner surface striate with two to three distinct longitudinal ridges representing the parietal placentae; the seeds are light brown to weak yellowish orange, suborbicular or irregular, flattened, from 2 to 4 mm in diameter, with a thickened edge

and a prominent, pointed micropyle. The calyx is gamosepalous, inferior, five-toothed, and sometimes attached to a long, straight peduncle. Capsicum has a characteristic odor, and is sternutatory.

**Powdered Capsicum:** A dark orange or dark reddish orange to strong yellowish brown powder

##### Microscopic

**Unground Capsicum:** The epicarp of Capsicum consists of mostly quadrangular or rectangular cells up to 80  $\mu$ m in length and up to 20  $\mu$ m deep, arranged in regular rows, with thickened and cutinized outer and radial walls, the surface of the cuticle finely striated, the radial walls somewhat wavy (African Chillies), or of polygonal, quadrangular, triangular, or irregular cells up to 76  $\mu$ m in length and up to 30.5  $\mu$ m deep (Tabasco Pepper), or up to 125  $\mu$ m in length and up to 38  $\mu$ m deep (Louisiana Long Pepper), or up to 76  $\mu$ m in length and up to 38  $\mu$ m deep (Louisiana Sport Pepper), with cuticularized outer and radial walls, the latter usually prominently beaded. The mesocarp consists of thin-walled parenchyma (African Chillies), or an outer hypodermis of tangentially elongated collenchymatous cells (Louisiana Long Pepper and Tabasco Pepper), or of from one to three rows of hypodermal cells with cuticularized walls (Louisiana Sport Pepper), a broad middle zone of thin-walled parenchyma containing yellow to red chromoplasts, oil droplets, and elaioplasts, occasionally microcrystals, and traversed by vascular bundles, and an inner zone consisting of a layer of giant cells. The endocarp consists of a layer of elongated cells, some of them very thin-walled and containing chromoplasts and others in large oval areas with thickened, beaded, lignified walls. Epidermal cells of the seed are irregular in outline and up to 342  $\mu$ m in length, and have very sinuous, contorted, lignified walls, the cells from the edge of the seed being much thicker walled than those from the flat surface of the seed. The embryo is curved and embedded in the endosperm, the latter consisting of small-celled parenchyma containing fixed oil droplets and aleurone grains.

**Powdered Capsicum:** It shows numerous fragments of thin-walled parenchyma containing oil globules and orange, red, or yellow chromoplasts; and fragments of epicarp with either striated, rectangular cells arranged in parallel series (African Chillies), or with polygonal, triangular, or irregular cells, with or without beaded walls. The endocarp contains stone cells with slightly wavy, lignified walls and broad lumina. Numerous fragments of spermoderm composed of stone cells are present, showing in surface view, deeply sinuate, greatly thickened and lignified vertical walls containing numerous pore canals. Fragments of small-celled parenchyma of the endosperm containing fixed oil and aleurone grains, the latter up to 5.5  $\mu$ m in diameter, are also present, as well as occasional fibrovascular elements and calyx tissues.

- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter** (561): NMT 1%, other than stems and calyces, the proportion of which does not exceed 3%

#### • NONVOLATILE ETHER-SOLUBLE EXTRACTIVE

**Analysis:** Dry a sample of Capsicum, taken as directed under *Articles of Botanical Origin* (561), *Sampling*, over phosphorus pentoxide for NLT 12 h. Extract 2.0 g of dried Capsicum with anhydrous ether for 20 h in a continuous extraction apparatus. Transfer the ether solution to a tared porcelain dish, and allow it to evaporate spontaneously. Dry the residue, still in the tared dish, over phosphorus pentoxide for 18 h, and weigh to obtain the weight of the total ether extractive. Then heat the dish gradually up to 105°, until a constant weight is obtained.

**Acceptance criteria:** NLT 12% of nonvolatile ether-soluble extractive

- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash (561):** NMT 1.25%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. A few drops of chloroform may be added from time to time to prevent attack by insects.
- **LABELING:** Label each container to indicate which variety of Capsicum is contained therein.

### Capsicum Oleoresin

#### DEFINITION

Capsicum Oleoresin is an alcoholic extract of the dried ripe fruits of *Capsicum annum* var. *minimum* and small fruited varieties of *C. frutescens* (Fam. Solanaceae). It contains NLT 8.0% of total capsaicins [capsaicin ( $C_{18}H_{27}NO_3$ ), dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ), and nordihydrocapsaicin ( $C_{17}H_{27}NO_3$ )].

[**CAUTION**—Capsicum Oleoresin is a powerful irritant, and even in minute quantities produces an intense burning sensation when it comes in contact with the eyes and tender parts of the skin. Care should be taken to protect the eyes and to prevent contact of the skin with Capsicum Oleoresin.]

#### IDENTIFICATION

- **A.**

**Sample:** 0.5 g of Capsicum Oleoresin

**Analysis:** To the *Sample*, add 5 mL of water and 10 mL of a mixture of water, 0.2 M potassium chloride, and 0.2 N hydrochloric acid (135:50:13). Mix. Add 5.0 mL of 0.5 M sodium nitrite and 5.0 mL of 0.02 M sodium tungstate, and mix. Heat the *Sample* at 55°–60° for 15 min, allow to cool, and filter. To the filtrate add 10 mL of 1 N sodium hydroxide.

**Acceptance criteria:** A bright yellow color is produced (presence of capsaicin).

#### ASSAY

- **PROCEDURE**

**Mobile phase:** Methanol and 2% acetic acid (56:44). Pass through a filter of 0.5- $\mu$ m or finer pore size.

**Standard solution:** 0.5 mg/mL of USP Capsaicin RS in methanol. Pass a portion of this solution through a filter of 0.2- $\mu$ m pore size, and use the filtrate as the *Standard solution*.

**Sample solution:** 10 mg/mL of Capsicum Oleoresin in methanol. Pass a portion of this solution through a filter of 0.2- $\mu$ m pore size, and use the filtrate as the *Sample solution*.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *Sample solution*  
[NOTE—The relative retention times for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin are about 0.9, 1.0, and 1.6, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.2 between nordihydrocapsaicin and capsaicin, *Sample solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0% in repeated injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of total capsaicins in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak areas for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Capsaicin RS in the *Standard solution* (mg/mL)

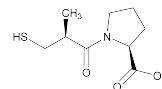
$C_U$  = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NLT 8.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that if separation occurs, it should be warmed and mixed before use.
- **USP REFERENCE STANDARDS (11)**  
USP Capsaicin RS

### Captopril



$C_9H_{15}NO_3S$  217.29  
L-Proline, 1-[(2S)-3-mercapto-2-methyl-1-oxopropyl]-.  
1-[(2S)-3-Mercapto-2-methylpropionyl]-L-proline  
[62571-86-2].

» Captopril contains not less than 97.5 percent and not more than 102.0 percent of  $C_9H_{15}NO_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Captopril RS

USP Captopril Disulfide RS

**Identification, Infrared Absorption (197K).**

**Specific rotation (781S):** between  $-125^\circ$  and  $-134^\circ$ .

*Test solution:* 10 mg per mL, in dehydrated alcohol.

**Loss on drying (731)**—Dry it in vacuum at 60° for 3 hours; it loses not more than 1.0% of its weight.

**Residue on ignition (281):** not more than 0.2%.

**Heavy metals, Method II (231):** 0.003%.

**Related compounds**—

**Mobile phase**—Prepare a filtered and degassed mixture of a 9 in 100 solution of tetrahydrofuran in methanol and a 1 in 2000 solution of phosphoric acid in water (33:67). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Dissolve USP Captopril RS, USP Captopril Disulfide RS, and 3-acetylthio-2-methylpropanoic acid in methanol to obtain a stock solution containing about 0.1 mg of each per mL. Quantitatively dilute a portion of this stock solution with methanol to obtain a solution containing about 10  $\mu$ g of each Reference Standard per mL.

**Standard solution**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Captopril Disulfide RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of 10  $\mu$ g per mL.

**Test solution**—[NOTE—Use low-actinic glassware.] Transfer 50 mg of Captopril, accurately weighed, to a 25-mL volumetric flask. Dissolve the specimen in methanol, dilute with methanol to volume, mix, and use the solution promptly.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution* (about 20 µL), and record the peak responses as directed for *Procedure*: the relative retention times are about 0.32 for captopril, 0.42 for 3-acetylthio-2-methylpropanoic acid, and 1.0 for captopril disulfide; and the resolution, *R*, between captopril and 3-acetylthio-2-methylpropanoic acid is not less than 3.0.

**Procedure**—[NOTE—Use the peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of captopril disulfide in the portion of Captopril taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in µg per mL, of USP Captopril Disulfide RS in the *Standard solution*;  $C_U$  is the concentration, in µg per mL, of Captopril in the *Test solution*; and  $r_U$  and  $r_S$  are the captopril disulfide peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% of the captopril disulfide is found. Compare the peak responses, excluding those of the solvent, captopril, and captopril disulfide, in the chromatogram of the *Test solution* with the main peak response in the chromatogram of the *Standard solution*: the peak response of each impurity does not exceed 40% of the main peak response in the chromatogram of the *Standard solution* (0.2%), and the sum of the impurity peak responses does not exceed the main peak response in the chromatogram of the *Standard solution* (0.5%).

#### Assay—

**0.1 N Potassium iodate titrant**—Dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in water to make 1000.0 mL.

**Procedure**—Dissolve about 300 mg of Captopril, accurately weighed, in 100 mL of water in a suitable glass-stoppered flask, add 10 mL of 3.6 N sulfuric acid, 1 g of potassium iodide, and 2 mL of starch TS. Titrate with 0.1 N Potassium iodate titrant to a faint blue endpoint that persists for not less than 30 seconds. Perform a blank determination (see *Titrimetry* <541>), and make any necessary correction. Each mL of 0.1 N Potassium iodate titrant is equivalent to 21.73 mg of  $C_9H_{15}NO_3S$ .

### Captopril Oral Solution

» Captopril Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ). Prepare Captopril Oral Solution 0.75 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>). See also *Captopril Oral Suspension*:

Captopril powder . . . . .	75 mg
Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , a sufficient quantity to make . . . . .	100 mL

Add Captopril powder and about 10 mL of Vehicle to a mortar, and mix. Add the Vehicle in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough Vehicle to bring to final volume, and mix well.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store in a cold place.

**Labeling**—Label it to state the beyond-use date.

**USP Reference standards** <11>—

USP Captopril RS

**pH** <791>: between 3.8 and 4.3.

**Beyond-use date:** 7 days after the day on which it was compounded.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (11:9) containing 0.5 mL of phosphoric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve USP Captopril RS in water to obtain a solution having a known concentration of 7.5 µg per mL.

**Assay preparation**—Agitate the container of Oral Solution for 30 minutes on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 seconds. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm analytical column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the retention time is about 5.0 minutes; and the relative standard deviation for replicate injections is not more than 0.9%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of captopril ( $C_9H_{15}NO_3S$ ) in the volume of Oral Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Captopril RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Captopril Oral Suspension

» Captopril Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of captopril

(C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S). Prepare Captopril Oral Suspension 0.75 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795). See also *Captopril Oral Solution*):

Captopril .....	75 mg
Vehicle: a mixture of Vehicle for Oral Solution, (regular or sugar-free), <i>NF</i> and Vehicle for Oral Suspension, <i>NF</i> (1:1), a sufficient quantity to make .....	100 mL

If using Tablets place the required number of Captopril Tablets in a suitable mortar and comminute to a fine powder, or add Captopril powder to the mortar. Add about 10 mL of the Vehicle, and mix to a uniform paste. Add the Vehicle in small portions, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient Vehicle to bring to final volume, and mix well.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store in a cold place.

**Labeling**—Label it to state that it is to be well shaken, and to state the beyond-use date.

**USP Reference standards** (11)—

USP Captopril RS

**pH** (791): between 3.8 and 4.3.

**Beyond-use date:** 7 days after the day on which it was compounded.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (11:9) containing 0.5 mL of phosphoric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve USP Captopril RS in water to obtain a solution having a known concentration of 7.5 µg per mL.

**Assay preparation**—Agitate the container of Oral Suspension for 30 minutes on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 seconds. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm analytical column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the retention time is about 5.0 minutes, and the relative standard deviation for replicate injections is not more than 0.9%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S) in the volume of Oral Suspension taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Captopril RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Captopril Tablets

» Captopril Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Captopril RS

USP Captopril Disulfide RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Transfer a portion of powdered Tablets, equivalent to about 100 mg of captopril, to a conical flask. Add 25 mL of methanol, stir for 30 minutes using a magnetic stirrer, and centrifuge. Use the clear supernatant.

**Standard solution:** 4 mg per mL, in methanol.

**Application volume:** 50 µL, as streaks.

**Developing solvent system:** a mixture of toluene, glacial acetic acid, and methanol (75:25:1).

**Procedure**—Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with a freshly prepared mixture of 1 volume of ammonium hydroxide and 6 volumes of a solution of 0.04% 5,5'-dithiobis(2-nitrobenzoic acid) in methanol.

**Dissolution** (711)—[NOTE—Completely deaerate the *Dissolution Medium* to minimize exposure of captopril to air, and analyze the samples immediately.]

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 1:** 50 rpm.

**Time:** 20 minutes.

**Procedure**—Determine the amount of C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S dissolved by employing UV absorption at the wavelength of maximum absorbance at about 205 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Captopril RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S is dissolved in 20 minutes.

**Uniformity of dosage units** (905): meets the requirements.

**Limit of captopril disulfide**—[NOTE—Protect solutions from exposure to air. Use within 8 hours of preparation.]

**Mobile phase**—Proceed as directed in the *Assay*.

**System suitability solution**—Dissolve suitable quantities of USP Captopril RS and USP Captopril Disulfide RS in *Mobile phase* to obtain a solution having known concentrations of about 1 mg per mL and 0.05 mg per mL, respectively.

**Standard solution**—Dissolve an accurately weighed quantity of USP Captopril Disulfide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the



powder, equivalent to about 25 mg of captopril, to a suitable centrifuge tube. Add 25.0 mL of *Mobile phase*, sonicate for 15 minutes, and centrifuge. Use the clear supernatant as the *Test solution*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1 with about 15% hydrocarbon load. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for captopril and 1.0 for captopril disulfide; the resolution, *R*, between captopril and captopril disulfide in the *System suitability solution* is not less than 2.0; and the relative standard deviation for replicate injections of the *Standard solution* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of captopril disulfide in the portion of Tablets taken by the formula:

$$2500C / W(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Captopril Disulfide RS in the *Standard solution*; *W* is the quantity, in mg, of captopril in the portion of Tablets taken to prepare the *Test solution*, based on the labeled amount per Tablet; and *r<sub>U</sub>* and *r<sub>S</sub>* are the captopril disulfide peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

**Assay**—[NOTE—Protect solutions from exposure to air. Use within 8 hours of preparation.]

**Mobile phase**—Prepare a filtered and degassed mixture of 550 mL of methanol and 450 mL of water containing 0.50 mL of phosphoric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve suitable quantities of USP Captopril RS and USP Captopril Disulfide RS in *Mobile phase* to obtain a solution having known concentrations of about 1 mg per mL and 0.05 mg per mL, respectively.

**Assay preparation**—Transfer not fewer than 20 Tablets to a suitable volumetric flask, add *Mobile phase* to fill the flask to about half of its capacity, and sonicate for 15 minutes. Dilute with *Mobile phase* to volume, shake by mechanical means for 15 minutes, and filter. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 1 mg of captopril per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1 with about 15% hydrocarbon load. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for captopril and 1.0 for captopril disulfide; the resolution, *R*, between captopril and captopril disulfide is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S) in the portion of Tablets taken by the formula:

$$(L / D)C(r_U / r_S)$$

in which *L* is the labeled amount, in mg, of captopril in each Tablet; *D* is the concentration, in mg per mL, of captopril in the *Assay preparation* based on the labeled quantity per Tablet and the extent of dilution; *C* is the con-

centration, in mg per mL, of USP Captopril RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the captopril peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Captopril and Hydrochlorothiazide Tablets

» Captopril and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S) and hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Benzothiadiazine Related Compound A RS  
4-Amino-6-chloro-1,3-benzenedisulfonamide.  
C<sub>6</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 285.73

USP Captopril RS

USP Captopril Disulfide RS

USP Hydrochlorothiazide RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation* obtained as directed in the *Assay*.

**Dissolution** <711>—

*Medium*: 0.1 N hydrochloric acid; 900 mL.

*Apparatus 1*: 50 rpm.

*Times*: 20 minutes for captopril; 30 minutes for hydrochlorothiazide.

**Procedure**—Determine the amounts of C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S and C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub> dissolved, employing the procedure set forth in the *Assay*. Use filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, and compare with a *Standard solution* having known concentrations of USP Captopril RS and USP Hydrochlorothiazide RS in the same medium.

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S) is dissolved in 20 minutes, and not less than 60% (*Q*) of the labeled amount of hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Limit of captopril disulfide**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and phosphoric acid (550:450:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Dissolve an accurately weighed quantity of USP Captopril Disulfide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 15 µg per mL.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of captopril, to a 50-mL volumetric flask, add about 20 mL of *Mobile phase*, and sonicate for 15 minutes, with occasional shaking. Dilute with *Mobile phase* to volume, mix, and centrifuge. Use the clear supernatant as the *Test solution*.

**System suitability solution**—Prepare a solution in *Mobile phase* containing about 0.0075 mg per mL of USP Captopril RS and USP Hydrochlorothiazide RS, and 0.015 mg per mL of USP Captopril Disulfide RS.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector

and a column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.3 for captopril and 1.0 for captopril disulfide. The resolution,  $R$ , between the captopril and captopril disulfide peaks is not less than 4.0, and both peaks are resolved from the hydrochlorothiazide peak. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the captopril disulfide peaks. Calculate the percentage of captopril disulfide in the portion of Tablets taken by the formula:

$$(5C/W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Captopril Disulfide RS in the *Standard solution*;  $W$  is the quantity, in mg, of captopril in the portion of Tablets taken to prepare the *Test solution*, based on the labeled amount per Tablet; and  $r_U$  and  $r_S$  are the captopril disulfide peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

#### Limit of benzothiadiazine related compound A—

*Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

**Test solution**—Use the Assay preparation

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the benzothiadiazine related compound A peaks. Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken by the formula:

$$(5C/W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution*;  $W$  is the quantity, in mg, of hydrochlorothiazide in the portion of Tablets taken to prepare the *Test solution*, based on the labeled amount per Tablet; and  $r_U$  and  $r_S$  are the benzothiadiazine related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and phosphoric acid (750:250:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Captopril RS and USP Hydrochlorothiazide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.3 mg of USP Hydrochlorothiazide RS per mL and about 0.3/  $J$  mg of USP Captopril RS per mL,  $J$  being the ratio of the labeled amount, in mg, of captopril to the labeled amount, in mg, of hydrochlorothiazide per Tablet.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of hydrochlorothiazide, to a 50-mL volumetric flask, add *Mobile phase*, and sonicate for 15 minutes with occasional shaking. Dilute with *Mobile phase* to volume, mix, and centrifuge.

**System suitability solution**—Prepare a solution in *Mobile phase* containing about 0.3 mg each of USP Captopril RS, USP Hydrochlorothiazide RS, and USP Benzothiadiazine Related Compound A RS per mL.

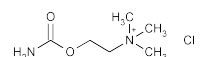
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for benzothiadiazine related compound A, 0.5 for hydrochlorothiazide, and 1.0 for captopril. The resolution,  $R$ , between the void volume and the benzothiadiazine related compound A peak is not less than 1.7, the resolution,  $R$ , between the benzothiadiazine related compound A and hydrochlorothiazide peaks is not less than 1.8, and the resolution,  $R$ , between the captopril and hydrochlorothiazide peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of captopril ( $C_9H_{15}NO_3S$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the corresponding analyte peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carbachol



$C_6H_{15}ClN_2O_2$  182.65  
Ethanaminium, 2-[(aminocarbonyl)oxy]-*N,N,N*-trimethyl-, chloride.  
Choline chloride, carbamate [51-83-2].

» Carbachol contains not less than 99.0 percent and not more than 101.0 percent of  $C_6H_{15}ClN_2O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carbachol RS

**Identification**—

A: *Infrared Absorption* (197K).

B: A solution (1 in 20) meets the requirements for the silver nitrate precipitate test for *Chloride* (191).

**Melting range** (741): between 200° and 204°, with some decomposition.

**Loss on drying** (731)—Dry about 1 g, accurately weighed, at 105° for 2 hours: it loses not more than 2.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Ordinary impurities** (466)—

**Test solution**: a mixture of methanol and water (4:1).

**Standard solution**: a mixture of methanol and water (4:1).

*Eluant:* alcohol.

*Visualization:* 16.

**Assay**—Dissolve about 400 mg of Carbachol, accurately weighed, in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.27 mg of  $C_6H_{15}ClN_2O_2$ .

## Carbachol Intraocular Solution

» Carbachol Intraocular Solution is a sterile solution of Carbachol in an aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_6H_{15}ClN_2O_2$ . It contains no preservatives or antimicrobial agents.

**Packaging and storage**—Preserve in tight containers, at controlled room temperature, and protect from freezing.

**Labeling**—Label it to indicate that it is for single-dose intraocular use only, and that the unused portion is to be discarded.

**USP Reference standards** (11)—  
USP Carbachol RS

**Identification**—To 5 mL of Intraocular Solution, add 4 or 5 drops of saturated (filtered) ammonium reineckate solution: a pink precipitate is formed that is soluble in acetone; the acetone solution is red.

**Sterility** (71): meets the requirements.

**pH** (791): between 5.0 and 7.5.

**Assay**—

*Hypochlorite reagent and Standard preparation*—Prepare as directed in the Assay under Carbachol Ophthalmic Solution.

*Assay preparation*—Dilute, if necessary, an accurately measured volume of Intraocular Solution quantitatively and stepwise with water to obtain a solution containing about 100  $\mu$ g of carbachol per mL.

*Procedure*—Proceed as directed for Procedure in the Assay under Carbachol Ophthalmic Solution.

## Carbachol Ophthalmic Solution

» Carbachol Ophthalmic Solution is a sterile solution of Carbachol in an isotonic, aqueous medium. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_6H_{15}ClN_2O_2$ . It may contain suitable preservatives and antimicrobial agents.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Carbachol RS

**Identification**—Dilute the test article to a concentration of about 1 mg of carbachol per mL. Add 5 mL of ammonium reineckate solution (1 in 30), and shake vigorously for 1 minute: a red precipitate soluble in acetone is formed.

**Sterility** (71): meets the requirements.

**pH** (791): between 5.0 and 7.0.

**Assay**—

*Hypochlorite reagent*—Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, allow to stand for 30 minutes, then mix equal volumes of the resulting solution and 1 N sodium hydroxide. Prepare fresh daily.

*Standard preparation*—Dissolve a suitable quantity of USP Carbachol RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 100  $\mu$ g per mL.

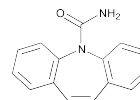
*Assay preparation*—Dilute an accurately measured volume of Ophthalmic Solution quantitatively and stepwise with water to obtain a solution containing about 100  $\mu$ g of carbachol per mL.

*Procedure*—Transfer 2.0-mL portions each of the Assay preparation and the Standard preparation, and of water to provide a blank, to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid, and mix. Treat each as follows. Add 4.0 mL of Hypochlorite reagent, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 minutes, accurately timed. Add 2.0 mL of phenol solution (1 in 200), rinsing the walls of the flask with the solution and with additional small portions of water. Mix, and allow to stand for 5 minutes. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to assure complete acidification of all contents, then mix. Add 1.0 mL of potassium iodide solution (3 in 1000), mix, and allow to stand for 5 minutes. Add 3.0 mL of starch TS, mix, transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume. Concomitantly determine the absorbances of the solutions from the Assay preparation and the Standard preparation in 1-cm cells at the wavelength of maximum absorbance at about 590 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of  $C_6H_{15}ClN_2O_2$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.001 CD(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Carbachol RS in the Standard preparation, D is the dilution factor used in the Assay preparation, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

## Carbamazepine



$C_{15}H_{12}N_2O$  236.27

5*H*-Dibenz[*b,f*]azepine-5-carboxamide.

5*H*-Dibenz[*b,f*]azepine-5-carboxamide [298-46-4].

» Carbamazepine contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{12}N_2O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carbamazepine RS

USP Carbamazepine Related Compound A RS  
10,11-Dihydrocarbamazepine.

USP Carbamazepine Related Compound B RS  
Iminostilbene.

**Identification, Infrared Absorption** (197M).

**X-ray diffraction** (941)—The X-ray diffraction pattern conforms to that of USP Carbamazepine RS, similarly determined.

**Acidity**—Add 2.0 g to 40.0 mL of water, mix for 15 minutes, and filter through paper. To a 10.0-mL aliquot of the solution so obtained add 1 drop of phenolphthalein TS, and titrate with 0.01 N sodium hydroxide VS from a 10-mL buret. Perform a blank determination, and make any necessary correction. Not more than 1.0 mL of 0.010 N sodium hydroxide is required for each 1.0 g of Carbamazepine.

**Alkalinity**—To a 10.0-mL aliquot of the solution prepared in the test for *Acidity* add 1 drop of methyl red TS, and titrate with 0.01 N hydrochloric acid VS from a 10-mL buret. Perform a blank determination, and make any necessary correction. Not more than 1.0 mL of 0.010 N hydrochloric acid is required for each 1.0 g of Carbamazepine.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%, a 2.0-g test specimen being used.

**Chloride** (221)—Boil 1.0 g with 20.0 mL of water for 10 minutes, cool, again adjust the volume, and filter: a 10.0-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

**Heavy metals, Method II** (231): 0.001%.

**Related compounds—**

*Mobile phase and System suitability solution*—Proceed as directed in the Assay.

*Standard solution*—Dissolve accurately weighed quantities of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 0.02 mg per mL of each component. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of methanol and water (50:50) to volume, and mix.

*Test solution*—Transfer about 100 mg of Carbamazepine, accurately weighed, to a 50-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, add about 20 mL of water, and shake. Allow the mixture to cool to room temperature, and dilute with water to volume.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between carbamazepine related compound A and carbamazepine is not less than 1.70; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantities, in mg, of carbamazepine related compound A and carbamazepine related compound B in the portion of Carbamazepine taken by the formula:

$$100C(r_i / r_{Si})$$

in which  $C$  is the concentration, in mg per mL, of carbamazepine related compound A or carbamazepine related compound B in the *Standard solution*; and  $r_i$  and  $r_{Si}$  are the peak responses obtained for either carbamazepine related compound A or carbamazepine related compound B from the *Test solution* and the corresponding peak obtained from the *Standard solution*, respectively. Calculate the quantities, in

mg, of all other impurities found in the portion of Carbamazepine taken by the formula:

$$100C(r_i / r_s)$$

in which  $r_i$  is the peak response for any other impurity; and  $r_s$  is the peak response for carbamazepine obtained from the *Standard solution*: not more than 0.2% of any individual impurity is found; and the total of all impurities (including carbamazepine related compound A and carbamazepine related compound B) is not more than 0.5%.

**Assay—**

*Mobile phase*—Prepare a 1000-mL mixture of water, methanol, and tetrahydrofuran (85:12:3), add 0.22 mL of formic acid, mix, then add 0.5 mL of triethylamine, and mix. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve accurately weighed quantities of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 0.1 and 0.5 mg per mL, respectively. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Carbamazepine RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume.

*Assay preparation*—Transfer about 100 mg of Carbamazepine, accurately weighed, to a 50-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dissolve in and dilute with a mixture of methanol and water (1:1) to volume.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation* and the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between carbamazepine related compound A and carbamazepine in the *System suitability solution* is not less than 1.70; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{15}H_{12}N_2O$  in the portion of Carbamazepine taken by the formula:

$$500C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Carbamazepine RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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## Carbamazepine Oral Suspension

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» Carbamazepine Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, protected from freezing and from excessive heat.

**USP Reference standards** (11)—

USP Carbamazepine RS

**Identification, Infrared Absorption** (197S)—

**Solution**—Place 5 mL of Oral Suspension in a separator containing 20 mL of 0.1 N sodium hydroxide, and extract with 25 mL of chloroform. Pass the extract through anhydrous sodium sulfate supported on filter paper into a beaker. Wash the anhydrous sodium sulfate with 10 mL of chloroform, and add the washing to the extract. Evaporate the chloroform extract with the aid of a stream of nitrogen to dryness. Dissolve the residue in 10 mL of methylene chloride.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 100 cfu per g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

**Uniformity of dosage units** (90S)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay**—

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under Carbamazepine.

**Assay preparation**—Transfer an accurately measured volume of freshly mixed Oral Suspension, equivalent to about 200 mg of carbamazepine, to a 100-mL volumetric flask, add about 70 mL of methanol, shake by mechanical means for about 30 minutes, sonicate for about 2 minutes, dilute with methanol to volume, and mix. Allow the solution to stand for about 10 minutes, transfer 10.0 mL of the clear solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. This is the final *Assay preparation*. For system suitability determination, transfer 10.0 mL of this solution to a suitable container, add 10.0 mL of *System suitability solution*, and mix.

**Procedure**—Proceed as directed in the Assay under Carbamazepine. Calculate the quantity, in mg, of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the portion of Oral Suspension taken by the formula:

$$10C(r_u / r_s)$$

in which the terms are as defined therein.

## Carbamazepine Tablets

» Carbamazepine Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{15}H_{12}N_2O$ .

**Packaging and storage**—Preserve in tight containers, preferably of glass. Dispense Carbamazepine Tablets in a container labeled "Store in a dry place. Protect from moisture."

**Labeling**—The labeling indicates the *Dissolution* test with which the product complies.

**USP Reference standards** (11)—

USP Carbamazepine RS

**Identification**—Boil, in a 50-mL beaker, a quantity of powdered Tablets, equivalent to about 250 mg of carbamazepine, with 15 mL of acetone for 5 minutes. Filter while hot into a second beaker, using two 5-mL portions of hot ace-

tone to effect transfer. Evaporate with the aid of nitrogen to about 5 mL, and cool in an ice bath until crystals are formed. Filter the crystals, wash with 3 mL of cold acetone, and dry in vacuum at 70° for 30 minutes: the crystals so obtained respond to the *Identification* test under Carbamazepine.

**Dissolution** (711)—

FOR PRODUCTS LABELED AS 100-MG CHEWABLE TABLETS—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** water containing 1% sodium lauryl sulfate; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_{15}H_{12}N_2O$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 288 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Carbamazepine RS in the same *Medium*. [NOTE—A volume of methanol not exceeding 1% of the final total volume of the Standard solution may be used to dissolve the carbamazepine.]

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{15}H_{12}N_2O$  is dissolved in 60 minutes. Use *Acceptance Table 1*, with the following exceptions: at  $S_2$ , no unit is less than  $Q - 5\%$ ; at  $S_3$ , no unit is less than  $Q - 10\%$ ; and not more than 2 of the 24 units are less than  $Q - 5\%$ .

FOR PRODUCTS LABELED AS 200-MG TABLETS—

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus, and Procedure**—Proceed as directed for Test 1.

**Times and Tolerances:** between 45% and 75% of the labeled amount of  $C_{15}H_{12}N_2O$  is dissolved in 15 minutes; not less than 75% (Q) of the labeled amount of  $C_{15}H_{12}N_2O$  is dissolved in 60 minutes. Use *Acceptance Table 2*, with the following exceptions: at 15 minutes—at  $L_2$ , no unit is more than 5% outside the stated range; at  $L_3$ , no unit is more than 10% outside the stated range; and not more than 2 of the 24 units are more than 5% outside the stated range. At 60 minutes—at  $L_2$ , no unit is less than  $Q - 5\%$ ; at  $L_3$ , no unit is less than  $Q - 10\%$ ; and not more than 2 of the 24 units are less than  $Q - 5\%$ .

**TEST 3**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium, Apparatus, and Procedure**—Proceed as directed for Test 1.

**Times and Tolerances:** between 60% and 85% of the labeled amount of  $C_{15}H_{12}N_2O$  is dissolved in 15 minutes; not less than 75% (Q) of the labeled amount of  $C_{15}H_{12}N_2O$  is dissolved in 60 minutes. Use *Acceptance Table 2*, with the following exceptions: at 15 minutes—at  $L_2$ , no unit is more than 5% outside the stated range; at  $L_3$ , no unit is more than 10% outside the stated range; and not more than 2 of the 24 units are more than 5% outside the stated range. At 60 minutes—at  $L_2$ , no unit is less than  $Q - 5\%$ ; at  $L_3$ , no unit is less than  $Q - 10\%$ ; and not more than 2 of the 24 units are less than  $Q - 5\%$ .

**Uniformity of dosage units** (90S): meet the requirements.

**Water, Method III** (921)—Finely powder 20 Tablets, and accurately weigh about 1.5 g of the powder into a dry, tared evaporating dish. Dry at 120° for 2 hours: it loses not more than 5.0% of its weight.

**Assay**—

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Carbamazepine.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of carbamazepine, to a 50-mL volumetric flask, add about 40 mL of methanol, sonicate for about 15 minutes, allow to cool to room temperature, dilute with methanol to volume, mix, and filter, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.

**Procedure**—Proceed as directed in the Assay under *Carbamazepine*. Calculate the quantity, in mg, of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which the terms are as defined therein.

## Carbamazepine Extended-Release Tablets

» Carbamazepine Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ).

**Packaging and storage**—Preserve in tight container, and store at controlled room temperature.

**USP Reference standards** (11)—  
USP Carbamazepine RS

**Identification**—

**A: Ultraviolet Absorption** (197U)—

**Solution**—Use the *Test solution* prepared as directed in the test for *Uniformity of dosage units*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

**Medium:** water; 900 mL, 1800 mL for 400-mg Tablets.

**Apparatus 1:** 100 rpm.

**Times:** 3, 6, 12, and 24 hours.

**Procedure**—Determine the amount of  $C_{15}H_{12}N_2O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Carbamazepine RS in the same *Medium*.

**Tolerances**—The percentages (*Q*) of the labeled amount of  $C_{15}H_{12}N_2O$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 10% and 35%
6	between 35% and 65%
12	between 65% and 90%
24	not less than 75%

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Carbamazepine RS in methanol to obtain a solution containing 2 mg per mL.

**Standard solution**—Quantitatively dilute an accurately measured volume of *Standard stock solution* with methanol to obtain a solution having a known concentration of 10 µg per mL.

**Test solution**—Finely powder 1 Tablet, and quantitatively transfer the powder, with the aid of methanol, to a 100-mL volumetric flask. Add about 70 mL of methanol, and shake by mechanical means for 60 minutes. Sonicate for 15 minutes, and dilute with methanol to volume. Allow to stand for 10 to 15 minutes. Dilute a portion of the clear solution quantitatively, and stepwise if necessary, with methanol to obtain a solution containing about 10 µg of carbamazepine per mL.

**Procedure**—Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* by employing UV absorption at the wavelength of maximum absorbance at about 284 nm, using methanol as a blank. Calculate the quantity, in mg, of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the Tablet taken by the formula:

$$(LC / D)(A_U / A_S)$$

in which *L* is the labeled quantity, in mg, of carbamazepine in the Tablet; *C* is the concentration, in µg per mL, of USP Carbamazepine RS in the *Standard solution*; *D* is the concentration, in µg per mL, of the *Test solution*, based on the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively.

**Water, Method 1a** (921): not more than 5.0%.

**Limit of residual solvents**—

**Standard solution**—Dissolve accurately measured quantities of methanol and methylene chloride in dimethylformamide to obtain a solution having known concentrations of about 5 µg of each per mL.

**Test solution**—Finely powder 10 Tablets, and quantitatively transfer the powder to a 50-mL volumetric flask. Add about 30 mL of dimethylformamide, shake by mechanical means for 60 minutes, and sonicate for 2 minutes. Dilute with dimethylformamide to volume, and mix. Centrifuge a portion of the solution at about 2500 rpm for 20 minutes, and use the clear supernatant.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 3-m glass column containing 0.2% phase G39 on support S7. The injection port and detector temperatures are maintained at about 170° and 300°, respectively. The column temperature is programmed as follows. Initially it is maintained at 75° for 10 minutes, then increased at a rate of 20° per minute to 155°, and maintained at 155° for 30 minutes. The carrier gas is helium, flowing at a rate of about 10 mL per minute.

**Procedure**—Separately inject equal volumes (about 2 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the amount, in µg, of methylene chloride and methanol in each Tablet taken by the formula:

$$5C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of methylene chloride or methanol in the *Standard solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution*, respectively: not more than 23 µg of methylene chloride per Tablet is found; and not more than 100 µg of methanol per Tablet is found.

**Chromatographic purity**—Not more than 0.2% of any individual impurity is found; and not more than 0.5% of total impurities is found, the results from both *Test 1* and *Test 2* being used.

## TEST 1—

**Mobile phase and Chromatographic system**—Prepare as directed in the Assay.

**System suitability solution**—Dissolve suitable quantities of phenytoin and USP Carbamazepine RS in methanol to obtain a solution containing about 0.6 and 0.2 mg per mL, respectively. Dilute this solution quantitatively, and stepwise if necessary, with methanol to obtain a solution containing about 60 µg of phenytoin and 20 µg of USP Carbamazepine RS per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Carbamazepine RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 4 µg per mL.

**Test solution**—Use the Assay stock preparation.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Test solution and the Standard solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(C_S / C_T)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Carbamazepine RS in the Standard solution;  $C_T$  is the concentration, in mg per mL, of carbamazepine in the Test solution;  $r_i$  is the peak response of each impurity obtained from the Test solution; and  $r_s$  is the peak response for carbamazepine obtained from the Standard solution.

## TEST 2—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and acetonitrile (10:7:3). Make adjustments if necessary (see System Suitability under Chromatography <621>).

**System suitability solution**—Dissolve suitable quantities of iminostilbene and USP Carbamazepine RS in methanol to obtain a solution containing about 12.5 and 5.0 µg per mL, respectively.

**Standard solution**—Use the Standard solution, prepared as directed for Test 1.

**Test solution**—Use the Assay stock preparation.

**Chromatographic system** (see Chromatography <621>)—Prepare as directed in the Assay. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.3 for carbamazepine and 1.0 for iminostilbene; the resolution,  $R$ , between carbamazepine and iminostilbene is not less than 10.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Proceed as directed for Test 1.

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and methylene chloride (600:450:45). Make adjustments if necessary (see System Suitability under Chromatography <621>).

**Internal standard solution**—Prepare a solution of phenytoin in methanol containing about 600 µg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Carbamazepine RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 200 µg per mL. Pipet 10.0 mL of this solution into a conical flask, add 10.0 mL of Internal standard solution, mix, and filter. This solution contains about 100 µg of USP Carbamazepine RS per mL.

**System suitability solution**—Pipet equal volumes of Internal standard solution and Standard preparation into a suitable flask, and mix.

**Assay stock preparation**—Finely powder 10 Tablets, and quantitatively transfer the powder, with the aid of alcohol,

to a volumetric flask of such volume that when the solution is diluted to volume a final concentration estimated to be about 4 mg of carbamazepine per mL is obtained. Shake by mechanical means for 60 minutes. Sonicate for 15 minutes, and dilute with methanol to volume. Allow to stand for 10 to 15 minutes, then filter a portion of the supernatant, and use the clear filtrate.

**Assay preparation**—Transfer 5.0 mL of the Assay stock preparation to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pipet 10.0 mL of this solution into a conical flask, add 10.0 mL of Internal standard solution, mix, and filter.

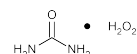
**Chromatographic system** (see Chromatography <621>)—The liquid chromatograph is equipped with a 230-nm detector, a 4.6-mm × 30-cm guard column that contains 7-µm packing L7, and a 3.9-mm × 30-cm column that contains packing L1. [NOTE—Wash the column with 50 to 100 mL of methanol before and after use.] The flow rate is about 2 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.8 for phenytoin, and 1.0 for carbamazepine; the resolution,  $R$ , between phenytoin and carbamazepine is not less than 2.8; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of carbamazepine ( $C_{15}H_{12}N_2O$ ) in each Tablet taken by the formula:

$$0.004(CV)(R_U / R_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Carbamazepine RS in the Standard preparation;  $V$  is the volume, in mL, of the volumetric flask used to prepare the Assay stock preparation; and  $R_U$  and  $R_S$  are the peak response ratios of carbamazepine to the internal standard obtained from the Assay preparation and the Standard preparation, respectively.

## Carbamide Peroxide



$CH_6N_2O_3$  94.07

Urea, compd. with hydrogen peroxide (1:1).

Urea compound with hydrogen peroxide (1:1) [124-43-6].

» Carbamide Peroxide contains not less than 96.0 percent and not more than 102.0 percent of  $CH_6N_2O_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

**Identification—**

**A:** Mix 1 mL of a solution (1 in 10) of it with 1 mL of nitric acid: a white, crystalline precipitate is formed.

**B:** A solution of it (1 in 10) responds to the tests for Peroxide <191>.

**Assay**—Transfer about 100 mg of Carbamide Peroxide, accurately weighed, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, insert the stopper, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the

endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of  $\text{CH}_6\text{N}_2\text{O}_3$ .

## Carbamide Peroxide Topical Solution

» Carbamide Peroxide Topical Solution is a solution in anhydrous glycerin of Carbamide Peroxide or of carbamide peroxide prepared from hydrogen peroxide and Urea. It contains not less than 78.0 percent and not more than 110.0 percent, by weight, of the labeled amount of  $\text{CH}_6\text{N}_2\text{O}_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

### Identification—

**A:** Mix 1 mL with 1 mL of nitric acid: a white, crystalline precipitate is formed.

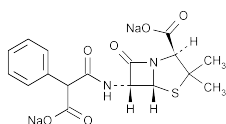
**B:** It responds to the tests for *Peroxide* (191).

**Specific gravity** (841): between 1.245 and 1.272.

**pH** (791): between 4.0 and 7.5.

**Assay**—Transfer an accurately weighed quantity of Topical Solution, equivalent to about 100 mg of carbamide peroxide, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of  $\text{CH}_6\text{N}_2\text{O}_3$ .

## Carbenicillin Disodium



$\text{C}_{17}\text{H}_{16}\text{N}_2\text{Na}_2\text{O}_6\text{S}$  (anhydrous) 422.36

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(carboxyphenylacetyl)amino]-3,3-dimethyl-7-oxo-, disodium salt, [2S-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-

N-(2-Carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo.[3.2.0]-hept-6-yl)-2-phenylmalonamic acid disodium salt [4800-94-6].

» Carbenicillin Disodium has a potency equivalent to not less than 770  $\mu\text{g}$  of carbenicillin ( $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6\text{S}$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards (11)—

USP Carbenicillin Monosodium Monohydrate RS

USP Endotoxin RS

**Identification**—It responds to the tests for *Sodium* (191).

**pH** (791): between 6.5 and 8.0, in a solution containing 10 mg of carbenicillin per mL.

**Water, Method I** (921): not more than 6.0%.

**Other requirements**—Where the label states that Carbenicillin Disodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Carbenicillin for Injection*. Where the label states that Carbenicillin Disodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Carbenicillin for Injection*.

### Assay—

**Assay preparation**—Dissolve a suitable quantity of Carbenicillin Disodium, accurately weighed, in *Buffer No. 1*, and dilute quantitatively with *Buffer No. 1* to obtain a solution having a convenient concentration of carbenicillin.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer No. 1* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Carbenicillin for Injection

» Carbenicillin for Injection contains an amount of Carbenicillin Disodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ( $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6\text{S}$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

### USP Reference standards (11)—

USP Carbenicillin Monosodium Monohydrate RS

USP Endotoxin RS

**Identification**—It responds to the tests for *Sodium* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.05 USP Endotoxin Unit per mg of carbenicillin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g being aseptically dissolved in 200 mL of *Fluid A*.

**pH** (791): between 6.5 and 8.0, in the solution constituted as directed in the labeling.

**Water, Method I** (921): not more than 6.0%.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and *Constituted Solutions and Labeling* under *Injections* (1).

### Assay—

**Assay preparation 1** (where it is packaged for dispensing and where the package is represented as being a single-dose container)—Constitute Carbenicillin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and dilute quantitatively with *Buffer No. 1* to obtain a solution having a convenient concentration of carbenicillin.

**Assay preparation 2** (where the label states the quantity of carbenicillin in a given volume of constituted solution)—Constitute Carbenicillin for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer No. 1* to obtain a solution having a convenient concentration of carbenicillin.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer No. 1* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.



## Carbenicillin Indanyl Sodium

$C_{26}H_{25}N_2NaO_6S$  516.54

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[3-[(2,3-dihydro-1*H*-inden-5-yl)oxy]-1,3-dioxo-2-phenylpropyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-

1-(5-Indanyl)(2*S*,5*R*,6*R*)-*N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-2-phenylmalonamate monosodium salt [26605-69-6].

» Carbenicillin Indanyl Sodium has a potency equivalent to not less than 630  $\mu$ g and not more than 769  $\mu$ g of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. For periods up to 18 months, store at controlled room temperature.

**USP Reference standards** (11)—  
USP Carbenicillin Indanyl Sodium RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** It responds to the tests for *Sodium* (191).

**pH** (791): between 5.0 and 8.0, in a solution containing 100 mg per mL.

**Water, Method I** (921): not more than 2.0%.

### Assay—

**Mobile phase**—Prepare a buffer of 0.0009 M tetrabutylammonium hydrogen phosphate and 0.05 M dibasic sodium phosphate as follows. Dissolve 604 mg of tetrabutylammonium phosphate and 26.8 g of dibasic sodium phosphate in 1800 mL of water, adjust with phosphoric acid to a pH of 3.8, and dilute with water to 2000 mL. Prepare a filtered and degassed mixture of this buffer and acetonitrile (116:84), allow to stand for 1 hour, and if necessary readjust with phosphoric acid to a pH of 3.8. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Diluting solvent**—Prepare a mixture of acetonitrile and 0.005 M monobasic potassium phosphate (85:15).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Carbenicillin Indanyl Sodium RS quantitatively in *Diluting solvent* to obtain a solution having a known concentration of about 250  $\mu$ g per mL. This *Standard preparation* contains the equivalent of about 222  $\mu$ g of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) per mL.

**Assay preparation**—Transfer about 125 mg of Carbenicillin Indanyl Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) in each

mg of the Carbenicillin Indanyl Sodium taken by the formula:

$$0.5(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Carbenicillin Indanyl Sodium RS, calculated on the anhydrous basis, in the *Standard preparation*; P is the assigned potency, in  $\mu$ g per mg, of USP Carbenicillin Indanyl Sodium RS; W is the quantity, in mg, of Carbenicillin Indanyl Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the carbenicillin indanyl peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carbenicillin Indanyl Sodium Tablets

» Carbenicillin Indanyl Sodium Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Carbenicillin Indanyl Sodium RS

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of carbenicillin, with 10 mL of a solvent mixture consisting of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (200:100:75:25:1.5). Shake the mixture for 5 minutes, and dilute 1 volume of it with 9 volumes of the solvent mixture. Apply 10  $\mu$ L each of this solution and of a solution of USP Carbenicillin Indanyl Sodium RS in the same solvent mixture containing 1 mg of carbenicillin per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (400:300:75:25:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and heat the plate at 80° for 30 minutes. Allow the plate to cool, and expose it to iodine vapors in a closed chamber for about 30 seconds. Spray the plate with a reagent consisting of a 1 in 100 solution of ferric chloride in 0.1 N hydrochloric acid, potassium ferricyanide solution (1 in 100), and methanol (4:4:3): the principal spots from the test solution and the Standard solution are blue on a yellow-green background, and the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution ( $R_f$  about 0.5).

### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) equivalent dissolved from the difference between UV absorbances at the wavelengths of maximum and minimum absorbance at about 267 nm and 254 nm, respectively, of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Carbenicillin Indanyl Sodium RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{18}N_2O_6S$  equivalent is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 2.0%.

#### Assay—

**Mobile phase, Diluting solvent, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay under Carbenicillin Indanyl Sodium.

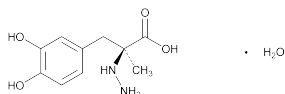
**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 111 mg of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ), to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under Carbenicillin Indanyl Sodium. Calculate the quantity, in mg, of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) in the portion of Tablets taken by the formula:

$$0.5CP(r_U / r_S)$$

in which the terms are as defined therein.

## Carbidopa



$C_{10}H_{14}N_2O_4 \cdot H_2O$  244.24

$C_{10}H_{14}N_2O_4$  226.23

Benzenepropanoic acid,  $\alpha$ -hydrazino-3,4-dihydroxy- $\alpha$ -methyl-, monohydrate, (S)-;  
(-)-L- $\alpha$ -Hydrazino-3,4-dihydroxy- $\alpha$ -methylhydrocinnamic acid monohydrate [38821-49-7].  
Anhydrous [28860-95-9].

#### DEFINITION

Carbidopa contains NLT 98.0% and NMT 102.0% of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ).

#### IDENTIFICATION

- A. INFRARED ABSORPTION** (197M)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Alcohol and 0.05 M monobasic sodium phosphate, adjusted with phosphoric acid to a pH of 2.7 (5:95)

**System suitability solution:** 0.1 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Methyldopa RS in *Mobile phase*

**Standard solution:** 0.5 mg/mL of USP Carbidopa RS in *Mobile phase*. [NOTE—Use gentle heat and ultrasonication, if necessary, to dissolve.]

**Sample solution:** 0.5 mg/mL of Carbidopa in *Mobile phase*

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

##### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for methyldopa and carbidopa are about 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 0.9 between methyldopa and carbidopa, *System suitability solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ) in the portion of Carbidopa taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carbidopa RS, as the monohydrate, in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carbidopa in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

#### IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS, Method II** (231): NMT 10 ppm
- LIMIT OF METHYLDOPA AND CARBIDOPA RELATED COMPOUND A**

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Impurity standard solution:** 2.5  $\mu$ g/mL of USP Methyldopa RS and 2.5  $\mu$ g/mL of USP Carbidopa RS in *Mobile phase*

#### Analysis

**Samples:** *Sample solution* and *Impurity standard solution*  
[NOTE—The relative retention times for methyldopa, carbidopa, and carbidopa related compound A are about 0.8, 1.0, and 1.8 respectively.]

Calculate the percentage of methyldopa in the portion of Carbidopa taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of methyldopa from the *Sample solution*

$r_S$  = peak response of methyldopa from the *Impurity standard solution*

$C_S$  = concentration of USP Methyldopa RS in the *Impurity standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of the *Sample solution* ( $\mu$ g/mL)

Calculate the percentage of carbidopa related compound A in the portion of Carbidopa taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of carbidopa related compound A from the *Sample solution*

$r_S$  = peak response of carbidopa from the *Impurity standard solution*

$C_S$  = concentration of USP Carbidopa RS in the *Impurity standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** NMT 0.5% of methyldopa, and NMT 0.5% of carbidopa related compound A

#### SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 10 mg/mL, in 0.7 g/mL of aluminum chloride solution (prepared using the hexahydrate form of the aluminum salt) that has been filtered and adjusted with 0.25 N sodium hydroxide to a pH of 1.5

**Acceptance criteria:**  $-21.0^\circ$  to  $-23.5^\circ$ , calculated as the monohydrate

• **Loss on Drying** (731)

**Analysis:** Heat 1 g in a suitable vacuum drying apparatus at 100° and a pressure of NMT 5 mm of mercury to constant weight. Cool, and weigh.

**Acceptance criteria:** 6.9%–7.9%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Carbidopa RS  
USP Methyldopa RS

## Carbidopa and Levodopa Tablets

» Carbidopa and Levodopa Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and of levodopa ( $C_9H_{11}NO_4$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Carbidopa RS

USP Levodopa RS

**Identification**—Transfer a portion of powdered Tablets, equivalent to about 10 mg of carbidopa, to a 100-mL volumetric flask containing about 50 mL of 0.05 N hydrochloric acid. Agitate for 20 minutes, add methanol to volume, mix, and filter or centrifuge. Separately prepare 2 Standard solutions containing 0.1 mg per mL of USP Carbidopa RS and USP Levodopa RS, respectively, in a solvent prepared by mixing equal volumes of 0.05 N hydrochloric acid and methanol. Apply 20  $\mu$ L of the test solution and 20  $\mu$ L of each Standard solution at separate points to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram using a solvent system consisting of a mixture of acetone, chloroform, *n*-butanol, glacial acetic acid, and water (60:40:40:40:35) until the solvent front has moved about 15 cm. Air-dry, spray uniformly with about 0.5 mL of ninhydrin reagent (prepared by dissolving 0.3 g of ninhydrin in 100 mL of *n*-butanol acidified with 3 mL of glacial acetic acid), and heat at 105° for about 10 minutes: the solution under test exhibits two spots (red-dish brown for levodopa and yellow-orange for carbidopa) having  $R_f$  values that correspond to those exhibited by the Standard solutions.

**Dissolution** (711)—

**Medium:** 0.1 N hydrochloric acid; 750 mL.

**Apparatus 1:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amounts of carbidopa and levodopa in solution in filtered portions of the solution under test, in comparison with a Standard solution having known concentrations of USP Carbidopa RS and USP Levodopa RS in the same medium, as directed for *Procedure* in the Assay.

**Tolerances**—Not less than 80% (Q) of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Sodium 1-decanesulfonate solution**—Dissolve 0.24 g of sodium 1-decanesulfonate in 1 L of water.

**Mobile phase**—Mix 11.04 g of monobasic sodium phosphate and 950 mL of water in a beaker. Add 1.3 mL of *Sodium 1-decanesulfonate solution*, and adjust with phosphoric acid to a pH of 2.8. Transfer to a 1-L volumetric flask, dilute with water to volume, and pass through a membrane filter.

**Standard preparation**—Transfer about 50 mg of USP Levodopa RS, accurately weighed, to a 100-mL volumetric flask. Add an accurately weighed quantity of USP Carbidopa RS, which is in a ratio with USP Levodopa RS that corresponds with the ratio of carbidopa to levodopa in the Tablets. Add 10 mL of 0.1 N phosphoric acid. Warm gently to dissolve the standards. Dilute with water to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of levodopa, to a 100-mL volumetric flask, add 10 mL of 0.1 N phosphoric acid, dilute with water to volume, and mix.

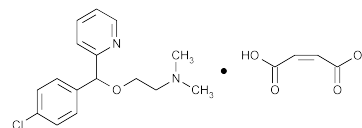
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate, about 2 mL per minute, is adjusted until the retention times for levodopa and carbidopa are about 4 minutes and 11 minutes, respectively. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%; and the resolution factor between levodopa and carbidopa is not less than 6.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of carbidopa ( $C_{10}H_{14}N_2O_4$ ) in the portion of Tablets taken by the formula:

$$(100C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Carbidopa RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the carbidopa peak obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of levodopa ( $C_9H_{11}NO_4$ ) by the same formula, reading the terms to refer to levodopa instead of carbidopa.

## Carbinoxamine Maleate



$C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  406.86

Ethanamine, 2-[(4-chlorophenyl)-2-pyridinylmethoxy]-*N,N*-dimethyl-, (*Z*)-2-butenedioate (1:1).

2-[*p*-Chloro- $\alpha$ -[2-(dimethylamino)ethoxy]benzyl]pyridine maleate (1:1) [3505-38-2].

» Carbinoxamine Maleate, dried at 105° for 2 hours, contains not less than 98.0 percent and not more than 102.0 percent of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Carbinoxamine Maleate RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 50 µg per mL.

*Medium:* methanol.

Absorptivities at 260 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 116° and 121°, determined after drying.

**pH** (791): between 4.6 and 5.1, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Ordinary impurities** (466)—

*Test solution:* chloroform.

*Standard solution:* chloroform.

*Eluant:* a mixture of cyclohexane, chloroform, and diethylamine (75:15:10).

*Visualization:* 1.

**Assay**—Dissolve about 400 mg of Carbinoxamine Maleate, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.34 mg of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

## Carbinoxamine Maleate Tablets

» Carbinoxamine Maleate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Carbinoxamine Maleate RS

**Identification**—A solution of carbinoxamine maleate (1 in 50,000) in dilute sulfuric acid (1 in 70) prepared from the Tablets as directed under *Salts of Organic Nitrogenous Bases* (501) exhibits an absorbance maximum at  $263 \pm 2$  nm. The absorptivity at 263 nm is within 7.0% of that of a 1 in 50,000 solution of USP Carbinoxamine Maleate RS in dilute sulfuric acid (1 in 70), similarly measured.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 260 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Carbinoxamine Maleate RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a 100-mL volumetric flask, add 10.0 mL of water, and shake by mechanical means for 15 minutes. Dilute with methanol

to volume, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with a mixture of methanol and water (9:1) to obtain a solution containing about 40 µg of carbinoxamine maleate per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Carbinoxamine Maleate RS, in the same medium having a known concentration of about 40 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 260 nm, with a suitable spectrophotometer, using a mixture of methanol and water (9:1) as the blank. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of carbinoxamine maleate in the Tablet; *C* is the concentration, in µg per mL, of USP Carbinoxamine Maleate RS in the Standard solution; *D* is the concentration, in µg per mL, of carbinoxamine maleate in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—Weigh and finely powder not less than 30 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of carbinoxamine maleate, to a separator, add 35 mL of water and 3 g of sodium bicarbonate, and mix. Extract with five 20-mL portions of chloroform, filtering the extracts through a pledget of cotton. Evaporate the combined chloroform extracts on a steam bath just to dryness, dissolve the residue in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.05 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 10.17 mg of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

## Carbol-Fuchsin Topical Solution

» Prepare Carbol-Fuchsin Topical Solution as follows:

Basic Fuchsin . . . . .	3 g
Phenol . . . . .	45 g
Resorcinol . . . . .	100 g
Acetone . . . . .	50 mL
Alcohol . . . . .	100 mL
Purified Water, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the Basic Fuchsin in a mixture of the Acetone and Alcohol, and add to this solution the Phenol and Resorcinol previously dissolved in 725 mL of Purified Water. Then add sufficient Purified Water to make the product measure 1000 mL, and mix.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Specific gravity** (841): not less than 0.990 and not more than 1.050.

**Alcohol content** (611): between 7.0% and 10.0% of  $C_2H_5OH$ .

## Carbon Dioxide

CO<sub>2</sub> 44.01  
Carbon dioxide [124-38-9].

### DEFINITION

Carbon Dioxide contains NLT 99.0%, by volume, of carbon dioxide (CO<sub>2</sub>).

[NOTE—The following tests are designed to reflect the quality of Carbon Dioxide in both its vapor and liquid phases, which are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the specimens for the tests with the least possible release of Carbon Dioxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination or change of the specimens.]

### IDENTIFICATION

- A.**  
**Sample:** 100 ± 5 mL, released from the vapor phase of the contents of the container  
**Analysis:** Pass the *Sample* through a carbon dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.  
**Acceptance criteria:** The indicator change extends throughout the entire indicating range of the tube.

### ASSAY

#### PROCEDURE

[NOTE—Sampling for this Assay may be done from the vapor phase for convenience, but this method results in more residual volume. If the specification of 1 mL is exceeded from the vapor phase, a liquid specimen may be taken.]

**Sample:** 100.0 mL of specimen taken from the liquid phase, as directed in the test for *Nitrogen Dioxide*

**Analysis:** Assemble a 100-mL gas buret, provided with a leveling bulb and two-way stopcock, and a gas absorption pipet of suitable capacity by connecting the pipet to one of the buret outlets. Fill the buret with slightly acidified water (turned pink with methyl orange), and fill the pipet with potassium hydroxide solution (1 in 2). By manipulation of the leveling bulb and leveling water, draw the potassium hydroxide solution to fill the pipet and capillary connection up to the stopcock. Fill the buret with the leveling water, and draw it through the other stopcock opening in such a manner that all gas bubbles are eliminated from the system. Draw the *Sample* into the buret. By raising the leveling bottle, force the measured specimen into the pipet. The absorption may be facilitated by rocking the pipet or by flowing the specimen between pipet and buret. Draw any residual gas into the buret, and measure its volume.

**Acceptance criteria:** NMT 1.0 mL of gas remains (NLT 99.0%, by volume, of CO<sub>2</sub>).

### IMPURITIES

#### NITROGEN DIOXIDE

**Sample:** 550 ± 50 mL, obtained as directed in the *Analysis*

**Analysis:** Arrange the container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized specimen plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 2.5 ppm

#### LIMIT OF AMMONIA

**Sample:** 1050 ± 50 mL of the gas obtained as directed in the test for *Nitrogen Dioxide*

**Analysis:** Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except pass 1050 ± 50 mL of this gas through an ammonia detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 0.0025%

#### LIMIT OF HYDROGEN SULFIDE

**Sample:** 1050 ± 50 mL, released from the vapor phase

**Analysis:** Pass 1050 ± 50 mL, released from the vapor phase, through a hydrogen sulfide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 1 ppm

#### LIMIT OF NITRIC OXIDE

**Sample:** 550 ± 50 mL, released from the vapor phase

**Analysis:** Pass 550 ± 50 mL, released from the vapor phase, through a nitric oxide–nitrogen dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 2.5 ppm

#### CARBON MONOXIDE

**Sample:** 1050 ± 50 mL, released from the vapor phase of the contents of the container

**Analysis:** Pass 1050 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon monoxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 0.001%

#### SULFUR DIOXIDE

**Sample:** 1050 ± 50 mL, obtained as directed in the test for *Nitrogen Dioxide*

**Analysis:** Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except to pass 1050 ± 50 mL through a sulfur dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 5 ppm

### SPECIFIC TESTS

#### WATER DETERMINATION

**Analysis:** Flush the regulator that has been flushed with 5 L or more of the gas specimen. Pass 50 ± 5 L, released from the vapor phase, through a water vapor detector tube connected to the regulator with a minimum length of metal or polyethylene tubing. Measure the gas passing through the detector tube with a gas flowmeter set at a flow rate of 2 L/min.

**Acceptance criteria:** NMT 150 mg/m<sup>3</sup>

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in cylinders.

## Carbon Monoxide C 11

» Carbon Monoxide C 11 is a colorless, odorless, non-irritating gas, suitable for administration by inhalation, in which a portion of the molecules are labeled with radioactive <sup>11</sup>C. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>11</sup>C expressed in MBq (or in mCi) at the time indicated in the labeling.

**Specific activity:** dependent upon the amount of radioactivity to be inhaled, but not more than 1.23 mmoles of carbon monoxide per volume.

**Packaging and storage**—Dispense the gas either continuously or batchwise, and preserve in a single-dose container that is adequately shielded. It may also be trapped either on activated charcoal at  $-196^{\circ}$  or on a molecular sieve at  $-72^{\circ}$ .

**Labeling**—The label must include the following: the time and date of calibration; the amount of  $^{11}\text{C}$  as carbon monoxide expressed as total MBq (mCi) at time of calibration; the expiration time and date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{11}\text{C}$  is 20.39 minutes. Each container to hold  $^{11}\text{CO}$  shall be independently labeled to indicate lot number and/or batch number. The labeling states that a microbiological filter (0.22  $\mu\text{m}$ ) is to be in place to remove any possible particulate matter that could be carried through to the final product.

**Radionuclide identification** (see *Radioactivity* <821>)—

**A:** Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent upon geometry and detector efficiency.

**B:** Radio-gas chromatography using a molecular sieve chromatographic column to determine the absence of  $^{13}\text{N}_2$  using a suitable radioactivity detector and mass detector.

**Radionuclidic purity**—A multichannel analyzer is used to count all radioactivity from 40 to 2,500 KeV to determine the absence of radiation, other than at 0.511 MeV and 1.022 MeV, over a period of 4 hours. Possible impurities could be  $^{13}\text{N}_2$  ( $t_{1/2} = 9.97$  min, this gamma ray spectrum is indistinguishable from  $^{11}\text{C}$ , (B+) 491 KeV),  $^{10}\text{C}$  ( $t_{1/2} = 19$  sec, 718.3 KeV (100%)),  $^{14}\text{O}$  ( $t_{1/2} = 70$  sec, 2312.7 KeV (99.4%)).  $^{11}\text{C}$ CO should contain no more than 10% impurities at the time of inhalation.

**Radiochemical purity and mass determination**—

[NOTE—This pharmaceutical may be synthesized by different methods and may therefore contain different impurities. Additional validated tests relevant to the synthetic procedure may be necessary in order to assure radiochemical purity of the final product.] Confirm by radio-gas chromatography. The gas stream, either directly from the target, or after initial chemical processing, is directed to an injection loop valve of a gas chromatograph and two precalibrated columns, a molecular sieve that allows separation of carbon monoxide from the different air components ( $\text{O}_2$ ,  $\text{N}_2$ , and  $\text{CH}_4$ ), and a column containing support S3 and of sufficient length to separate  $\text{CO}_2$  from  $\text{N}_2$  and  $\text{CO}$  (which co-elute) and  $\text{CO}_2$  from  $\text{NO}_2$  at room temperature. A simple radioactivity detector and a thermal conductivity detector (or equivalent) are required for the mass determination of carbon monoxide. The radiochemical purity is not less than 98%. Mass analysis of the gas-air mixture must demonstrate levels of carbon monoxide less than 1.23 mmoles in the entire dose which is the upper limit for a single-bolus inhalation.

**Assay for radioactivity**—Determine the radioactivity, in MBq (or mCi), by use of a calibrated system as directed under *Radioactivity* <821>.

## Flumazenil C 11 Injection

» Flumazenil C 11 Injection is a sterile solution, suitable for intravenous administration, of Flumazenil in which a portion of the molecules are labeled at the N-position with radioactive  $^{11}\text{C}$ . It contains not less than 90.0 percent and not more than 110 percent of the labeled amount of

$^{11}\text{C}$  expressed in MBq (or mCi) at the time indicated in the labeling. Its specific activity is not less than 14.8 GBq (400 mCi) per  $\mu\text{mol}$ . It may contain suitable buffers.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* <1>: the time and date of calibration; the amount of  $^{11}\text{C}$  as [N-methyl- $^{11}\text{C}$ ]Flumazenil, expressed as MBq (or mCi); the specific activity, expressed as MBq (or mCi) per  $\mu\text{mol}$ ; the concentration, expressed as MBq (or mCi) per mL, at the date and time of calibration; the expiration date and time; the lot or batch number; the name and quantity of any added preservative or stabilizer; and the statements, "Caution—Radioactive Material" and "Do not use if cloudy or if it contains particulate matter." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{11}\text{C}$  is 20 minutes.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Flumazenil RS

**Radionuclide identification** <821>—Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent upon geometry and detector efficiency.

**Bacterial endotoxins** <85>—It contains not more than 175/V USP Endotoxin Unit per mL, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** <791>: between 4.5 and 8.5.

**Radionuclidic purity** <821>—Using a multichannel analyzer, count all radioactivity from 40 to 2,500 keV to determine the absence of radiation, other than at 0.511 MeV and 1.022 MeV, over a period of 4 hours. Determine the half-life (20 minutes) by a suitable detector system.

**Chemical purity**—

**Mobile phase**—Transfer 1.2 g of monobasic sodium phosphate to a 1000-mL volumetric flask, dissolve in 500 mL of deionized distilled water, and dilute with deionized distilled water to volume to obtain a stock solution. Transfer 300 mL of acetonitrile to another 1000-mL volumetric flask, and dilute with the stock solution to volume. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve an accurately weighed quantity of USP Flumazenil RS in acetonitrile to obtain a solution having a known concentration of 1 mg of flumazenil per mL. Dilute a portion of this solution quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu\text{g}$  of flumazenil per mL.

**Test solution**—Pipet an accurately measured volume of Injection into a suitable container, and dilute with *Mobile phase* to obtain a solution containing about 0.1 mL of flumazenil per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. The column temperature is maintained at  $20^{\circ}$ . Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 100 theoretical plates; the tailing factor is not more than 1.1; and the relative standard deviation for replicate injections is not more than 3.2%.

**Procedure**—Inject about 20  $\mu\text{L}$  of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Separately calculate the percentage of

each impurity in the portion of the Injection taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 0.2% of any individual impurity is found, and not more than 0.9% of total impurities is found.

#### Radiochemical purity <821>—

*Mobile phase and System suitability solution*—Prepare as directed in the test for *Chemical purity*.

*Chromatographic system*—Proceed as directed in the test for *Chemical purity*, except that the liquid chromatograph is also equipped with a suitable collimated radiation detector (see *Radioactivity* <821>).

*Procedure*—Inject about 20  $\mu$ L of the Injection into the chromatograph, record the chromatogram, and measure responses for the major peaks. The radioactivity under the main peak is not less than 98% of the total radioactivity measured.

#### Specific activity—

*Mobile phase and System suitability solution*—Proceed as directed in the test for *Chemical purity*.

*Chromatographic system*—Proceed as directed in the test for *Chemical purity*, except that the liquid chromatograph is also equipped with a suitable collimated radiation detector (see *Radioactivity* <821>).

*Procedure*—Calculate the specific activity, in MBq (or mCi) per  $\mu$ mol, of Injection by the formula:

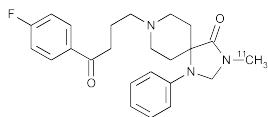
$$3.03(C_R P_R) / C$$

in which  $C_R$  is the radioactivity content, in MBq (or mCi) per mL, as determined in the *Assay for radioactivity*;  $P_R$  is the radiochemical purity (in %), as determined in the test for *Radiochemical purity*; and  $C$  is the concentration, in  $\mu$ g per mL, of flumazenil in the Injection as determined in the test for *Chemical purity*. The specific activity is not less than 400 mCi per  $\mu$ mol.

**Other requirements**—It meets the requirements under *Injections* <1>, except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility* <71>, the latter test being started on the day following final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** <821>—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity, in MBq (or mCi) per mL, of Injection by use of a calibrated system.

## Mespiperone C 11 Injection



» Mespiperone C 11 Injection is a sterile, isotonic solution, suitable for intravenous administration, of 3-*N*-[ $^{11}\text{C}$ ] methylspiperone in which a portion of the molecules are labeled with radioactive  $^{11}\text{C}$ . It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{11}\text{C}$  expressed in GBq (or mCi) at the time

indicated in the labeling. Its specific activity is not less than 18.5 GBq (500 mCi) per  $\mu$ mol.

#### Specific activity—

*Mobile phase and Standard solution*—Prepare as directed in the test for *Chemical purity*.

*Chromatographic system*—Proceed as directed in the test for *Chemical purity*.

*Procedure*—Calculate the specific activity, in MBq (or mCi) per  $\mu$ mol, of Mespiperone C 11 Injection by the formula:

$$3.11(C_R P_R) / C$$

in which  $C_R$  is the radioactivity content, in MBq (or mCi) per mL, as determined in the *Assay for radioactivity*;  $P_R$  is the radiochemical purity (in %), as determined in the test for *Radiochemical purity*, and  $C$  is the concentration (in  $\mu$ g per mL) of 3-methylspiperone in the Injection, as determined in the test for *Chemical purity*.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following: the time and date of calibration; the amount of  $^{11}\text{C}$  as methylspiperone expressed as total GBq (or mCi) at time of calibration; the expiration time and date; the lot or batch number; and the statements, "Caution—Radioactive Material" and "Do not use if cloudy or if it contains particulate matter." The labeling indicates that in making dosage calculations correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{11}\text{C}$  is 20 minutes.

#### USP Reference standards <11>—

USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* <821>)—Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent on geometry and detector efficiency.

**Bacterial endotoxins** <85>—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, in which  $V$  is the maximum recommended total dose, in mL, at the expiration time.

**pH** <791>: between 4.5 and 7.

**Radionuclidic purity** <821>—Using a multichannel analyzer, count all radioactivity from 40 to 2500 KeV to determine the absence of radiation, other than at 0.511 MeV and 1.022 MeV, over a period of 4 hours. Determine the half-life (20 minutes) by a suitable detector system.

#### Chemical purity—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and 0.05 M monobasic potassium phosphate (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard solution*—Dissolve an accurately weighed quantity of 3-methylspiperone hydrochloride in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*Test solution*—Pipet an accurately measured volume of Injection into a suitable container, and dilute with *Mobile phase* to obtain a solution containing about 0.1 mg per mL.

*Chromatographic system* (see *Chromatography* <621>). The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. For simultaneous analysis of radiochemical purity, a suitable radioactivity detector (see *Radioactivity* <821>) is coupled to the system. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 100 theoretical plates, the tailing factor for the analyte peak is not

more than 1.1, and the relative standard deviation for replicate injections is not more than 3.2%.

**Procedure**—Inject about 20  $\mu\text{L}$  of the *Test Solution* into the chromatograph, record the chromatogram, and measure the peak responses. Separately calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response obtained for each impurity, and  $r_s$  is the sum of the responses of all of the peaks: not more than 0.2% of any individual impurity is found, and the sum of all impurities is not more than 0.9%.

**Radiochemical purity** (821)—Proceed as directed in the test for *Chemical purity*, except that the liquid chromatograph is also equipped with a suitable collimated radioactivity detector. The radioactivity under the main peak is not less than 98% of the total radioactivity measured.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day following final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity, in GBq (or mCi) per mL, of Injection by use of a calibrated system.

## Methionine C 11 Injection

» Methionine C 11 Injection is a sterile isotonic solution, suitable for intravenous administration of  $[\text{C}^{11}]$ methionine, in which a portion of the molecules are labeled with radioactive  $^{11}\text{C}$ . It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{11}\text{C}$  expressed in MBq (or in mCi) at the time indicated in the labeling. It may contain preservatives and stabilizers.

**Specific activity:** not less than 37.0 GBq (1.0 Ci) per mmol.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following: the time and date of calibration; the amount of  $^{11}\text{C}$  as methionine expressed as total MBq (or mCi) per mL at time of calibration; the expiration time and date; the name and quantity of any added preservative or stabilizer; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{11}\text{C}$  is 20.4 minutes. Each container to hold  $^{11}\text{C}$  methionine shall be independently labeled to indicate lot number and batch number. The labeling states that a microbiological filter (0.22  $\mu\text{m}$ ) is to be in place to remove any possible particulate matter that could be carried through to the final product.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent upon geometry and detector efficiency.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum recommended total dose, in mL, at the expiration time.

**pH** (791): between 6.0 and 8.0.

**Radionuclidic purity** (821)—Using a suitable gamma-ray spectrometer, determine the absence of radiation other than at 0.511 MeV, over a period of 20 minutes. Determine the half-life (20.41 minutes) by a suitable detector system.

**Chemical purity**—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.008 M copper acetate and 0.017 M L-proline. Adjust with 0.030 M sodium acetate to a pH of 5. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of DL-methionine in *Mobile phase* and dilute quantitatively, and stepwise if necessary, to obtain a solution containing 0.1 mg per mL.

**Test solution**—Pipet a volume of Injection into a suitable container, and dilute with *Mobile phase* to obtain a solution containing about 0.1 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 0.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for D-methionine and 2.4 for L-methionine; the resolution,  $R$ , between the D- and L-enantiomers is not less than 1.5; the column efficiency is not less than 1400 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Inject a volume (about 10  $\mu\text{L}$ ) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of D-methionine in the portion of Injection taken by the formula:

$$100(r_i / r_s)$$

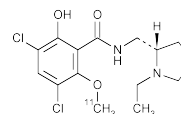
in which  $r_i$  is the peak response for D-methionine, and  $r_s$  is the sum of the responses of all of the peaks: not more than 4% of D-enantiomer is found.

**Radiochemical purity**—Proceed as directed under *Chemical purity*. The radioactivity of the main peak is not less than 98% of the total radioactivity measured.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the tests for *Sterility* (71) and *Bacterial Endotoxins* (85), these tests being started on the day of final manufacture, and except that it is not subject to the recommendation of *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity, in GBq (Ci) per mL, of the Injection by use of a calibrated system.

## Raclopride C 11 Injection



» Raclopride C 11 Injection is a sterile solution, suitable for intravenous administration, of



raclopride, in which a portion of the molecules are labeled at the O-methyl position with radioactive  $^{11}\text{C}$ . It contains not less than 90.0 percent and not more than 110 percent of the labeled amount of  $^{11}\text{C}$  expressed in MBq (or mCi) at the time indicated in the labeling. Its specific activity is not less than 18.5 Gbq (500 mCi) per  $\mu\text{mol}$ . It may contain suitable buffers.

#### Specific activity—

*Mobile phase and Standard solution*—Prepare as directed in the test for *Chemical purity*.

*Chromatographic system*—Proceed as directed in the test for *Radiochemical purity*.

*Procedure*—Calculate the specific activity, in GBq (or mCi) per  $\mu\text{mol}$ , of Injection by the formula:

$$3.47(C_r P_r) / C$$

in which  $C_r$  is the radioactivity content, in MBq (or mCi) per mL, as determined in the *Assay for radioactivity*;  $P_r$  is the radiochemical purity (in %), as determined in the test for *Radiochemical purity*; and  $C$  is the concentration (in  $\mu\text{g}$  per mL) of raclopride in the Injection, as determined in the test for *Chemical purity*.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling under Injections* (1): the time and date of calibration; the amount of  $^{11}\text{C}$  as [O-methyl- $^{11}\text{C}$ ]raclopride, expressed as total megabecquerels (or millicuries); the specific activity, expressed as megabecquerels (or millicuries) per  $\mu\text{mol}$ ; and the concentration, expressed as megabecquerels (or millicuries) per mL, at the date and time of calibration; the expiration date and time; the lot or batch number; the name and quantity of any added preservative or stabilizer; and the statements, "Caution—Radioactive Material" and "Do not use if cloudy or if it contains particulate matter." The labeling indicates that in making dosage calculations correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{11}\text{C}$  is 20 minutes.

#### USP Reference standards (11)—

USP Endotoxin RS

**Radionuclide identification** (821)—Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent upon geometry and detector efficiency.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL, in which  $V$  is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 4.5 and 7.

**Radionuclidic purity** (821)—Using a multichannel analyzer, count all radioactivity from 40 to 2500 keV to determine the absence of radiation, other than at 0.511 MeV and 1.022 MeV, over a period of 4 hours. Determine the half-life (20 minutes) by a suitable detector system.

#### Chemical purity—

*Mobile phase*—Add 840  $\mu\text{L}$  of phosphoric acid to 500 mL of deionized distilled water in a 1000-mL volumetric flask. Add 270 mL of acetonitrile, dilute with deionized distilled water to volume, filter, and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of raclopride (as the tartrate salt) in water to obtain a solution having a known concentration of about 1 mg of raclopride per mL. Dilute a portion of this solution quantita-

tively with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu\text{g}$  of raclopride per mL.

*Test solution*—Prepare a solution by quantitatively diluting an accurately measured volume of Injection, equivalent to about 37 MBq (1 mCi) of radioactivity, with 10 parts of *Mobile phase*, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L9. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.75 for O-desmethyleraclopride and 1.0 for raclopride; the resolution,  $R$ , between acetate and carbonate is not less than 1.5; the column efficiency determined from the analyte peak is not less than 85 theoretical plates; and the relative standard deviation for replicate injections is not more than 10.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the raclopride peaks. Calculate the concentration, in  $\mu\text{g}$  per mL, of raclopride ( $\text{C}_{15}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_3$ ) in the portion of Injection taken by the formula:

$$C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of raclopride in the *Standard solution*; and  $r_U$  and  $r_S$  are the raclopride peak responses obtained from the *Test solution* and the *Standard solution*, respectively. In the chromatogram of the *Test solution*, the area of the peak with a retention time of about 6 minutes (raclopride) is not less than 98% of the total area of all peaks.

#### Radiochemical purity—

*Mobile phase and Standard solution*—Prepare as directed in the test for *Chemical purity*.

*Chromatographic system*—Proceed as directed in the test for *Chemical purity*, except that the liquid chromatograph is also equipped with a suitable collimated radiation detector (see *Radioactivity* (821)).

*Procedure*—Inject about 20  $\mu\text{L}$  of the Injection into the chromatograph, record the chromatogram, and measure the areas of the responses for the major peaks. The radioactivity under the main peak is not less than 95% of the total area of all peaks observed, and its retention time is within 10% of that obtained for the *Standard solution*, similarly chromatographed.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day following final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly under Radioactivity* (821)), determine the radioactivity, in MBq (or mCi) per mL, of Injection by use of a calibrated system.

## Sodium Acetate C 11 Injection



» Sodium Acetate C 11 Injection is a sterile solution, suitable for intravenous administration, of Sodium Acetate in which a portion of the carboxyl molecules are labeled with radioactive  $^{11}\text{C}$ . It contains not less than 90.0 percent and not

more than 110.0 percent of the labeled amount of  $^{11}\text{C}$  expressed in MBq (or in  $\mu\text{Ci}$  or mCi) at the time indicated in the labeling. It may contain suitable buffers.

**Specific activity:** not less than 3.7 GBq (100 mCi) per  $\mu\text{mol}$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{11}\text{C}$  as labeled sodium acetate expressed as total MBq (or mCi) and the concentration as megabecquerels per mL (or as millicuries per mL), on the date and time of calibration; the expiration date and time; the lot or batch number; the name and quantity of any added preservative or stabilizer; an indication on the labeling that states, "Do not use if cloudy or if it contains particulate matter;" and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{11}\text{C}$  is 20 minutes.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (821)—Its gamma-ray spectrum is identical to that of a specimen of  $^{11}\text{C}$  in that it exhibits a positron annihilation peak at 0.511 MeV and possibly a sum peak of 1.022 MeV, dependent upon geometry and detector efficiency.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 4.5 and 8.5.

**Radionuclidic purity** (821)—A multichannel analyzer is used to count all radioactivity from 40 to 2,500 keV to determine the absence of radiation, other than at 0.511 MeV and 1.022 MeV, over a period of 4 hours. Determine the half-life by a suitable detector system.

**Chemical purity**—

**Mobile phase**—Add 14 mL of 0.5 N sulfuric acid to 500 mL of water in a 1000-mL volumetric flask. Add 100 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Reference solution**—Dissolve an accurately weighed quantity of sodium acetate in water to obtain a solution having a known concentration of about 1 mg per mL. Quantitatively dilute a portion of this solution with *Mobile phase* to obtain a solution having a known concentration of about 20  $\mu\text{g}$  per mL.

**Test solution**—Prepare a solution by quantitatively diluting an accurately measured volume of Injection, equivalent to about 1 mCi of radioactivity with 10 parts of *Mobile phase*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 7.8-mm  $\times$  10-cm column that contains packing L9. The flow rate is about 1 mL per minute. Chromatograph the *Reference solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 minutes for acetate and 1.0 minute for carbonate; the resolution,  $R$ , between acetate and carbonate is not less than 1.4; the column efficiency is not less than 85 theoretical plates; and the relative standard deviation for replicate injections is not more than 10%.

**Procedure**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Reference solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the acetate peaks. Calculate the concentration,

in  $\mu\text{g}$  per mL, of sodium acetate in the Injection by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in g per mL, of sodium acetate in the *Reference solution*; and  $r_U$  and  $r_S$  are the acetate peak responses obtained from the *Test solution* and the *Reference solution*, respectively.

**Radiochemical purity**—

**Mobile phase and Reference solution**—Proceed as directed under *Chemical purity*.

**Chromatographic system**—Proceed as directed under *Chemical purity* except that the liquid chromatograph is also equipped with a suitable collimated radiation detector (see *Radioactivity* (821)).

**Procedure**—Inject about 30  $\mu\text{L}$  of the Injection into the chromatograph, record the chromatogram, and measure the areas for the major peaks. The radioactivity under the acetate C 11 peak is not less than 95% of the total area of all peaks observed, and its retention time is within  $\pm 10\%$  of that obtained for the *Reference solution*, similarly chromatographed.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day following final manufacture, and except that it is not subject to the recommendation of *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity in MBq (mCi) per mL, of the Injection by use of a calibrated system.

## Urea C 13

» Urea C 13 contains not less than 99.0 percent and not more than 100.5 percent of  $^{13}\text{CH}_4\text{N}_2\text{O}$ .

**Packaging and storage**—Preserve in well-closed containers at room temperature.

**USP Reference standards** (11)—  
USP Urea C 13 RS

**Limit of biuret**—

**Standard solution**—Dissolve an accurately weighed quantity of biuret in water, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.033 mg per mL.

**Test solution**—Transfer 100.0 mg of Urea C 13 to a test tube, and dissolve in 3 mL of water.

**Procedure**—To the *Test solution* and to 3 mL of the *Standard solution* add 3 mL of sodium hydroxide solution (10 in 100) and about 3 drops of copper sulfate solution (0.5 in 100), mix, and allow to stand for 5 minutes. Any reddish violet color in the *Test solution* is not more intense than that obtained from the *Standard solution*: not more than 0.1% of biuret is found.

**Isotopic purity**—

**Test solution**—Prepare a solution in methanol containing about 12 mg of Urea C 13 per mL.

**Chromatographic system** (see *Chromatography* (621) and *Mass Spectrometry* (736))—The gas chromatograph is connected to a mass spectrometer, and is equipped with a 0.25-mm  $\times$  15-m capillary column coated with a 0.1- $\mu\text{m}$  film of phase G47. The injection port is maintained at a temperature of 250°, the detector is maintained at a temperature of 200°, and the transfer line to the mass spectrometer is maintained at a temperature of 265°. Helium is

used as the carrier gas. The mass spectrometer is operated in a single-ion response mode. The electron energy is 70 eV.

**Procedure**—Inject about 1 µL of the *Test solution* into the gas chromatograph, record the total ion chromatogram, and combine all of the mass spectra scans across the entire major peak. Record the peak intensities at mass-to-charge ratios of 60, 61, 62, and 63. Calculate the percentage of carbon that is C 13 in the portion of Urea C 13 taken by the formula:

$$100[(I_{61} + I_{63})/(I_{60} + I_{61} + I_{63})]$$

in which  $I_{60}$ ,  $I_{61}$ , and  $I_{63}$  are the relative peak intensities at mass-to-charge ratios of 60, 61, and 63, respectively: not less than 99% is found. Calculate the percentage of oxygen that is O 18 in the portion of Urea C 13 taken by the formula:

$$100[(I_{62} + I_{63})/(I_{60} + I_{61} + I_{62} + I_{63})]$$

in which  $I_{62}$  is the relative peak intensity at a mass-to-charge ratio of 62, and the other terms are as defined above: not more than 15% is found.

**Other requirements**—It meets the requirements for *Identification tests A and B*, *Melting range*, *Residue on ignition*, *Alcohol-insoluble matter*, *Chloride*, *Sulfate*, and *Heavy metals* under *Urea*.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (89:10:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Biuret stock solution**—Transfer about 15 mg of biuret, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability preparation**—Transfer about 25 mg of urea, accurately weighed, to a 10-mL volumetric flask. Pipet 1.0 mL of *Biuret stock solution* into the flask, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Urea C 13 RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 2 mg per mL.

**Assay preparation**—Transfer about 100 mg of Urea C 13, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L8. The flow rate is about 0.8 mL per minute. Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between urea and biuret is not less than 1.5; and the relative standard deviation for replicate injections is not more than 1%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $^{13}\text{CH}_4\text{N}_2\text{O}$  in the portion of Urea C 13 taken by the formula:

$$(M_U / M_S)50C(r_U / r_S)$$

in which  $M_U$  and  $M_S$  are the molecular weights of Urea C 13 and USP Urea C 13 RS, respectively;  $C$  is the concentration, in mg per mL, of USP Urea C 13 RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Urea C 13 for Oral Solution

» Urea C 13 for Oral Solution is a dry powder prepared from Urea C 13. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of urea C 13 ( $^{13}\text{CH}_4\text{N}_2\text{O}$ ). It contains no preservatives.

**Packaging and storage**—Preserve in sterile, well-closed containers.

**Labeling**—Label it to indicate that the solution is to be discarded if particulate matter is visible after reconstitution. [NOTE—It is to be reconstituted with *Sterile Purified Water*.]

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 1000 cfu per g, the total combined molds and yeasts count does not exceed 100 cfu per g, and it meets the requirements for the absence of *Salmonella* species and *Escherichia coli*.

**Completeness of solution** (641): meets the requirements, a solution in carbon dioxide-free water containing 100 mg per mL being used.

**Other requirements**—It meets the requirements for *Identification tests A and B* under *Urea* and for the *Assay* under *Urea C 13*, and for packaged solids under *Uniformity of Dosage Units* (905).

## Urea C 14 Capsules



» Urea C 14 Capsules contain  $^{14}\text{CH}_4\text{N}_2\text{O}$  in which a portion of the molecules are labeled with radioactive  $^{14}\text{C}$  to provide 0.04 MBq (or 1 µCi) of radioactivity per capsule. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{14}\text{C}$  expressed as MBq (or µCi).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Expiration date**—The expiration date is not later than two years from the date of manufacture.

**Labeling**—Label it to include the following: the amount of  $^{14}\text{C}$ , expressed in MBq (or µCi) per Capsule at the time of calibration; the expiration date; the total radioactivity per container; and the statement, "Caution—Radioactive material."

**Radionuclide identification** (821)—A solution of 1 or more Capsules in 1 N hydrochloric acid when tested using a liquid scintillation counter produces beta emission having a 49 keV mean and a 156 keV max.

**Dissolution** (711)—

*Medium*: simulated gastric fluid TS; 500 mL.

*Apparatus 1*: 50 rpm.

*Time*: 10 minutes.

**Procedure**—Determine the background levels of  $^{14}\text{C}$  with a 1-mL portion of the solution under test using a liquid scintillation counter.

**Tolerances**: not less than 80% (Q) of the labeled amount of  $^{14}\text{C}$  is dissolved in 10 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Radionuclidic purity** (821)—Determine the radionuclidic purity of a solution of 1 or more Capsules in water using a liquid scintillation counter: not less than 99.9% of the radioactivity is present as C 14.

**Radiochemical purity**—

*Adsorbent*: 0.25-mm layer of chromatographic cellulose.

*Test solution*—Open 2 Capsules and place them in a suitable container, add 8 mL of methanol, and mix.

*Reference solution*: 40 mg of urea per mL, in water.

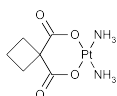
*Application volume*: 20  $\mu$ L of the *Test solution* and 4  $\mu$ L of the *Reference solution*.

*Developing solvent system*: *n*-butanol saturated with water.

*Procedure*—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Locate the spots on the plate by spraying with Ehrlich's reagent. Determine the radioactivity distribution with a suitable radiation detector (see *Radioactivity* (821)), and obtain the  $R_f$  value: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that obtained from the *Reference solution*, and the radioactivity of the  $^{14}\text{C}$  band is not less than 90% of the total radioactivity.

**Assay for radioactivity** (821)—Prepare a solution of 1 or more Capsules in 1 N hydrochloric acid. Using a liquid scintillation counter, determine the radioactivity, in MBq (or mCi) per mL by use of a calibrated system.

## Carboplatin



$\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Pt}$  371.25

Platinum, diammine[1,1-cyclobutanedicarboxylato(2-)- $\text{O},\text{O}'$ ]-, (SP-4-2).

*cis*-Diammine(1,1-cyclobutanedicarboxylato)platinum [41575-94-4].

» Carboplatin contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Pt}$ , calculated on the anhydrous basis.

**Caution**: Great care should be taken in handling Carboplatin because it is a suspected carcinogen.

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** (11)—

USP Carboplatin RS

**Identification**, *Infrared Absorption* (197K).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 5.0 and 7.0, in a solution in water containing 10 mg per mL.

**Water**, *Method I* (921): not more than 0.5%, anhydrous formamide being used as the solvent.

**Transmittance**—Transfer about 100 mg of Carboplatin, accurately weighed, to a 10-mL volumetric flask, dissolve in 6 mL of water, dilute with water to volume, and mix. Determine the percent transmittance in 1-cm cells at a wavelength of 440 nm, using water as the blank: not less than 97% transmittance is observed.

**Water-insoluble matter**—Transfer about 1 g of carboplatin, accurately weighed, to a 150-mL beaker. Add 100 mL of water, and dissolve by stirring with a stirring bar for 30 minutes. With aid of suction, filter through a tared filtering cru-

cible. Rinse the beaker with water, and transfer the rinsings to the crucible. Dry the crucible at  $130 \pm 10^\circ$  to constant weight: not more than 0.5% is found.

**Limit of 1,1-cyclobutanedicarboxylic acid**—

*Reagent A*—Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 N sodium hydroxide to a pH of 7.55.

*Mobile phase*—Add 20 mL of *Reagent A* to a mixture of 880 mL of water and 100 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of 1,1-cyclobutanedicarboxylic acid in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 2.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*System suitability solution*—Mix 1.0 mL of the *Standard solution* with 1.0 mL of *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Transfer about 50 mg of Carboplatin, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.0-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections (about 100  $\mu$ L) of the *System suitability solution*, record the chromatograms, and measure the peak responses: the relative retention times are about 0.65 for carboplatin and 1.0 for 1,1-cyclobutanedicarboxylic acid; the column efficiency, determined from the 1,1-cyclobutanedicarboxylic acid peak, is not less than 1500 theoretical plates; the resolution,  $R$ , between carboplatin and 1,1-cyclobutanedicarboxylic acid peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 10%.

*Procedure*—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for the 1,1-cyclobutanedicarboxylic acid peaks. Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin taken by the formula:

$$5(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of 1,1-cyclobutanedicarboxylic acid in the *Standard solution*,  $W$  is the weight, in mg, of Carboplatin taken to prepare the *Test solution*, and  $r_U$  and  $r_S$  are the peak responses for 1,1-cyclobutanedicarboxylic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% is found.

**Chromatographic purity**—

*Mobile phase*, *Chromatographic system*, and *Procedure*—Proceed as directed in the *Assay*.

*Standard solution*—Quantitatively dilute a volume of the *Standard preparation*, prepared as directed in the *Assay*, with water to obtain a solution having a known concentration of about 2.5  $\mu$ g of USP Carboplatin RS per mL.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The sum of the peak responses, excluding the carboplatin and 1,1-cyclobutanedicarboxylic acid responses, from the *Test solution*, is not more than 2 times the carboplatin response from the *Standard solution*, and no single peak response is greater than that of the carboplatin peak from the *Standard solution*: not more than 0.25% of

any individual impurity is found, and the sum of all impurities is not more than 0.5%.

**Platinum content**—[NOTE—Thoroughly cleanse all glassware with nitric acid and rinse with water to prevent “mirroring” of platinum precipitate.] Transfer about 0.25 g of Carboplatin, accurately weighed, to a 600-mL beaker. Add 400 mL of water, and slowly dissolve by heating almost to the boiling point, stirring frequently with a glass rod. Proceed as directed in the test for *Platinum content* under *Cisplatin*, beginning with “When solution is complete.” The weight of the platinum so obtained is between 52.0% and 53.0% of the carboplatin taken, calculated on the anhydrous basis.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Carboplatin RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of about 1 mg per mL. [NOTE—Use this solution within 2 hours.]

**Assay preparation**—Transfer about 50 mg of Carboplatin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. [NOTE—Use this solution within 2 hours.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.0-mm × 30-cm column that contains packing L8. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 3.0, the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.5, and the relative standard deviation for replicate injections is not more than 1.2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_6H_{12}N_2O_4Pt$  in the portion of Carboplatin taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Carboplatin RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carboplatin for Injection

» Carboplatin for Injection is a sterile, lyophilized mixture of Carboplatin and Mannitol. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_{12}N_2O_4Pt$ .

**Caution:** Great care should be taken in handling Carboplatin because it is a suspected carcinogen.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>, protected from light.

#### USP Reference standards <11>—

USP Carboplatin RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

#### Identification—

**Spray reagent**—Add 5.6 g of stannous chloride to 10 mL of hydrochloric acid, and stir for 5 minutes. [NOTE—It is not necessary that all of the solids dissolve.] Add 90 mL of water and 1 g of potassium iodide, and stir. Prepare this solution fresh daily.

**Standard solution**—Prepare a solution in water containing 10 mg of USP Carboplatin RS per mL.

**Test solution**—Dissolve the contents of 1 container in water to obtain a solution containing 10 mg of carboplatin per mL.

**Procedure**—Separately apply 10  $\mu$ L each of the *Standard solution* and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a chromatographic chamber lined with filter paper and equilibrated for 2 hours with a mixture of acetone and water (80:20). Develop the chromatogram until the solvent front has moved about 10 cm from the origin. Remove the plate from the chamber, and air-dry at room temperature for 2 hours. Spray with the *Spray reagent*, and heat at 110° for 10 minutes: the principal spot from the *Test solution* corresponds in appearance and  $R_f$  value to that obtained from the *Standard solution*.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Bacterial endotoxins** <85>—It contains not more than 0.54 USP Endotoxin Unit per mg of carboplatin.

**pH** <791>: between 5.0 and 7.0, in a solution constituted as directed in the labeling, *Sterile Water for Injection* being used.

**Water, Method I** <921>—Proceed as directed in the test for *Water* under *Cisplatin for Injection*: not more than 3.0% is found.

**Uniformity of dosage units** <905>: meets the requirements.

#### Limit of 1,1-cyclobutanedicarboxylic acid—

**Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed for *Limit of 1, 1-cyclobutanedicarboxylic acid* under *Carboplatin*.

**Standard solution**—Dissolve an accurately weighed quantity of 1,1-cyclobutanedicarboxylic acid in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution**—Quantitatively dissolve the contents of 1 container in *Mobile phase* to obtain a solution having a concentration of 1 mg of carboplatin per mL. [NOTE—Complete the chromatographic analysis of the solution within 2 hours.]

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the 1,1-cyclobutanedicarboxylic acid peaks. Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin for Injection taken by the formula:

$$100(CV / L)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of 1,1-cyclobutanedicarboxylic acid in the *Standard solution*;  $V$  is the volume, in mL, of the constituted container contents;  $L$  is the labeled quantity, in mg, of carboplatin per container; and  $r_U$  and  $r_S$  are the peak responses for 1,1-cyclobutanedicarboxylic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is found.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Carboplatin.

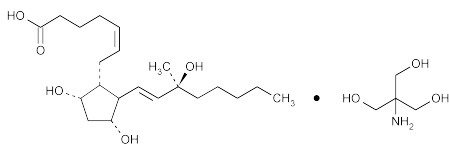
*Assay preparation*—Quantitatively dissolve the contents of 1 container in water to obtain a solution having a concentration of 1 mg per mL. [NOTE—Complete chromatographic analysis of this solution within 2 hours.]

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Assay preparation and the Standard preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_6H_{12}N_2O_4Pt$  in the portion of Carboplatin for Injection taken by the formula:

$$CV(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Carboplatin RS in the Standard preparation; V is the volume, in mL, of the constituted container contents; and  $r_U$  and  $r_S$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Carboprost Tromethamine



$C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$  489.64

Prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-15-methyl-, (5Z,9 $\alpha$ ,11 $\alpha$ ,13E,15S)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).

(Z)-7-[(1R,2R,3R,5S)-3,5-Dihydroxy-2-[(E)-(3S)-3-hydroxy-3-methyl-1-octenyl]cyclopentyl]-5-heptenoic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).

(15S)-15-Methylprostaglandin F<sub>2 $\alpha$</sub>  tromethamine [58551-69-2].

» Carboprost Tromethamine contains not less than 95.0 percent and not more than 105.0 percent of  $C_{25}H_{47}NO_8$ , calculated on the dried basis.

**Caution**—Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it.

**Packaging and storage**—Preserve in well-closed containers, and store in a freezer.

**USP Reference standards** (11)—

USP Carboprost Tromethamine RS

**Identification, Infrared Absorption** (197M).

**Specific rotation** (781S): between +18° and +24°.

*Test solution*: 10 mg per mL, in alcohol.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 50° for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Limit of 15R-epimer and 5-trans isomer—**

*Mobile phase, Internal standard preparation, Citrate buffer, and Chromatographic system*—Proceed as directed in the Assay. To evaluate the system suitability requirements, use the Standard preparation prepared as directed in the Assay.

*Test solution*—Use the Assay preparation.

*Procedure*—Inject a volume (about 25  $\mu$ L) of the Test solution into the chromatograph, record the chromatogram,

and measure the peak areas. Calculate the percentage of the 15R-epimer (as the tromethamine salt) in the portion of Carboprost Tromethamine taken by the formula:

$$100r_A / (r_A + r_B + r_C)$$

in which  $r_A$  is the area of any peak at a relative retention time of 0.7;  $r_B$  is the area of any peak at a relative retention time of 1.0; and  $r_C$  is the area of any peak at a relative retention time of about 1.2. Not more than 2.0% is found. Calculate the percentage of the 5-trans isomer (as the tromethamine salt) in the portion of Carboprost Tromethamine taken by the formula:

$$100r_C / (r_A + r_B + r_C)$$

in which the terms are as defined above. Not more than 3.0% is found.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of methylene chloride, 1,3-butanediol, and water (992:7:0.5). Make adjustments if necessary (see System Suitability under Chromatography (621)).

*Internal standard preparation*—Using the Mobile phase, prepare a solution containing about 7 mg of guaifenesin per mL.

*Citrate buffer*—Dissolve 10.5 g of citric acid in about 75 mL of water. Add 5 N sodium hydroxide slowly to adjust to a pH of 4.0, and dilute with water to 100 mL.

*Standard preparation*—Transfer about 5 mg of USP Carboprost Tromethamine RS, accurately weighed, to a stoppered, 50-mL centrifuge tube. Add 20.0 mL of methylene chloride and 2 mL of Citrate buffer. Shake the stoppered tube for about 10 minutes, and centrifuge. Remove and discard the top (aqueous) layer, and transfer a 4.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial. Evaporate with the aid of a stream of nitrogen to dryness. Add 100  $\mu$ L of a freshly prepared solution of  $\alpha$ -bromo-2'-acetonaphthone in acetonitrile (1 in 50). Swirl to wash down the sides of the vial. Add 50  $\mu$ L of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100), swirl again, and place the vial in a suitable heating device maintained at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 mL of Internal standard preparation, mix, and pass the resulting solution through a fine-porosity filter. Protect the filtered solution from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

*Assay preparation*—Proceed as directed for Standard preparation, except to use Carboprost Tromethamine in place of USP Carboprost Tromethamine RS.

*Chromatographic system* (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm stainless steel column that contains 10- $\mu$ m packing L3. The flow rate is about 1.8 mL per minute. Chromatograph the Standard preparation, and record the responses as directed for Procedure: the relative retention times for guaifenesin and the 2-naphthacyl ester of carboprost are about 0.6 and 1.0, respectively; the resolution,  $R$ , between guaifenesin and the 2-naphthacyl ester of carboprost is not less than 4.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of

$C_{25}H_{47}NO_8$  in the portion of Carboprost Tromethamine taken by the formula:

$$W(R_U / R_S)$$

in which  $W$  is the weight, in mg, of USP Carboprost Tromethamine RS taken to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the 2-naphthacyl ester of carboprost to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carboprost Tromethamine Injection

» Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in aqueous solution, which may also contain Benzyl Alcohol, Sodium Chloride, and Tromethamine. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carboprost ( $C_{21}H_{36}O_5$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store in a refrigerator.

### USP Reference standards (11)—

USP Carboprost Tromethamine RS  
USP Endotoxin RS

### Identification, Infrared Absorption (197K)—

**Test specimen**—Extract a volume of Injection, equivalent to about 2.5 mg of carboprost tromethamine, with 1.5 to 2 times its volume of chloroform. Discard the chloroform layer, and acidify the aqueous layer with 3 to 5 drops of hydrochloric acid. Extract the acidified solution with an equivalent volume of chloroform. Filter the chloroform layer through a pledget of cotton, and concentrate it to a volume of less than 1 mL. Combine the resulting solution with 150 to 180 mg of potassium bromide. Dry the potassium bromide mixture in vacuum overnight, and prepare a pellet from the dried mixture.

**Bacterial endotoxins** (85): not more than 714.3 USP Endotoxin Units per mg of carboprost tromethamine.

**pH** (791): between 7.0 and 8.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Mobile phase and Citrate buffer**—Prepare as directed in the *Assay* under *Carboprost Tromethamine*.

**Internal standard preparation**—Using the *Mobile phase*, prepare a solution containing about 3 mg of guaifenesin per mL.

**Standard preparation**—Using an accurately weighed quantity of USP Carboprost Tromethamine RS, prepare a Standard solution containing about 0.332 mg of USP Carboprost Tromethamine RS per mL and 9 mg of benzyl alcohol per mL. Transfer 2.0 mL of the solution so obtained into a stoppered centrifuge tube. Add 20.0 mL of methylene chloride and 1.0 mL of *Citrate buffer*, shake the stoppered tube for about 10 minutes, and centrifuge. Remove and discard the top (aqueous) layer, transfer an 8.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial, and evaporate the solution with the aid of a stream of nitrogen. [NOTE—The residue does not evaporate to dryness because of the presence of benzyl alcohol.] Add 100  $\mu$ L of a freshly prepared solution of  $\alpha$ -bromo-2'-acetonaphthone in acetonitrile (1 in 50), and swirl to wash down the sides of the vial. Add 50  $\mu$ L of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100). Swirl again, and place the vial in a

suitable heating device maintained at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 mL of *Internal standard preparation*, mix, and pass the resulting solution through a fine-porosity filter. Protect the filtered solution from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

**Assay preparation**—Pipet an accurately measured volume of Injection, equivalent to about 500  $\mu$ g of carboprost, to a stoppered, 50-mL centrifuge tube. Proceed as directed for *Standard preparation*, beginning with "Add 20.0 mL of methylene chloride".

**Chromatographic system**—Proceed as directed in the *Assay* under *Carboprost Tromethamine*. To evaluate the system suitability requirements, use the *Standard preparation* prepared as directed in the *Assay* under *Carboprost Tromethamine*.

**Procedure**—Proceed as directed in the *Assay* under *Carboprost Tromethamine*. Calculate the quantity, in  $\mu$ g, of carboprost ( $C_{21}H_{36}O_5$ ) in each mL of Injection taken by the formula:

$$(368.51/489.64)C(R_U / R_S)$$

in which 368.51 and 489.64 are the molecular weights of carboprost and carboprost tromethamine, respectively;  $C$  is the concentration, in  $\mu$ g per mL, of USP Carboprost Tromethamine RS in the Standard solution used to prepare the *Standard preparation*; and the other terms are as defined therein.

## Carboxymethylcellulose Sodium

Cellulose carboxymethyl ether sodium salt [9004-32-4].

### DEFINITION

Carboxymethylcellulose Sodium is the sodium salt of a polycarboxymethyl ether of cellulose. It contains NLT 6.5% and NMT 9.5% of sodium (Na), calculated on the dried basis.

### IDENTIFICATION

#### • A. PROCEDURE

**Sample solution:** Add 1 g of powdered Carboxymethylcellulose Sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced.

**Analysis:** To 1 mL of the *Sample solution*, diluted with an equal volume of water in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer.

**Acceptance criteria:** A red-purple color develops at the interface.

#### • B. PROCEDURE

**Sample solution:** Use the *Sample solution* from *Identification* test A.

**Analysis:** To 5 mL of the *Sample solution*, add an equal volume of barium chloride TS.

**Acceptance criteria:** A fine, white precipitate is formed.

#### • C. IDENTIFICATION TESTS—GENERAL, Sodium (191):

A portion of the *Sample solution* meets the requirements.

**Sample solution:** Use the *Sample solution* from *Identification* test A.

### ASSAY

#### • PROCEDURE

**Sample solution:** Transfer to a beaker 500 mg of Carboxymethylcellulose Sodium, add 80 mL of glacial acetic acid, heat the mixture in a boiling water bath for 2 h, and cool to room temperature.

**Analysis:** Titrate the *Sample solution* with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.

**Acceptance criteria:** NLT 6.5% and NMT 9.5% of Na, on the dried basis

## IMPURITIES

### Inorganic Impurities

- **HEAVY METALS, Method II <231>:** NMT 20 ppm, adding 1 mL of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue

## SPECIFIC TESTS

### • ROTATIONAL RHEOMETER METHODS <912>

**Analysis:** Determine the viscosity in a water solution at the concentration stated on the label. Using undried Carboxymethylcellulose Sodium, weigh the amount that, on the dried basis, will provide 200 g of solution of the stated concentration. Add the substance in small amounts to 180 mL of stirred water contained in a tared, wide-mouth bottle, continue stirring rapidly until the powder is well wetted, add sufficient water to make the mixture weigh 200 g, and allow to stand, with occasional stirring, until solution is complete. Adjust the temperature to  $25 \pm 0.2^\circ$ , and determine the viscosity, using a rotational type of viscometer, making certain that the system reaches equilibrium before taking the final reading.

**Acceptance criteria:** The viscosity of solutions of 2% or higher concentration is NLT 80.0% and NMT 120.0% of that stated on the label; the viscosity of solutions of less than 2% concentration is NLT 75.0% and NMT 140.0% of that stated on the label.

- **PH <791>:** 6.5–8.5 in a solution (1 in 100)
- **LOSS ON DRYING <731>:** Dry a sample at  $105^\circ$  for 3 h: it loses NMT 10.0% of its weight.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate the viscosity in solutions of stated concentrations.

# Carboxymethylcellulose Sodium Paste

## DEFINITION

Carboxymethylcellulose Sodium Paste contains NLT 16.0% and NMT 17.0% of carboxymethylcellulose sodium.

## IDENTIFICATION

- **A.**

**Sample solution:** Digest a quantity of Paste, equivalent to 1 g of carboxymethylcellulose sodium, with 50 mL of water until the solution is virtually complete. Filter.

**Analysis:** To 30 mL of the *Sample solution* add 3 mL of hydrochloric acid. Filter the solution, and save the filtrate for *Identification* test C.

**Acceptance criteria:** A white precipitate is formed.
- **B.**

**Sample solution:** The remainder of the *Sample solution* prepared in *Identification* test A

**Analysis:** To the *Sample solution* add an equal volume of barium chloride TS.

**Acceptance criteria:** A fine, white precipitate is formed.
- **C. IDENTIFICATION TESTS—GENERAL, Sodium <191>:** The filtrate from *Identification* test A meets the requirements of the tests.

## ASSAY

### • PROCEDURE

**Sample:** 2 g

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid in dioxane VS

**Endpoint detection:** Potentiometric

**Analysis:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 75 mL of glacial acetic acid, attach a condenser, and reflux for 2 h. Cool, transfer the mixture to a 250-mL beaker with the aid of small volumes of glacial acetic acid. Titrate with *Titrant*. Each mL of 0.1 N perchloric acid is equivalent to 29.67 mg of carboxymethylcellulose sodium.

**Acceptance criteria:** 16.0%–17.0%

## IMPURITIES

### • HEAVY METALS, Method II <231>

**Test preparation:** Prepare as directed in the chapter, using 400 mg of Paste and adding 1 mL of hydroxylamine hydrochloride solution (200 mg/mL) to the solution of the residue.

**Acceptance criteria:** NMT 50 µg/g

## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>:** The total bacterial count does not exceed  $10^3$  cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

### • CONSISTENCY

**Apparatus:** A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of  $30^\circ$ ; the point of the tip is truncated to a diameter of  $0.381 \pm 0.025$  mm; the base of the tip is  $8.38 \pm 0.05$  mm in diameter; and the length of the tip is  $14.94 \pm 0.05$  mm.

The remaining portion of the cone has an angle of  $90^\circ$ , is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are  $100 \pm 6$  mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

**Sample:** Paste

**Analysis:** Place the required number of containers in an oven, bring them and a quantity of the *Sample* to a temperature of  $82 \pm 2.5^\circ$ , and pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to  $25 \pm 2.5^\circ$  over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at  $25 \pm 0.5^\circ$ . If the room temperature is below  $23.5^\circ$  or above  $26.5^\circ$ , adjust the temperature of the cone to  $25 \pm 0.5^\circ$  by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm.

Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than  $\pm 3\%$ .

**Acceptance criteria:** The final average of the trials is 30.0–36.0 mm, indicating a consistency value of between 300 and 360.



- **LOSS ON DRYING** (731)  
Analysis: Dry at 105° for 3 h.  
Acceptance criteria: NMT 2.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and avoid prolonged exposure to temperatures exceeding 30°.

**Carboxymethylcellulose Sodium Tablets**

» Carboxymethylcellulose Sodium Tablets contain an amount of sodium (Na) equivalent to not less than 6.5 percent and not more than 9.5 percent of the labeled amount of carboxymethylcellulose sodium.

**Packaging and storage**—Preserve in tight containers.

**Identification**—Digest a quantity of powdered Tablets, equivalent to about 1 g of carboxymethylcellulose sodium, with 50 mL of water until solution is virtually complete, and filter: the filtrate responds to the following tests.

A: To about 30 mL of the solution add 3 mL of hydrochloric acid: a white precipitate is formed.

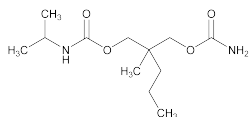
B: To the remainder of the solution add an equal volume of barium chloride TS: a fine, white precipitate is formed.

C: The filtrate from *Identification* test A responds to the tests for *Sodium* (191).

**Disintegration** (701): 2 hours.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of carboxymethylcellulose sodium, add 80 mL of glacial acetic acid, heat the mixture on a steam bath for 2 hours, cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.

**Carisoprodol**

$C_{12}H_{24}N_2O_4$  260.33  
(±)-2-Methyl-2-propyl-1,3-propanediol carbamate isopropylcarbamate [78-44-4].

**DEFINITION**

Carisoprodol contains NLT 98.0% and NMT 102.0% of  $C_{12}H_{24}N_2O_4$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution* as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Diluent:** Acetonitrile and water (50:50)

**Solution A:** Acetonitrile and water (25:75)

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	100	0
36	80	20
51	80	20
52	100	0
60	100	0

**System suitability solution:** 0.125 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*

**Standard solution:** 2.5 mg/mL of USP Carisoprodol RS in *Diluent*

**Sample solution:** 2.5 mg/mL of Carisoprodol in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 25 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor:** NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the carisoprodol peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carisoprodol from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carisoprodol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS, Method II** (231): NMT 10 ppm

• **ORGANIC IMPURITIES**

**Diluent, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 10 μg/mL of USP Carisoprodol RS in *Diluent*

**Sample solution:** 50 mg/mL of Carisoprodol in *Diluent*.  
[NOTE—Sonication may be used to aid dissolution.]

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*  
[NOTE—See *Table 2* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor:** NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation:** NMT 5.0% for the carisoprodol peak, 3 replicate injections of *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Identify the specified impurities using the relative retention times given in *Table 2*.  
Calculate the percentage of each impurity in the portion of Carisoprodol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carisoprodol in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprodol related compound A <sup>a</sup>	0.19	0.06	0.1
Meprobamate	0.24	0.08	0.5
Carisoprodol monocarbamate <sup>b</sup>	0.86	1.4	0.1
Carisoprodol	1.0	—	—
Any other unknown individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

<sup>a</sup> 2-Hydroxymethyl-2-methylpentyl carbamate.

<sup>b</sup> N-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

**SPECIFIC TESTS**

- LOSS ON DRYING (731):** Dry a sample in vacuum at 60° for 3 h; it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers at room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Carisoprodol RS
  - USP Carisoprodol Related Compound A RS
  - 2-Hydroxymethyl-2-methylpentyl carbamate,  $C_8H_{17}NO_3$  175.23
  - USP Meprobamate RS

## Carisoprodol Tablets

**DEFINITION**

Carisoprodol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ).

**IDENTIFICATION**

- A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

**PROCEDURE**

**Diluent:** Acetonitrile and water (50:50)

**Mobile phase:** Acetonitrile and water (25:75)

**System suitability solution:** 0.1 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*

**Standard solution:** 2.5 mg/mL of USP Carisoprodol RS in *Diluent*

**Sample solution:** Nominally 2.5 mg/mL in *Diluent* prepared as follows. Transfer an amount equivalent to the label claim of carisoprodol from powdered Tablets (NLT 20) to a suitable volumetric flask, and fill 50% of the flask volume with *Diluent*. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the *Sample solution*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 15 cm; 4-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 25 μL

**Run time:** 1.5 times the retention time of carisoprodol

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor:** NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the carisoprodol peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carisoprodol from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carisoprodol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

**DISSOLUTION (711)**

**Medium:** 0.05 M phosphate buffer, pH 6.9 (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 5 units of α-amylase per mL; 900 mL

[NOTE—Use only freshly prepared solutions containing α-amylase; and equilibrate the *Medium* at 37° for NMT 1 h before beginning the *Dissolution* test.]

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Mobile phase:** Acetonitrile and water (40:60)

**System suitability solution:** 2.4 mg/mL of 2-methyl-2-propyl-1,3-propanediol and 3.4 mg/mL of USP Carisoprodol RS in *Mobile phase*

**Standard solution:** 0.4 mg/mL of USP Carisoprodol RS in *Medium*

[NOTE—A volume of acetonitrile not exceeding 2% of the final total volume of solution may be used to aid in dissolving the carisoprodol.]

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm × 30-cm; packing L1

**Temperature:** 30 ± 1° for column and detector

**Flow rate:** 2 mL/min

**Injection size:** 150 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-methyl-2-propyl-1,3-propanediol and carisoprodol are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between 2-methyl-2-propyl-1,3-propanediol and carisoprodol, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for 3 replicate injections of the *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Record the peak responses, and measure the heights for the major peaks.

Calculate the percentage of the labeled amount of carisoprodol (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of carisoprodol (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 10 µg/mL of USP Carisoprodol RS in *Diluent*

**Sample solution:** Nominally 10 mg/mL in *Diluent* prepared as follows. Transfer an amount equivalent to four times the label claim of carisoprodol from powdered Tablets (NLT 20 Tablets) to a suitable volumetric flask, and fill 50% of the flask volume with *Diluent*. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the *Sample solution*.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, *System Suitability solution*

**Tailing factor:** NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation:** NMT 5.0% for the carisoprodol peak for 3 replicate injections of the *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Identify the specified impurities using the relative retention times given in *Table 1*.

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carisoprodol in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*).

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprodol related compound A <sup>a</sup>	0.19	0.06	0.75
Meprobamate	0.24	0.08	0.65
Carisoprodol monocarbamate <sup>b</sup>	0.86	1.4	0.20
Carisoprodol	1.0	—	—
Any other unknown degradation product	—	1.0	0.20
Total impurities	—	—	1.25

<sup>a</sup> 2-Hydroxymethyl-2-methylpentyl carbamate.

<sup>b</sup> N-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>
  - USP Carisoprodol RS
  - USP Carisoprodol Related Compound A RS
  - 2-Hydroxymethyl-2-methylpentyl carbamate.
  - C<sub>8</sub>H<sub>17</sub>NO<sub>3</sub> 175.23

### Carisoprodol and Aspirin Tablets

» Carisoprodol and Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of carisoprodol (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) and aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Aspirin RS

USP Carisoprodol RS

USP Salicylic Acid RS

**Identification**—The retention times of the aspirin peak and the carisoprodol peak in the chromatogram of the *Assay preparation* correspond to those of the aspirin peak and the carisoprodol peak in the chromatogram of the *Aspirin and carisoprodol standard preparation*, as obtained under *Assay for aspirin and carisoprodol and limit of free salicylic acid*.

**Dissolution** (711)—

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Time: 45 minutes.

**Mobile phase**—Mix 510 mL of methanol, previously filtered through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity, and 490 mL of glacial acetic acid solution (1 in 50), similarly filtered, and degas.

**Standard preparation**—Transfer about 90 mg of USP Aspirin RS and 90 mg of USP Carisoprodol RS, both accurately weighed, to a 250-mL volumetric flask, *J* being the ratio of the labeled amount, in mg, of carisoprodol to that of aspirin. Add 5 mL of acetonitrile, previously filtered through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity, swirl to dissolve, dilute with water to volume, and mix.

**Resolution solution**—Dissolve salicylic acid in *Standard preparation* to obtain a solution containing about 0.36 mg of salicylic acid per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. Maintain the detector and the column at  $30 \pm 1^\circ$ . The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the aspirin and salicylic acid peaks, and between the carisoprodol and salicylic acid peaks, is not less than 1.5; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 300  $\mu\text{L}$ ) of the *Standard preparation* and the solution under test, previously filtered, into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.4 for aspirin and 1.0 for carisoprodol. Calculate the quantity, in mg, of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) dissolved by the formula:

$$0.9C(r_U / r_S)$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained for aspirin from the solution under test and the *Standard preparation*, respectively. Calculate the quantity, in mg, of carisoprodol ( $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4$ ) dissolved by the same formula, except to read "USP Carisoprodol RS" where "USP Aspirin RS" is specified, and "carisoprodol" where "aspirin" is specified.

**Tolerances**—Not less than 75% (*Q*) of the labeled amounts of  $\text{C}_9\text{H}_8\text{O}_4$  and  $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4$  are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to aspirin and to carisoprodol.

**Assay for aspirin and carisoprodol and limit of free salicylic acid**—

**Mobile phase**—Mix 5 mL of glacial acetic acid and 500 mL of water, and pass through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity. Add 360 mL of the filtrate to 640 mL of methanol, similarly filtered, mix, and degas. Make adjust-

ments if necessary (see *System Suitability* under *Chromatography* (621)).

**Solvent mixture**—Prepare a mixture of water, acetonitrile, and glacial acetic acid (59:40:1).

**Aspirin and carisoprodol standard preparation**—Transfer about 80 mg of USP Aspirin RS and 80 mg of USP Carisoprodol RS, both accurately weighed, to a 25-mL volumetric flask, *J* being the ratio of the labeled amount, in mg, of carisoprodol to that of aspirin. Add about 15 mL of *Solvent mixture*, swirl for 5 minutes, and sonicate for 25 to 30 seconds. Dilute with *Solvent mixture* to volume, and mix.

**Salicylic acid standard preparation**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 16  $\mu\text{g}$  per mL.

**Resolution solution**—Dissolve salicylic acid in *Aspirin and carisoprodol standard preparation* to obtain a solution containing about 0.5 mg of salicylic acid per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 100-mL volumetric flask. Add about 50 mL of *Solvent mixture*, swirl for 5 minutes, sonicate for 25 to 30 seconds, shake by mechanical means for 30 minutes, dilute with *Solvent mixture* to volume, and mix. Pass a portion of this solution through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity, and use the filtrate as the *Assay preparation*. [NOTE—Use within 8 hours.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector, a 313-nm detector, and a 4.6-mm  $\times$  25-cm column that contains packing L7. Maintain the refractive index detector and the column at  $30 \pm 1^\circ$ . The flow rate is about 1 mL per minute. Chromatograph the *Aspirin and carisoprodol standard preparation* and the *Resolution solution*, and record the peak responses as directed under *Procedure*, using the refractive index detector: the resolution, *R*, between the solvent and aspirin peaks in the chromatogram of the *Resolution solution* is not less than 1.2; the resolution, *R*, between the aspirin and salicylic acid peaks is not less than 1.5; and the relative standard deviation for replicate injections of the *Aspirin and carisoprodol standard preparation* is not more than 2.0%. Chromatograph the *Salicylic acid standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Aspirin and carisoprodol standard preparation*, the *Salicylic acid standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks, using the refractive index detector for the *Aspirin and carisoprodol standard preparation*, the 313-nm detector for the *Salicylic acid standard preparation*, and both detectors for the *Assay preparation*. The relative retention times are about 0.6 for aspirin, 0.7 for salicylic acid, and 1.0 for carisoprodol. Calculate the quantity, in mg, of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Aspirin and carisoprodol standard preparation*; and  $r_U$  and  $r_S$  are the peak responses, with the use of the refractive index detector, obtained for aspirin from the *Assay preparation* and the *Aspirin and carisoprodol standard preparation*, respectively. Calculate the quantity, in mg, of carisoprodol ( $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4$ ) in the portion of Tablets taken by the same formula, except to read "USP Carisoprodol RS" where "USP Aspirin RS" is specified, and "carisoprodol" where "as-

pirin" is specified. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$10(C/a)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Salicylic Acid RS in the *Salicylic acid standard preparation*;  $a$  is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $r_U$  and  $r_S$  are the peak responses, with the use of the 313-nm detector, obtained for salicylic acid from the *Assay preparation* and the *Salicylic acid standard preparation*, respectively: not more than 3.0% is found.

## Carisoprodol, Aspirin, and Codeine Phosphate Tablets

» Carisoprodol, Aspirin, and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of carisoprodol ( $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4$ ), aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ), and codeine phosphate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ).

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Aspirin RS  
USP Carisoprodol RS  
USP Codeine Phosphate RS  
USP Codeine N-Oxide RS  
 $\text{C}_{18}\text{H}_{21}\text{O}_4$  315.37  
USP Salicylic Acid RS

**Identification**—The retention times of the aspirin, carisoprodol, and codeine phosphate peaks in the chromatograms of the *Assay preparations* correspond to those of the *Standard preparations* obtained as directed in the *Assay for aspirin and carisoprodol and limit of free salicylic acid* and the *Assay for codeine phosphate*.

### Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Time: 45 minutes.

**Determination of dissolved aspirin and carisoprodol**—Proceed as directed in the test for *Dissolution* under *Carisoprodol and Aspirin Tablets*.

**Determination of dissolved codeine phosphate**—

**MOBILE PHASE**—Dissolve 2.2 g of docusate sodium and 0.8 g of ammonium nitrate in 550 mL of water, and filter through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity. Add 450 mL of similarly filtered acetonitrile to the filtrate, mix, and degas.

**STANDARD PREPARATION**—Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in water to obtain a solution having a known concentration of about 18  $\mu\text{g}$  per mL.

**CHROMATOGRAPHIC SYSTEM** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**PROCEDURE**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Standard preparation* and the solution under test, previously filtered, into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculate the quantity, in mg, of codeine phosphate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) dissolved by the formula:

$$(406.37 / 397.37)(0.9C)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for the codeine phosphate peaks from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $\text{C}_9\text{H}_8\text{O}_4$ ,  $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4$ , and  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$  are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to aspirin, to carisoprodol, and to codeine phosphate.

### Assay for aspirin and carisoprodol and limit of free salicylic acid—

**Mobile phase, Solvent mixture, Aspirin and carisoprodol standard preparation, Salicylic acid standard preparation, and Resolution solution**—Prepare as directed in the *Assay for aspirin and carisoprodol and limit of free salicylic acid* under *Carisoprodol and Aspirin Tablets*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 100-mL volumetric flask. Add about 50 mL of *Solvent mixture*, swirl for 5 minutes, sonicate for 25 to 30 seconds, shake by mechanical means for 30 minutes, dilute with *Solvent mixture* to volume, and mix. Pass a portion of this solution through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity, and use the filtrate as the *Assay preparation*.

**Chromatographic system and Procedure**—Proceed as directed in the *Assay for aspirin and carisoprodol and limit of free salicylic acid* under *Carisoprodol and Aspirin Tablets*.

### Assay for codeine phosphate—

**Mobile phase**—Dissolve 2.2 g of docusate sodium in 600 mL of methanol. Dissolve 0.8 g of ammonium nitrate in 400 mL of water. Mix these two solutions, adjust with glacial acetic acid to a pH of  $3.3 \pm 0.05$ , filter through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Solvent mixture**—Mix equal volumes of methanol and 0.01 N sulfuric acid.

**Standard preparation**—Dissolve accurately weighed quantities of USP Codeine Phosphate RS and USP Aspirin RS in *Solvent mixture*, with the aid of swirling for 5 minutes and sonication for 25 to 30 seconds, to obtain a solution having known concentrations of about 0.16 mg of codeine phosphate and 0.16/ mg of aspirin per mL,  $J$  being the ratio of the labeled amount, in mg, of aspirin to that of codeine phosphate.

**Resolution solution**—Transfer about 8 mg of USP Codeine Phosphate RS to a 50-mL volumetric flask containing about 6 mg of USP Codeine N-Oxide RS, dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 16 mg of codeine phosphate, to a 100-mL volumetric flask. Add about 50 mL of *Solvent mixture*, sonicate for 30 minutes, shake by mechanical means for about 30 minutes, dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the reso-

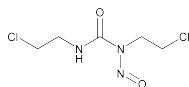
lution,  $R$ , between the codeine phosphate and codeine  $N$ -Oxide peaks is not less than 1.2; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for codeine  $N$ -Oxide and 1.0 for codeine phosphate. Calculate the quantity, in mg, of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(406.37)(397.37)(100C)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for the codeine phosphate peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carmustine



$C_5H_9Cl_2N_3O_2$  214.05  
Urea,  $N,N'$ -bis(2-chloroethyl)- $N$ -nitroso-;  
1,3-Bis(2-chloroethyl)-1-nitrosourea [154-93-8].

### DEFINITION

Carmustine contains NLT 98.0% and NMT 102.0% of  $C_5H_9Cl_2N_3O_2$ , calculated on the anhydrous and solvent-free basis.

**[CAUTION]**—Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197F)

**Sample:** Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

**Standard:** A similar preparation of USP Carmustine RS

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase:** Acetonitrile and water (3:7)

**Standard solution:** 1.5 mg/mL of USP Carmustine RS in acetonitrile

**Sample solution:** 1.5 mg/mL of Carmustine in acetonitrile

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Refrigerated autosampler temperature:** 4°–5°

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.9

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_5H_9Cl_2N_3O_2$  in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carmustine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Inorganic Impurities

- HEAVY METALS, Method II (231):** NMT 20 ppm

#### Organic Impurities

- PROCEDURE 1: LIMIT OF ETHER-INSOLUBLE SUBSTANCES**

[NOTE— Perform in a well-ventilated fume hood.]

**Analysis:** Transfer 1.0 g of sample to a suitable container containing 10 mL of anhydrous ether, stir for 5 min, and immediately filter through a tared glass-filtering crucible of medium pore size. Wash the container with an additional 10 mL of ether, and filter through the same glass-filtering crucible. Dry the crucible at 105° for 1 h. Cool in a desiccator and weigh.

**Acceptance criteria:** The weight of the residue does not exceed 0.1%.

- PROCEDURE 2: LIMIT OF CARMUSTINE RELATED COMPOUND A**

[NOTE— Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Carmustine standard solution:** Use the *Standard solution*, prepared as directed in the *Assay*.

**Standard stock solution:** 0.75 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

**Standard solution:** 0.0075 mg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard stock solution*

**System suitability solution 1:** 0.75  $\mu$ g/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard solution*

**System suitability solution 2:** Transfer 5.0 mL of *Carmustine standard solution* and 10.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 5.0 mL of this solution into a 50-mL volumetric flask, and dilute with acetonitrile to volume.

#### System suitability

**Samples:** *Carmustine standard solution*, *System suitability solution 1*, and *System suitability solution 2*

[NOTE— The relative retention times for carmustine related compound A and carmustine are 0.3 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 10 between carmustine related compound A and carmustine, *System suitability solution 2*

**Tailing factor:** NMT 1.9, *Carmustine standard solution*  
**Relative standard deviation:** NMT 5%, *System suitability solution 1*

### Analysis

[NOTE—Run the *Sample solution* at least 1.5 times the retention time of carmustine.]

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of carmustine related compound A from the *Sample solution*  
 $r_S$  = peak response of carmustine related compound A from the *Standard solution*  
 $C_S$  = concentration of carmustine related compound A in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- $r_U$  = peak response of any unspecified impurity from the *Sample solution*  
 $r_T$  = sum of all peak responses from the *Sample solution*

#### Acceptance criteria

Carmustine related compound A: NMT 0.5%

Any unspecified impurity: NMT 0.1%

#### • PROCEDURE 3: LIMIT OF 2-CHLOROETHYLAMINE

[NOTE— Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Standard solution 1 (0.2%):** 1.2 mg/mL of 2-chloroethylamine monohydrochloride in methanol. [NOTE— 1.2 mg/mL of 2-chloroethylamine monohydrochloride is equivalent to 0.8 mg/mL of 2-chloroethylamine.]

**Standard solution 2 (0.1%):** 0.4 mg/mL of USP Carmustine RS in methanol

**Sample solution:** 0.4 g/mL of Carmustine in methanol

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic plate (20-cm × 20-cm) coated with silica gel 60

**Application volume:** 1 µL

**Developing solvent system 1:** Ethyl acetate

**Developing solvent system 2:** Ethyl acetate and methanol (7:3)

**Spray reagent 1:** Diethylamine

**Spray reagent 2:** 0.1 N silver nitrate solution

#### Analysis

**Samples:** *Standard solution 1* (0.2%), *Standard solution 2* (0.1%), and *Sample solution*

Develop with *Developing solvent system 1* for 27 min, followed by air drying for 5 min. Develop again in *Developing solvent system 2* for 8 min, followed by air drying for 10 min. Spray the plate with *Spray reagent 1*, and heat the plate for 20 min in an oven at 100°. Allow the plates to cool to room temperature, and spray the plate with *Spray reagent 2*. Allow the plate to be exposed to UV light at 365 nm for 15 min. Examine the plate under UV light.

#### Acceptance criteria

**2-Chloroethylamine:** The spot for 2-chloroethylamine from the *Sample solution* is not more intense than the principal spot from *Standard solution 1* (0.2%).

**Any unspecified impurity:** Any spot if present in the chromatogram from the *Sample solution*, except the principal spot of carmustine and the spot of 2-chloroethylamine, is not more intense than the principal spot from *Standard solution 2* (0.1%).

#### • PROCEDURE 4: LIMIT OF 2-CHLOROETHANOL

**Standard solution:** 0.02 mg/mL of 2-chloroethanol in acetonitrile

**System suitability solution:** 0.01 mg/mL of 2-chloroethanol in acetonitrile, diluted from the *Standard solution*

**Sample solution:** 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m × 0.53-mm column bonded with a 1-µm film of phase G16

**Temperature**

**Injector:** 90°

**Detector:** 260°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	80	14
80	30	200	3

**Carrier gas:** Helium

**Flow rate:** 7 mL/min

**Injection size:** 5 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Relative standard deviation:** NMT 5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2-chloroethanol in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 2-chloroethanol from the *Sample solution*

$r_S$  = peak response of 2-chloroethanol from the *Standard solution*

$C_S$  = concentration of 2-chloroethanol in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

#### Acceptance criteria

**2-Chloroethanol:** NMT 0.1%

#### • PROCEDURE 5: LIMIT OF ACETALDEHYDE

**Standard solution:** 10 µg/mL of acetaldehyde in acetonitrile

**Sample solution:** 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m × 0.53-mm column bonded with a 5-µm film of phase G1

**Temperature**

**Injector:** 70°

**Detector:** 260°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	210	3

**Injector split ratio:** 15:1

**Carrier gas:** Helium

**Flow rate:** 3 mL/min

**Injection size:** 5 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetaldehyde in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaldehyde from the *Sample solution*

$r_S$  = peak response of acetaldehyde from the *Standard solution*

$C_S$  = concentration of acetaldehyde in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Acetaldehyde:** NMT 0.1%

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature between 2° and 8°.
- **USP REFERENCE STANDARDS (11)**  
USP Carmustine RS  
USP Carmustine Related Compound A RS  
1,3-Bis(2-chloroethyl) urea.  
 $C_5H_{10}Cl_2N_2O$  185.05

## Carmustine for Injection

**DEFINITION**

Carmustine for Injection is a sterile lyophilized preparation of carmustine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carmustine ( $C_5H_9Cl_2N_3O_2$ ).

**[CAUTION]**—Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197F)**

**Sample:** Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

**Standard:** A similar preparation of USP Carmustine RS

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase:** See the gradient table below.

Time (min)	Water (%)	Acetonitrile (%)
0	90	10
2.5	90	10
7	40	60
8.5	90	10
10.5	90	10

**Diluent:** Acetonitrile and water (1:3)

**Standard stock solution:** 2.0 mg/mL of USP Carmustine RS in acetonitrile

**Standard solution:** 0.2 mg/mL of USP Carmustine RS in *Diluent*, from *Standard stock solution*

**Impurity standard stock solution:** 0.1 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

**System suitability solution:** 0.2 mg/mL of USP Carmustine RS and 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Standard stock solution* and *Impurity standard stock solution*, respectively

**Sample stock solution:** 2.0 mg/mL of carmustine in acetonitrile, from Carmustine for Injection. [NOTE—Allow test vials to warm to room temperature in a desiccator for 1 h.]

**Sample solution:** 0.2 mg/mL of carmustine in *Diluent*, from the *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Refrigerated autosampler temperature:** 5°

**Column:** 4.6-mm × 7.5-cm; 3-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between carmustine related compound A and carmustine

**Tailing factor:** NMT 1.5 for the carmustine related compound A and carmustine peaks

**Relative standard deviation:** NMT 2.0% for the carmustine related compound A and carmustine peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of carmustine ( $C_5H_9Cl_2N_3O_2$ ) in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of carmustine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**IMPURITIES**

**Organic Impurities**

- **PROCEDURE: LIMIT OF CARMUSTINE RELATED COMPOUND A**

**Diluent, Impurity standard stock solution, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Impurity standard stock solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times [100 \times C_S/(C_U \times A)] \times 100$$



- $r_U$  = peak response of carmustine related compound A from the *Sample solution*  
 $r_S$  = peak response of carmustine related compound A from the *Standard solution*  
 $C_S$  = concentration of carmustine related compound A in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of carmustine in the *Sample solution* (mg/mL)  
 A = assay of Carmustine for Injection, as a percentage

**Acceptance criteria**

Carmustine related compound A: NMT 1.0%

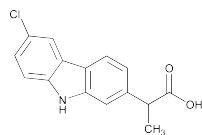
**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.95 USP Endotoxin Unit/mg of carmustine
- **STERILITY TESTS (71):** Meets the requirements
- **PH (791):** Between 4.0 and 6.8 in a constituted solution prepared as directed in the labeling
- **WATER DETERMINATION, Method I (921):** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve as described in *Injections (1)*, *Containers for Sterile Solids* at a temperature between 2° and 8°.
- **LABELING:** It meets the requirements in *Injections (1)*, *Labeling*.
- **CONSTITUTED SOLUTION:** At time of use, it meets the requirements in *Injections (1)*, *Constituted Solutions*.
- **USP REFERENCE STANDARDS (11)**  
 USP Carmustine RS  
 Urea, *N,N'*-bis(2-chloroethyl)-*N*-nitroso-;  
 1,3-Bis(2-chloroethyl)-1-nitrosourea.  
 $C_5H_9Cl_2N_3O_2$  214.05  
 USP Carmustine Related Compound A RS  
 1,3-Bis(2-chloroethyl) urea.  
 $C_5H_{10}Cl_2N_2O$  185.05  
 USP Endotoxin RS

## Carprofen



$C_{15}H_{12}ClNO_2$  273.71  
 9*H*-Carbazole-2-acetic acid, 6-chloro- $\alpha$ -methyl-, ( $\pm$ )-.  
 ( $\pm$ )-6-Chloro- $\alpha$ -methylcarbazole-2-acetic acid  
 [53716-49-7].

» Carprofen contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{12}ClNO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards (11)**—

USP Carprofen RS  
 USP Carprofen Related Compound A RS  
 Carbazole.  
 $C_{12}H_9N$  167.21

**Identification—**

**A:** *Infrared Absorption (197K).*

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying (731)**—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition (281):** not more than 0.1%.

**Heavy metals, Method II (231):** 0.002%.

**Limit of acetone and methylene chloride—**

**Standard solution**—Transfer about 5.0 g of acetone and 0.6 g of methylene chloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix. Pipet 1 mL of this solution into a 100-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix.

**Test solution**—Transfer about 500 mg of Carprofen, accurately weighed, to a 5-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix.

**Chromatographic system** (see *Chromatography (621)*)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm  $\times$  30-m capillary column coated with 3.0- $\mu$ m G43 stationary phase. The carrier gas is nitrogen, flowing at a rate of about 4.9 mL per minute. The split flow ratio is about 10:1. Initially the column temperature is maintained at 80° for 4 minutes, then is increased at a rate of 30° per minute to a temperature of 190°, and maintained at 190° for at least 3 minutes. The injection port temperature is maintained at 210°, and the detector temperature is maintained at 220°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: acetone elutes before methylene chloride; the resolution, *R*, between them is not less than 1.5; and the relative standard deviation for replicate injections, determined from the peak responses of acetone, is not more than 10.0%.

**Procedure**—Separately inject equal volumes (about 1  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each residual solvent in the portion of Carprofen taken by the formula:

$$0.5(C_S / W)(r_U / r_S)$$

in which  $C_S$  is the concentration, in  $\mu$ g per mL, of the individual residual solvent in the *Standard solution*; *W* is the weight, in mg, of Carprofen taken to prepare the *Test solution*;  $r_U$  is the peak response of the individual residual solvent in the *Test solution*; and  $r_S$  is the peak response of the individual residual solvent in the *Standard solution*: not more than 5000 ppm of acetone is found; and not more than 600 ppm of methylene chloride is found.

**Related compounds—**

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Inject about 10  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for all the peaks. Calculate the percentage of each related compound in the portion of Carprofen taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each individual peak other than the major peak of carprofen; and  $r_s$  is the sum of the peak responses: not more than 0.5% of each individual known related compound is found (see the relative retention times of these compounds in the table below); not more than 0.1% of each individual unknown related compound is found; and not more than 1.0% of total related compounds is found.

Known Related Compound	Approximate Relative Retention Time
Carprofen related compound A (carbazole)	0.9
2-[1,1-Dimethoxy-2-hydroxypropyl]-6-chlorocarbazole	1.3
2-[2-Chloropropionyl]-6-chloro-9-acetylcabazole	3.3

**Assay—**

*Mobile phase*—Prepare a mixture of acetonitrile, water, methanol, and glacial acetic acid (40:35:25:0.2).

*Carprofen related compound A solution*—[NOTE—Use low-actinic glassware.] Prepare a solution of USP Carprofen Related Compound A RS, accurately weighed, in *Mobile phase*, containing about 16 µg per mL, sonicating if necessary.

*Standard preparation*—[NOTE—Use low-actinic glassware.] Prepare a solution of USP Carprofen RS, accurately weighed, in *Mobile phase*, containing about 160 µg per mL, sonicating if necessary.

*System suitability solution*—[NOTE—Use low-actinic glassware.] Transfer 10 mL of *Carprofen related compound A solution* and 10 mL of *Standard preparation* into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Carprofen in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 160 µg per mL.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between carprofen and carprofen related compound A is not less than 2.0; the column efficiency for the carprofen peak is not less than 5000 theoretical plates; the tailing factor for the carprofen peak is not more than 2.0; and the relative standard deviation for replicate injections of carprofen is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of  $C_{15}H_{12}ClNO_2$  in the portion of Carprofen taken by the formula:

$$100P(C_S / C_U)(R_U / R_S)$$

in which  $P$  is the purity, in µg per mg, of USP Carprofen RS;  $C_S$  and  $C_U$  are the concentrations, in µg per mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $R_U$  and  $R_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Carprofen Tablets**

» Carprofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carprofen ( $C_{15}H_{12}ClNO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Carprofen RS

**Identification—**

**A:** *Infrared Absorption* <197K>—

*Reference specimen*—Mix about 2 mg of USP Carprofen RS with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the pellet immediately after preparation.

*Test specimen*—Grind into powder not fewer than 4 Tablets. Transfer the powder, equivalent to about 100 mg of carprofen, to a 125-mL separatory funnel. Add 30 mL of water and 3 drops of hydrochloric acid, and shake for about 5 minutes. Add about 30 mL of methylene chloride, and shake for another 5 minutes. Allow the phases to separate. Carefully drain and collect the lower methylene chloride layer through anhydrous sodium sulfate that is placed on a cotton pledget into a suitable container. Evaporate the methylene chloride on a steam bath with the aid of a stream of nitrogen to dryness. Dry the residue in vacuum at 60° for about 30 minutes. Mix about 2 mg of the dried residue with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the carprofen sample pellet immediately after preparation.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution** <711>—[NOTE—Use low-actinic volumetric flasks, dissolution vessels, and evaporation covers.]

*Medium*: 0.05 M phosphate buffer, pH 7.5 (prepared by dissolving 6.8 g of monobasic potassium phosphate in 600 mL of water, mixing, adding 18 mL of 2 N sodium hydroxide, mixing, diluting with water to 1000 mL, and adjusting with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of  $7.50 \pm 0.05$ ); 900 mL, degassed.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

Determine the amount of  $C_{15}H_{12}ClNO_2$  dissolved by employing the following method.

*Standard solution*—

FOR TABLETS LABELED TO CONTAIN 25 MG—Transfer about 25 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 10 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 75 MG—Transfer about 75 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 30 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 100 MG—Transfer about 100 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 40 mL of methanol. Dilute with *Medium* to volume, and mix.

*Test solution*—Pass a portion of the solution under test through a suitable 0.45-µm filter.

*System suitability solution*—Determine the absorbance of the *Standard solution*, as directed for *Procedure*, five times: the relative standard deviation is not more than 2.0%.

*Procedure*—Determine the amount of  $C_{15}H_{12}ClNO_2$  dissolved by measuring the absorbance of the *Test solution* in comparison with the appropriate *Standard solution* at the wavelength of maximum absorbance at about 300 nm, using a 0.5-cm cell for Tablets labeled to contain 25 mg, a 0.2-cm cell for Tablets labeled to contain 75 mg, and a 0.1-cm cell for Tablets labeled to contain 100 mg. Use *Medium* as the blank. Calculate the percentage of  $C_{15}H_{12}ClNO_2$  dissolved by the formula:

$$\frac{A_U \times W_S \times 100}{A_S \times LC}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $W_S$  is the weight, in mg, of USP Carprofen RS used to prepare the *Standard solution*; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{15}H_{12}ClNO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity*.

PROCEDURE FOR CONTENT UNIFORMITY—[NOTE—Use low-actinic glassware.]

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay*.

**Standard solution**—Prepare as directed for the *Standard preparation* in the *Assay*.

**Test solution**—Transfer 10 Tablets individually to 10 separate volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask, add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes or until the Tablets are completely disintegrated. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the individual solutions to 10 separate 50.0-mL volumetric flasks, dilute with *Mobile phase* to volume, and mix. Pass the solution through a polyvinylidene fluoride (PVDF) filter having a 0.45- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the percentage of the labeled content of  $C_{15}H_{12}ClNO_2$  in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which the terms are as defined therein.

#### Chromatographic purity—

**Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of 0.05  $\mu$ g of carprofen per mL.

**Sensitivity solution**—[NOTE—Use low-actinic glassware.] Quantitatively dilute the *Standard solution* with *Mobile phase* to obtain a solution containing about 0.005  $\mu$ g of carprofen per mL.

**Test solution**—Use the *Assay preparation*.

**Blank solution**—Transfer an accurately weighed portion of the Tablet base, equivalent to the weight of 1 Tablet, to a volumetric flask of the same calibrated volume as that used to prepare the *Test solution*. To each flask add *Mobile phase* to 80% of the calibrated volume. Sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard solution*, the *Sensitivity solution*, and

the *Test solution*, and record the peak responses as directed for *Procedure*: for the *Standard solution*, the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%; for the *Sensitivity solution*, the carprofen peak should be defined and integratable; for the *Test solution*, the resolution,  $R$ , between carprofen and the nearest impurity peak is not less than 2.0. After every six injections of any solution, inject a *Standard solution* in duplicate. The ratio of the average response of the duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

**Procedure**—Inject a volume (about 50  $\mu$ L) of the *Standard solution*, the *Test solution*, and the *Blank solution* into the chromatograph, record the chromatograms, and measure all the peak areas. Calculate the percentage of carprofen-related compounds in the portion of Tablets taken by the formula:

$$0.1(C_S / C_U)(r_i / r_S)$$

in which  $C_S$  is the concentration, in  $\mu$ g per mL, of carprofen in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of carprofen in the *Test solution*;  $r_i$  is the peak area of any peak other than carprofen obtained from the *Test solution*; and  $r_S$  is the peak area of carprofen obtained from the *Standard solution*: not more than 0.5% of any single impurity is found; and the sum of all impurities is not more than 2.0%. Disregard any peak also observed in the *Blank solution*.

#### Assay—

**Mobile phase**—Mix 500 mL of acetonitrile, 500 mL of water, and 1 mL of phosphoric acid. Degas before using. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay preparation**—[NOTE—Use low-actinic glassware.] Accurately weigh 20 Tablets, and calculate the average Tablet weight. Grind the Tablets into uniform powder. Transfer three accurately weighed portions of the powder, each equivalent to the weight of one Tablet, into three volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency for carprofen is not less than 4000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%. Inject the *Standard preparation* in duplicate after every 12 injections or

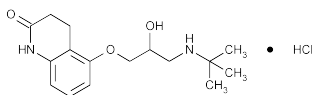
fewer of any other solution. The ratio of the average area of the duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of the labeled content of carprofen ( $C_{15}H_{12}ClNO_2$ ) in the portion of Tablets taken by the formula:

$$100(C_s / C_U)(r_U / r_s)$$

in which  $C_s$  and  $C_U$  are the concentrations, in mg per mL, of USP Carprofen RS in the *Standard preparation* and carprofen in the *Assay preparation*, respectively; and  $r_U$  and  $r_s$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carteolol Hydrochloride



$C_{16}H_{24}N_2O_3 \cdot HCl$  328.83

2-(1*H*)-Quinolinone, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-, monohydrochloride.  
5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydrocarbo-  
styryl monohydrochloride [51781-21-6].

» Carteolol Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of  $C_{16}H_{24}N_2O_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Carteolol Hydrochloride RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* water.

**C:** A solution (1 in 50) responds to the tests for *Chloride* (191).

**pH** (791): between 5.0 and 6.0, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231): not more than 0.002%.

**Arsenic, Method II** (211): 3 ppm.

**Chromatographic purity**—

*Standard solution A*—Prepare a solution of USP Carteolol Hydrochloride RS in methanol containing 0.5 mg per mL.

*Standard solution B*—Transfer 5.0 mL of *Standard solution A* to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Standard solution C*—Transfer 5.0 mL of *Standard solution B* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

*Test solution*—Transfer 250 mg of Carteolol Hydrochloride to a 10-mL volumetric flask, dissolve in methanol, using heat or sonication if necessary to achieve dissolution, dilute with methanol to volume, and mix.

**Procedure**—Apply separate 10- $\mu$ L portions of the *Test solution* and the *Standard solutions* to the starting line of a thin-

layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry. Line a chromatographic chamber with filter paper, and saturate the paper with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (50:20:1). Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot in the chromatogram obtained from the *Test solution* corresponds to that in the chromatogram obtained from *Standard solution A*. Compare the sizes and intensities of any spots other than the principal spot in the chromatogram obtained from the *Test solution* with those of the principal spots in the chromatograms obtained from the *Standard solutions*: no spot exceeds in size or intensity the principal spot in the chromatogram obtained from *Standard solution B* (0.2%), and the sum of all the impurity spots does not exceed 0.5%.

**Assay**—

**pH 6.0 Buffer**—Dissolve 1.34 g of dibasic sodium phosphate in about 1900 mL of water, adjust with 1 M phosphoric acid to a pH of  $6.0 \pm 0.05$ , dilute with water to 2000 mL and mix.

**Mobile phase**—Prepare a mixture of *pH 6.0 Buffer* and acetonitrile (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Increasing the proportion of *pH 6.0 Buffer* increases resolution.]

**Diluent**—Prepare a mixture of *pH 6.0 Buffer* and methanol (1:1).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Carteolol Hydrochloride RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of this stock solution to a 100-mL volumetric flask containing 5 mL of acetonitrile, dilute with water to volume, and mix. This solution contains about 0.1 mg of USP Carteolol Hydrochloride RS per mL.

**Resolution solution**—Transfer about 50 mg of *p*-acetotoluidide to a 100-mL volumetric flask, add 50 mL of acetonitrile, and swirl to dissolve. Dilute with water to volume, and mix. Transfer 10 mL of this solution and 10 mL of the stock solution used to prepare the *Standard preparation* to a second 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains about 0.05 mg of *p*-acetotoluidide and 0.1 mg of USP Carteolol Hydrochloride RS.

**Assay preparation**—Transfer about 100 mg of Carteolol Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask containing 5 mL of acetonitrile, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 252-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.8 for carteolol and 1.0 for *p*-acetotoluidide; and the resolution,  $R$ , between the carteolol peak and the *p*-acetotoluidide peak is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

mg, of  $C_{16}H_{24}N_2O_3 \cdot HCl$  in the portion of Carteolol Hydrochloride taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Carteolol Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the carteolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carteolol Hydrochloride Ophthalmic Solution

» Carteolol Hydrochloride Ophthalmic Solution is a sterile, aqueous, isotonic solution of Carteolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{24}N_2O_3 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carteolol Hydrochloride RS

**Identification**—

**A:** Prepare a test solution by diluting a suitable volume of Ophthalmic Solution with water, if necessary, to obtain a solution containing about 1 mg of carteolol hydrochloride per mL. Separately apply 10  $\mu$ L of the test solution and 10  $\mu$ L of a Standard solution of USP Carteolol Hydrochloride RS in water containing about 1 mg per mL to the starting line of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry. Line a chromatographic chamber with filter paper, and saturate the paper with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (50:20:1). Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wave-length UV light: the  $R_f$  value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the Standard solution.

**B:** The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.0 and 8.0.

**Assay**—

*pH 6.0 buffer, Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Carteolol Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5  $\mu$ m or finer, discarding the first 2 mL of the filtrate, and use the filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

mg, of  $C_{16}H_{24}N_2O_3 \cdot HCl$  in each mL of the Ophthalmic Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Carteolol Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the carteolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carteolol Hydrochloride Tablets

» Carteolol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{24}N_2O_3 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carteolol Hydrochloride RS

USP Dehydrocarteolol Hydrochloride RS

5-(3-*tert*-Butylamino-2-hydroxy)-propoxycarbostyryl hydrochloride.

$C_{16}H_{22}N_2O_3 \cdot HCl$  326.82

**Identification**—The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Mobile phase*—Dissolve 2.0 g of monobasic potassium phosphate in water to make 1000 mL of solution. Prepare a mixture of this solution and acetonitrile (600:400). Degas and pass through a filter having a porosity of 0.5  $\mu$ m or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Prepare a solution of USP Carteolol Hydrochloride RS in water having a known concentration of about 1.1L  $\mu$ g per mL,  $L$  being the labeled amount, in mg, of carteolol hydrochloride per Tablet.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 252-nm detector and a 3.9-mm  $\times$  30-cm column containing packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Pass a portion of the solution under test through a filter having a porosity of 1  $\mu$ m or finer, discarding the first 2 mL of the filtrate. Separately inject equal volumes (about 15  $\mu$ L) of the *Standard solution* and the test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{16}H_{24}N_2O_3 \cdot HCl$  dissolved by the formula:

$$0.9C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Carteolol Hydrochloride RS in the *Standard solution*, and  $r_U$  and  $r_S$  are the carteolol peak responses obtained from the test solution and the *Standard solution*, respectively.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{24}N_2O_3 \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of dehydrocarteolol hydrochloride—**

*pH 6.0 buffer, Mobile phase, and Diluent*—Proceed as directed in the Assay under *Carteolol Hydrochloride*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Dehydrocarteolol Hydrochloride RS quantitatively in *Diluent* to obtain a solution having a known concentration of about 1 µg per mL.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask, add about 50 mL of *Diluent*, and shake by mechanical means for 1 hour. Dilute with *Diluent* to volume, and mix. Pass about 5 mL of this solution through a filter having a 0.5-µm or finer porosity.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a fluorometric detector, with excitation at 300 nm and a 418-nm emission filter, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5%.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the dehydrocarteolol peak responses. Calculate the percentage of dehydrocarteolol hydrochloride in the portion of Tablets taken by the formula:

$$10(C/L)(W_A/W_T)(r_{ud}/r_{sd})$$

in which *C* is the concentration, in µg per mL, of USP Dehydrocarteolol Hydrochloride RS in the *Standard solution*; *L* is the labeled amount, in mg, of carteolol hydrochloride in each Tablet; *W<sub>A</sub>* is the average weight, in mg, of each Tablet; *W<sub>T</sub>* is the quantity, in mg, of the portion of Tablets taken to prepare the *Test solution*; and *r<sub>ud</sub>* and *r<sub>sd</sub>* are the dehydrocarteolol peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 1.0% is found.

**Assay—**

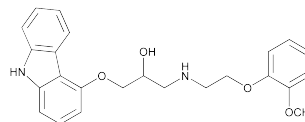
*pH 6.0 buffer, Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Carteolol Hydrochloride*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask. Add about 50 mL of *Diluent*, and shake by mechanical means for 1 hour. Add 5 mL of acetonitrile, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, discarding the first 2 mL of filtrate, and use the clear filtrate as the *Assay preparation*.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> · HCl in the portion of Tablets taken by the formula:

$$100C(r_u/r_s)$$

in which *C* is the concentration, in mg per mL, of USP Carteolol Hydrochloride RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the carteolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Carvedilol**C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>

406.47

2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-, (±)-; (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol [72956-09-3].

**DEFINITION**

Carvedilol contains NLT 98.0% and NMT 102.0% of C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>, calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Buffer:** 2.72 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid to a pH of 2.0.

**Mobile phase:** Acetonitrile and *Buffer* (31:69)

**System suitability solution:** 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound A RS in *Mobile phase*

**Standard solution:** 0.04 mg/mL of USP Carvedilol RS in *Mobile phase*

**Sample solution:** 0.04 mg/mL of Carvedilol in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 55°

**Flow rate:** 1 mL/min

**Run time:** 60 min

**Injection size:** 10 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 4.0 between carvedilol and carvedilol related compound A

**Tailing factor:** NMT 1.5 for the carvedilol peak

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carvedilol (C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of the sample taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

*r<sub>u</sub>* = peak response of carvedilol from the *Sample solution*

*r<sub>s</sub>* = peak response of carvedilol from the *Standard solution*

*C<sub>s</sub>* = concentration of carvedilol in the *Standard solution* (mg/mL)

*C<sub>u</sub>* = concentration of Carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** <281>: NMT 0.1% from 1 g

• **HEAVY METALS, Method II** <231>: NMT 10 ppm

• **ORGANIC IMPURITIES, PROCEDURE 1:** [NOTE—On the basis of the impurities present, perform either *Organic Impuri-*

ties, Procedure 1 or Organic Impurities, Procedure 2. Organic Impurities, Procedure 2 is recommended when carvedilol related compound F is a potential impurity.]  
**Buffer and Mobile phase:** Prepare as directed in the Assay.

**System suitability solution:** 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound C RS in *Mobile phase*

**Standard solution:** 1 µg/mL each of USP Carvedilol RS, USP Carvedilol Related Compound A RS, USP Carvedilol Related Compound B RS, USP Carvedilol Related Compound D RS, and USP Carvedilol Related Compound E RS, and 0.2 µg/mL of USP Carvedilol Related Compound C RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Carvedilol in *Mobile phase*

**Chromatographic system**  
 (See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** Dual wavelength, UV 220 and 240 nm. Use 220 nm for quantitating carvedilol related compound E, and use 240 nm for carvedilol and all other related compounds.

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 55°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** System suitability solution

**Suitability requirements**

**Resolution:** NLT 17 between carvedilol and carvedilol related compound C

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of carvedilol related compound A, carvedilol related compound B, carvedilol related compound C, carvedilol related compound D, carvedilol related compound E, and any other individual impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding related compound or any other impurity from the *Sample solution*

$r_S$  = peak response of the corresponding related compound from the *Standard solution*. To calculate the percentage of any other individual impurity use the peak response of carvedilol.

$C_S$  = concentration of the corresponding related compound in the *Standard solution* (mg/mL). To calculate the percentage of any other impurities for  $C_S$ , use the concentration of USP Carvedilol RS.

$C_U$  = concentration of Carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound E <sup>a</sup>	0.35	0.1
Carvedilol related compound A <sup>b</sup>	0.52	0.1
Carvedilol bisalkylpyrocatechol derivative (if present) <sup>c</sup>	0.70	0.15
Carvedilol	1.0	—
Carvedilol related compound C <sup>d</sup>	3.6	0.02
Carvedilol related compound D <sup>e</sup>	5.0	0.1
Carvedilol related compound B <sup>f</sup>	8.5	0.1
Any other individual impurity	—	0.10
Total impurities	—	0.5 <sup>g</sup>

<sup>a</sup> 2-(2-Methoxyphenoxy)ethyl amine.

<sup>b</sup> 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol.

<sup>c</sup> 3,3'-(2,2'-[1,2-Phenylenebis(oxy)])bis(ethane-2,1-diyl)bis(azanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>d</sup> 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)-amino)propan-2-ol.

<sup>e</sup> 4-(Oxiran-2-ylmethoxy)-9H-carbazole.

<sup>f</sup> 3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>g</sup> Disregard any impurity less than 0.01%.

#### • ORGANIC IMPURITIES, PROCEDURE 2

**Solution A:** Acetonitrile and trifluoroacetic acid (100:0.1)

**Solution B:** Trifluoroacetic acid and water (0.1:100)

**Diluent:** Acetonitrile, trifluoroacetic acid, and water (22:0.1:78)

**Mobile phase:** See Table 2.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	22	78
20	22	78
33	38	62
45	38	62
55	55	45
65	55	45
68	22	78
80	22	78

**System suitability solution:** 1.0 mg/mL of USP

Carvedilol System Suitability Mixture RS in *Diluent*

**Sample solution:** 1 mg/mL of Carvedilol in *Diluent*

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L68

**Column temperature:** 30°

**Flow rate:** 1.4 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** System suitability solution

**Suitability requirements**

**Resolution:** NLT 1.8 between carvedilol and carvedilol related compound F

**Analysis**

**Sample:** Sample solution

Calculate the percentage of each impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity in the *Sample solution*

$r_T$  = sum of all the peak responses in the *Sample solution*

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound A <sup>a</sup>	0.7	0.1
Carvedilol	1.0	—
Carvedilol related compound F <sup>b</sup>	1.2	0.1 <sup>c</sup>
N-Isopropylcarvedilol <sup>d</sup>	1.6	0.1
Carvedilol related compound C <sup>e</sup>	1.8	0.02
Carvedilol related compound B <sup>f</sup>	2.1	0.1
Biscarbazole <sup>g</sup>	3	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.5

<sup>a</sup> 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol.

<sup>b</sup> 1-(2-(2-Methoxyphenoxy)ethylamino)-3-(6,7,8,9-tetrahydro-5H-carbazol-4-yloxy)propan-2-ol.

<sup>c</sup> This impurity is quantitated using the procedure under *Organic Impurities, Procedure 3: Carvedilol Related Compound F*.

<sup>d</sup> 1-(H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]N-isopropylamino]-2-propanol.

<sup>e</sup> 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)propan-2-ol.

<sup>f</sup> 3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>g</sup> 1,3-Bis-(9H-carbazol-4-yloxy)-2-propanol.

• **ORGANIC IMPURITIES, PROCEDURE 3: CARVEDILOL RELATED COMPOUND F** (if present)

**Solution A:** Trifluoroacetic acid and water (0.5:100)

**Solution B:** Methanol and trifluoroacetic acid (100:0.5)

**Diluent:** Water and acetonitrile (1:1)

**Mobile phase:** *Solution A* and *Solution B* (65:35)

**System suitability solution:** 1.5 mg/mL of USP

Carvedilol System Suitability Mixture RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Carvedilol in *Diluent* prepared as follows. Use about 1.9 mL of *Diluent* per mg of the Carvedilol, and sonicate briefly to facilitate dissolution.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 30-mm; 3-μm packing L7

**Column temperature:** 40°

**Flow rate:** 2 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between carvedilol and carvedilol related compound F

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of carvedilol related compound F in the portion of the sample taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak response of carvedilol related compound F from the *Sample solution*

$r_T$  = sum of the peak responses of carvedilol and carvedilol related compound F from the *Sample solution*

$F$  = relative response factor, 1.1

Acceptance criteria: NMT 0.1%

**SPECIFIC TESTS**

- **LOSS ON DRYING** <731>: Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* by HPLC other than *Procedure 1* is used, then the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** <11>
  - USP Carvedilol RS
  - USP Carvedilol Related Compound A RS
    - 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol.
    - $C_{36}H_{43}N_3O_7$  629.74
  - USP Carvedilol Related Compound B RS
    - 3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).
    - $C_{39}H_{39}N_3O_6$  645.74
  - USP Carvedilol Related Compound C RS
    - 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)propan-2-ol.
    - $C_{31}H_{32}N_2O_4$  496.60
  - USP Carvedilol Related Compound D RS
    - 4-(Oxiran-2-ylmethoxy)-9H-carbazole.
    - $C_{15}H_{13}NO_2$  239.27
  - USP Carvedilol Related Compound E RS
    - 2-(2-Methoxyphenoxy)ethyl amine.
    - $C_9H_{13}NO_2$  167.21
  - USP Carvedilol System Suitability Mixture RS
    - Mixture of approximately 0.1% carvedilol related compound F (1-(2-(2-Methoxyphenoxy)ethylamino)-3-(2,3,4,9-tetrahydro-1H-carbazol-5-yloxy)propan-2-ol) in a matrix of carvedilol drug substance.

## Carvedilol Tablets

**DEFINITION**

Carvedilol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION** <197U>
  - Wavelength range:** 250–400 nm
  - Cell:** 0.2 cm
  - Sample solution:** 0.125 mg/mL of carvedilol prepared as follows. Place 10 Tablets in a 150-mL polypropylene tube, and disintegrate the Tablets in methanol (100 mL for the Tablet strengths 3.125, 6.25, and 25 mg, and 50 mL for the Tablet strength 12.5 mg) using a mechanical homogenizer. Transfer the homogenate to an appropriate volumetric flask, and dilute with methanol to volume. Pass through a suitable PTFE filter of 0.45-μm pore size.

**ASSAY**

• **PROCEDURE**

**Buffer:** Dissolve 0.7 g of anhydrous monobasic potassium phosphate in 500 mL of water, and add 10 mL of triethylamine. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Dissolve 1.04 g of sodium dodecyl sulfate in 150 mL of *Buffer* in a 2-L volumetric flask, and sonicate. Add 720 mL of acetonitrile, and dilute with water to volume. Pass through a nylon 66 filter of 0.2-μm pore size.

**Diluent:** Methanol and 1 M hydrochloric acid (9:1)

**Methanol solution:** Methanol and water (1:1)

**Standard solution:** 0.0125 mg/mL of USP Carvedilol RS prepared as follows. Dissolve a quantity of USP Carvedilol RS in a mixture of *Diluent* and water (9:1),



and sonicate until the solution is clear. Dilute with *Methanol solution* to obtain the required final concentration.

**Sample stock solution:** Transfer a portion of the powdered Tablets (NLT 20), equivalent to 25 mg of carvedilol, to a 100-mL volumetric flask. Add 10 mL of water, shake by hand, then add 70 mL of *Diluent*, and sonicate for 30 min. Shake on a mechanical shaker for about 30 min, and dilute with *Diluent* to volume to prepare a 0.25-mg/mL solution. Centrifuge an appropriate amount (about 50 mL) at 2000 rpm for 10 min.

**Sample solution:** 0.0125 mg/mL of carvedilol in *Methanol solution* from the *Sample stock solution*. Pass a portion of the solution through a suitable syringe filter of 0.45- $\mu$ m pore size, discard the first 5 mL, and use the filtrate as the *Sample solution*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  50-mm; packing L7

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Run time:** 30 min

**Injection size:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** 0.7% (7 mL/L) of hydrochloric acid, adjusted with 50% (w/w) sodium hydroxide to a pH of  $1.45 \pm 0.2$ ; 900 mL; deaerated

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard stock solution:** Transfer about 7 mg of USP Carvedilol RS to a 250-mL volumetric flask. Add 5 mL of methanol, and sonicate until dissolved. Cool to room temperature, dilute with *Medium* to volume, and mix well.

**Standard solution:** On the basis of the label claim and using the *Standard stock solution*, prepare a solution of USP Carvedilol RS in *Medium* having an appropriate concentration ( $C_S$ ), as shown in *Table 1*.

**Table 1**

Label Claim (mg)	$C_S$ (mg/mL)
25	0.028
12.5	0.014
6.25	0.007
3.125	0.0035

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Analytical wavelengths:** 285 and 380 nm

**Path length:** 1 cm

**Blank:** *Medium*

**Analysis:** Calculate the corrected absorbance of the *Standard solution* and the *Sample solution*:

$$A_{\text{corr}} = A_{285} - A_{380}$$

$A_{\text{corr}}$  = corrected absorbance of the *Standard solution* or the *Sample solution*

$A_{285}$  = absorbance of the *Standard solution* or the *Sample solution* at 285 nm

$A_{380}$  = absorbance of the *Standard solution* at 380 nm  
Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

$A_U$  = corrected absorbance from the *Sample solution*

$A_S$  = corrected absorbance from the *Standard solution*

$C_S$  = corrected concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Simulated gastric fluid without enzymes; 900 mL

**Apparatus 2, Time, Standard stock solution, Standard solution, Sample solution, and Analysis:** Proceed as directed in *Test 1*.

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** Simulated gastric fluid with pepsin, pH 1.45 (dissolve 12.0 g of sodium chloride and 19.2 g of purified pepsin (porcine origin, activity 800–2500 Units/mg of protein) in 18 mL of hydrochloric acid and sufficient water to make 6 L; adjust with hydrochloric acid to a pH of 1.45); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

**Mobile phase:** *Buffer* and acetonitrile (650:350)

**Standard stock solution:** 1.4 mg/mL of USP Carvedilol RS in methanol

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of ( $L/900$ ) mg/mL, where  $L$  is the Tablet label claim, in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-mm; 5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3500 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis:** Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905)**

**Buffer, Mobile phase, Diluent, Methanol solution, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** 0.25 mg/mL of carvedilol prepared as follows. Place 1 Tablet into a volumetric flask of appropriate size, based on the label claim. Add water to the flask up to about 10% of volume, and shake by hand to disintegrate the Tablet. Fill the flask up to 75% of volume with *Diluent*, and sonicate for 30 min to obtain complete disintegration. Shake on a mechanical shaker for 30 min, allow to cool, and dilute with *Diluent* to volume. Centrifuge an appropriate amount of this solution for 10 min at 2400 rpm, and transfer 4 mL of supernatant into a 100-mL volumetric flask. Fill the flask to about 85% of volume with *Methanol solution*, and sonicate for 20 min, with intermittent shaking. Dilute with *Methanol solution* to volume, and pass through a suitable syringe filter of 0.45- $\mu$ m pore size.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of carvedilol ( $C_{24}H_{26}N_2O_4$ ) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** Meet the requirements

**IMPURITIES**

• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, Diluent, Methanol solution, and Sample stock solution:** Prepare as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution* from the *Assay*.

**Standard solution:** 1.25  $\mu$ g/mL USP Carvedilol RS in a mixture of *Diluent* and water (1:1) from the *Standard stock solution*

**Sample solution:** Dilute with water to volume, 25 mL of the supernatant from the *Sample stock solution* in a 50-mL volumetric flask. Pass a portion of the solution through a suitable syringe filter of 0.45- $\mu$ m pore size.

**Chromatographic system:** Proceed as directed in the *Assay*, except for *Injection size*.

**Injection size:** 15  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of carvedilol from the *Standard solution*

$C_S$  = concentration of USP Carvedilol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 0.2% (specified or unspecified)

**Total impurities:** NMT 1.0%

[NOTE—Disregard any peaks with a relative retention time less than or equal to 0.04 and peaks with less than 0.05% of the nominal carvedilol peak response in the *Sample solution*.]

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers protected from moisture. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS (11)**  
 USP Carvedilol RS

## Casanthranol

» Casanthranol is obtained from *Cascara Sagrada*. It contains in each 100 g not less than 20.0 g of total hydroxyanthracene derivatives calculated on the dried basis, calculated as cascaroside A. Not less than 80 percent of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.

**Loss on drying (731)**—Dry it in vacuum at 80° for 16 hours: it loses not more than 10.0% of its weight.

**Residue on ignition (281):** not more than 4.0%.

**Heavy metals, Method II (231):** 0.0025%.

**Assay for total hydroxyanthracene derivatives**—[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrapment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

**Ferric chloride solution**—Dissolve 100 g of ferric chloride in water to make 100 mL.

**Assay solution**—Mix a portion of Casanthranol, and transfer an accurately weighed quantity of about 500 mg to a 100-mL volumetric flask. Add about 30 mL of 70 percent alcohol, swirl to dissolve, dilute with 70 percent alcohol to volume, and mix. Quickly filter through soft, rapid-flow filter paper, taking precautions to minimize loss by evaporation.

**Assay preparation**—Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water,

to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

**Procedure**—Pipet 10 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of total hydroxyanthracene derivatives in the portion of Casanthranol taken by the formula:

$$155A_U$$

in which  $A_U$  is the absorbance of the solution from the *Assay preparation*.

**Assay for cascarosides**—[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrapment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

**Ferric chloride solution and Assay solution**—Prepare as directed in the *Assay for total hydroxyanthracene derivatives*.

**Assay preparation**—Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

**Procedure**—Pipet 15 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory fun-

nel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance, against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of cascarosides in the portion of Casanthranol taken by the formula:

$$103.5A_U$$

in which  $A_U$  is the absorbance of the solution from the *Assay preparation*.

## Cascara Sagrada

### DEFINITION

Cascara Sagrada is the dried bark of *Frangula purshiana* (DC.) J. G. Cooper (syn. *Rhamnus purshiana* DC) (Fam. Rhamnaceae). It yields NLT 7.0% of total hydroxyanthracene derivatives, calculated as cascaroside A, and calculated on the dried basis. NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

[NOTE—Collect Cascara Sagrada not less than one year before use.]

### IDENTIFICATION

#### • A.

**Sample:** 100 mg of powdered Cascara Sagrada

**Analysis:** Add the *Sample* to 10 mL of hot water, shake the mixture occasionally until it is cold, filter, dilute the filtrate with water to 10 mL, and add 10 mL of 6 N ammonium hydroxide.

**Acceptance criteria:** An orange color is produced.

#### • B.

**Sample:** A portion of Cascara Sagrada

**Analysis:** Treat the *Sample* with 6 N ammonium hydroxide.

**Acceptance criteria:** It becomes red to reddish brown in color.

#### • C.

**Sample:** 100 mg of powdered Cascara Sagrada

**Analysis:** Macerate the *Sample* with 1 mL of alcohol, add 10 mL of water, boil the mixture, then cool, filter, and shake the filtrate with 10 mL of ether: a greenish-yellow ether layer separates. Shake 3 mL of the ether layer with 3 mL of 6 N ammonium hydroxide, and dilute the separated ammonia solution with 20 mL of water.

**Acceptance criteria:** A distinct orange-pink color remains.

### COMPOSITION

#### • CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrapment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Add 1 g of Cascara Sagrada to 70 mL of boiling water, boil for several min, with stirring. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter through suitable filter paper.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

Pipet 15 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*

$F$  = conversion factor, 138. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of of total hydroxyanthracene derivatives, calculated as cascaroside A:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = weight of Cascara Sagrada taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** NLT 7.0%, calculated on the dried basis

#### • CONTENT OF CASCAROSIDES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the

absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution and Sample stock solution:** Prepare as directed in the *Assay for Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

Proceed as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to evaporate a 20.0-mL portion of the methylene chloride solution instead of 15.0 mL.

**Sample:** *Sample solution*

Determine the absorbance and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*

$T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)

$F$  = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A on the dried basis

#### SPECIFIC TESTS

##### • BOTANIC CHARACTERISTICS

##### Macroscopic

**Cascara Sagrada:** The bark is usually in the form of flattened or transversely curved pieces, occasionally in quills of variable length and from 1 to 5 mm in thickness. The outer surface is brown, purplish brown, or brownish red, longitudinally ridged, with or without grayish or whitish lichen patches, sometimes with numerous lenticels and occasionally with moss attached.

The inner surface is longitudinally striate, light yellow, weak reddish brown, or moderate yellowish brown. The fracture is short with projections of phloem fiber bundles in the inner bark.

**Powdered Cascara Sagrada:** The powder is moderate yellowish brown to dusky yellowish orange.

#### Microscopic

**Cascara Sagrada:** The transverse section of the bark shows a yellowish-brown, purple, or reddish-brown cork of up to 10 or more rows of small cells; stone cells in yellowish, tangentially elongated groups of 20–50 cells in the cortex, pericycle, and outer phloem regions; phloem rays 1–4 cells wide, 15–25 cells deep, frequently diagonal or curved, forming converging groups; phloem fibers in small bundles, more or less surrounded by crystal fibers and located between the phloem rays; parenchyma with brown walls and containing starch grains and calcium oxalate crystals.

**Powdered Cascara Sagrada:** It shows numerous broken phloem fiber bundles with accompanying crystal fibers containing monoclinic prisms of calcium oxalate; stone cells more or less adherent, in small groups with thick, finely lamellated and porous walls; fragments of reddish-brown to yellow cork; masses of parenchyma and phloem ray cells colored reddish brown to orange upon the addition of a solution of an alkali; starch grains spheroidal, up to 8 µm in diameter; calcium oxalate in monoclinic prisms or rosette aggregates from 6 to 20 µm in diameter, occasionally up to 45 µm in diameter.

- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter** (561): NMT 4.0%
- **WATER DETERMINATION, Method III, Procedure for Articles of Botanical Origin** (921): Dry a sample at 105° for 5 h: it loses NMT 12.0% of its weight.

## Cascara Sagrada Extract

### DEFINITION

Cascara Sagrada Extract contains, in each 100 g, NLT 10.0 g and NMT 12.0 g of total hydroxyanthracene derivatives, of which NLT 50% consists of cascariosides, both calculated as cascarioside A.

Mix 900 g of Cascara Sagrada, in coarse powder, with 4000 mL of boiling water, and macerate the mixture for 3 h. Then transfer it to a percolator, allow it to drain, exhaust it by percolation, using boiling water as the menstruum, and collect 5000 mL of percolate. Evaporate the percolate to dryness, reduce the Extract to a fine powder, and, after assaying, add sufficient starch, dried at 100°, or other inert, nontoxic diluents to make the product contain, in each 100 g, 11 g of hydroxyanthracene derivatives. Mix the powders, and pass the Extract through a number 60 sieve.

### ASSAY

#### • CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Transfer 1 g of Extract to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow

to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, and dilute with water to volume, and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

**Analysis:** Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, and dilute with methylene chloride to volume. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5 mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*

$F$  = conversion factor, 155.2. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascarioside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = weight of Cascara Sagrada Extract taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** 10.0%–12.0% of total hydroxyanthracene derivatives, calculated as cascarioside A

#### • CONTENT OF CASCARIOSIDES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution and Sample stock solution:** Prepare as directed in the *Assay for Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

Pipet 25 mL of the *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Determine the absorbance and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*

$T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)

$F$  = conversion factor, 62.06. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 50% of total hydroxyanthracene derivatives calculated as cascaroside A

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.

## Cascara Tablets

#### DEFINITION

Cascara Tablets are prepared from Cascara Sagrada Extract.

They contain NLT 9.35% and NMT 12.65% of total hydroxyanthracene derivatives, calculated as cascaroside A, in the labeled amount of Cascara Sagrada Extract. NLT 50% of the hydroxyanthracene derivatives are cascarosides, calculated as cascaroside A.

#### STRENGTH

##### • CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrapment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 1 g of Cascara Sagrada Extract, to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride,

wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix.

Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol. Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*  
 $F$  = conversion factor, 206.9. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A, and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = nominal weight of Cascara Sagrada Extract in the portion of Tablets powder taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** 9.35%–12.65% in the labeled amount of Cascara Sagrada Extract, calculated as cascaroside A

#### • CONTENT OF CASCAROSIDES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions*.

**Ferric chloride solution and Sample stock solution:** Prepare as directed in *Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

Prepare as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to pipet 20 mL of *Sample solution*.

Determine the absorbance, and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract in the portion of Tablets powder taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*

$T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)

$F$  = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A, and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 50% of the content of total hydroxyanthracene derivatives are cascarosides, calculated as cascaroside A.

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701): 60 min
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers; if the Tablets are coated, well-closed containers may be used.

## Cascara Sagrada Fluidextract

#### DEFINITION

Prepare Cascara Sagrada Fluidextract as follows. To 1000 g of coarsely ground Cascara Sagrada add 3000 mL of boiling water, and allow to macerate in a suitable percolator for 2 h. Allow the percolation to proceed at a moderate rate, gradually adding boiling water until the drug is practically exhausted of its active principles. Evaporate the percolate on a water bath or in a vacuum still to NMT 800 mL. Cool, add 200 mL of alcohol and, if necessary, add sufficient water to make the product measure 1000 mL. Mix.

#### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I** (611): 18.0%–20.0% of  $C_2H_5OH$

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

## Aromatic Cascara Fluidextract

### DEFINITION

Prepare Aromatic Cascara Fluidextract as follows.

Cascara Sagrada, as a very coarse powder	1000 g
Magnesium Oxide	120 g
Suitable sweetening agent(s)	
Suitable essential oils(s)	
Suitable flavoring agent(s)	
Alcohol	200 mL
Purified Water, a sufficient quantity, to make	1000 mL

Mix the Cascara Sagrada with *Magnesium Oxide*, moisten it uniformly with 2000 mL of boiling water, and set it aside in a shallow container for 48 h, stirring occasionally. Pack it in a percolator, and percolate with boiling water until the material is exhausted. Evaporate the percolate, at a temperature not exceeding 100°, to 750 mL, and at once dissolve in it the flavoring agent(s). When the liquid has cooled, add the *Alcohol*, in which the sweetening agent(s) and oils have been dissolved, and add sufficient water to make the Aromatic Fluidextract measure 1000 mL. Mix.

### OTHER COMPONENTS

- **ALCOHOL DETERMINATION** (611): 18%–20% of  $C_2H_5OH$ , determined by the gas-liquid chromatographic method, using acetone as the internal standard

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

## Castor Oil

### DEFINITION

Castor Oil is the fixed oil obtained from the seed of *Ricinus communis* L. (Fam. Euphorbiaceae). It contains no added substances.

### IMPURITIES

- **HEAVY METALS, Method II** (231): NMT 10 ppm

### SPECIFIC TESTS

- **SPECIFIC GRAVITY** (841): 0.957–0.961
- **DISTINCTION FROM MOST OTHER FIXED OILS:** It is only partly soluble in solvent hexane (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of alcohol (foreign fixed oils).
- **FATS AND FIXED OILS, Free Fatty Acids** (401): The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization.
- **FATS AND FIXED OILS, Hydroxyl Value** (401)  
Free acid determination  
Sample: 10 g  
Titrimetric system  
(See *Titrimetry* (541).)  
Mode: Direct titration  
Titrant: 0.5 N alcoholic potassium hydroxide VS  
Endpoint detection: Visual  
Analysis: Transfer the *Sample* to a 250-mL conical flask, add 10 mL of pyridine that has been neutralized previously to phenolphthalein, and swirl to mix. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a

faint pink endpoint. Record the volume of *Titrant* consumed ( $V_A$ ).

### Hydroxyl value determination

Sample: 2 g

Blank: 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine

### Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 0.5 N alcoholic potassium hydroxide VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine, and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that has been neutralized previously to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Oil taken:

$$\text{Result} = \{[V_B + (W \times V_A/W_A) - V_T] \times M_r \times N\} / W$$

$V_B$  = volume of *Titrant* consumed by the *Blank* (mL)

$W$  = sample weight from the hydroxyl value determination (g)

$V_A$  = volume of *Titrant* consumed by the *Sample* in the free acid determination (mL)

$W_A$  = sample weight from the free acid determination (g)

$V_T$  = volume of *Titrant* consumed by the *Sample* in the hydroxyl value determination (mL)

$M_r$  = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq

$N$  = actual normality of the *Titrant*

Acceptance criteria: 160–168

- **FATS AND FIXED OILS, Iodine Value** (401): 83–88

- **FATS AND FIXED OILS, Saponification Value** (401): 176–182

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

## Castor Oil Capsules

### DEFINITION

Castor Oil Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of castor oil, calculated from the tests for *Weight Variation* and *Specific Gravity*.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197S)

Standard solution: 40 mg/mL of Castor Oil in chloroform

Sample solution: 40 mg/mL of the oil from Capsules in chloroform

Acceptance criteria: Meet the requirements

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements



**SPECIFIC TESTS**• **SPECIFIC GRAVITY** (841)

Sample: Capsule contents

Acceptance criteria: 0.957–0.961

• **FATS AND FIXED OILS, Free Fatty Acids** (401): The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization.• **FATS AND FIXED OILS, Hydroxyl Value** (401)

Free acid determination: Determine the amount of free acid in the Capsule contents.

Sample: 10 g of Capsule contents

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.5 N alcoholic potassium hydroxide VS

Endpoint detection: Visual

**Analysis 1:** Transfer the *Sample* to a 250-mL conical flask, add 10 mL of pyridine that has been neutralized previously to phenolphthalein, and swirl to mix. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint. Record the volume of *Titrant* consumed.

**Hydroxyl value determination:** Determine the hydroxyl value of the Capsule contents.

Sample: 2 g from the Capsule contents

Blank: 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 0.5 N alcoholic potassium hydroxide VS

Endpoint detection: Visual

**Analysis 2:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine, and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that previously has been neutralized to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Capsule contents taken:

$$\text{Result} = (M_r \times N/W) \times [B + (W \times A/C) - T]$$

$M_r$  = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq

$N$  = actual normality of the *Titrant*

$W$  = *Sample* weight for the hydroxyl determination (g)

$B$  = *Titrant* volume consumed by the *Blank* (mL)

$A$  = *Titrant* volume consumed by the *Sample* in the free acid determination (mL)

$C$  = *Sample* weight for the free acid determination (g)

$T$  = *Titrant* volume consumed by the *Sample* in the hydroxyl determination (mL)

**Acceptance criteria:** 160–168 in mg of KOH/g of Capsule content or hydroxyl value

• **FATS AND FIXED OILS, Iodine Value** (401)

Sample: Capsule contents

Acceptance criteria: 83–88

• **FATS AND FIXED OILS, Saponification Value** (401)

Sample: Capsule contents

Acceptance criteria: 176–182

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at controlled room temperature.

**Castor Oil Emulsion****DEFINITION**

Castor Oil Emulsion contains NLT 90.0% and NMT 120.0% of the labeled amount of Castor Oil.

**IDENTIFICATION**• **A.**

Sample: 10 mL of Emulsion well shaken

Analysis: Transfer the *Sample* to a 125-mL separator.

Add 10 mL of 1 N hydrochloric acid and 20 mL of solvent hexane. Shake vigorously for 2–3 min, allow the layers to separate, discard the aqueous phase, and filter the upper layer through anhydrous sodium sulfate into a small beaker. Evaporate the solvent on a steam bath, and to the residue add 1–2 drops of sulfuric acid.

**Acceptance criteria:** A red color indicates the presence of castor oil.

**ASSAY**• **PROCEDURE**

**Internal standard solution:** 12 mg/mL of di(2-ethylhexyl)phthalate in chloroform

**Standard solution:** Transfer 100 mg of castor oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.

**Sample solution:** Transfer an amount of Emulsion, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Prepare as directed in *Standard solution*, beginning with “Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid”.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 4-mm; packed with 4% liquid phase G25 on support S1

**Column conditioning:** Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.

**Temperature**

**Column:** 245°

**Injector:** 300°

**Detector:** 300°

**Flow rate:** Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an internal standard peak 8 min after introduction of the specimen.

**Carrier gas:** Helium

**Injection size:** 5 µL

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Measure the heights of the peaks due to castor oil and the internal standard.

Calculate the percentage of castor oil in the portion of Emulsion taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

$R_U$  = ratio of the heights of the peaks due to castor oil and internal standard, *Sample solution*

$R_S$  = ratio of the heights of the peaks due to castor oil and internal standard, *Standard solution*

$W_S$  = weight of castor oil taken to prepare the *Standard solution* (mg)

$W_U$  = nominal weight of castor oil in the amount of Emulsion taken to prepare the *Sample solution* (mg)

**Acceptance criteria:** 90.0%–120.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Aromatic Castor Oil

**DEFINITION**

Aromatic Castor Oil is Castor Oil containing suitable flavoring agents. It contains NLT 95.0% of castor oil.

**ASSAY**

• **PROCEDURE**

**Internal standard solution:** 12 mg/mL of di(2-ethylhexyl)phthalate in chloroform

**Standard solution:** Transfer 100 mg of Castor Oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask, through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.

**Sample solution:** Transfer an amount of Aromatic Castor Oil, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Proceed as directed for the *Standard solution*, beginning with “Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid...”.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.8-m × 4-mm column packed with 4% liquid phase G25 on support S1

**Column conditioning:** Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.

**Temperature**

**Column:** 245°

**Injector:** 300°

**Detector:** 300°

**Flow rate:** Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an internal standard peak 8 min after introduction of the specimen.

**Carrier gas:** Helium

**Injection size:** 5 µL

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Measure the heights of the peaks due to castor oil and the *Internal standard solution*.

Calculate the percentage of castor oil in the portion of Aromatic Castor Oil taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

$R_U$  = ratio of the heights of the peaks due to castor oil and the internal standard, *Sample solution*

$R_S$  = ratio of the heights of the peaks due to castor oil and the internal standard, *Standard solution*

$W_S$  = weight of Castor Oil taken to prepare the *Standard solution* (mg)

$W_U$  = nominal weight of castor oil in the sample of Aromatic Castor Oil taken to prepare the *Sample solution* (mg)

**Acceptance criteria:** NLT 95.0%

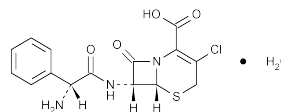
**OTHER COMPONENTS**

- **ALCOHOL DETERMINATION, Method I <611>:** NMT 4.0% of C<sub>2</sub>H<sub>5</sub>OH

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Cefaclor



C<sub>15</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub>S · H<sub>2</sub>O 385.82

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-, monohydrate, [6R-[6α,-7β(R\*)]]-

(6R,7R)-7-[(R)-2-Amino-2-phenylacetamido]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

3-Chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid monohydrate [70356-03-5].  
Anhydrous 367.81 [53994-73-3].

» Cefaclor has a potency of not less than 950 µg and not more than 1020 µg of C<sub>15</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub>S per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak for cefaclor in the chromatogram of the *Assay* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 3.0 and 4.5, in an aqueous suspension containing 25 mg per mL.

**Water, Method I** (921): between 3.0% and 6.5%.

**Related compounds**—

**Solvent**—Dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5.

**Blank solution**—Use the *Solvent*.

**Solution A**—Dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.0.

**Solution B**—Prepare a mixture of *Solution A* and acetonitrile (550:450), degassing for not more than 2 minutes.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Reducing the acetonitrile content increases the retention time of cefaclor and increases the resolution between cefaclor, delta-3 isomer and cefaclor.]

**Standard solution**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Solvent* to obtain a solution having a known concentration of about 0.05 mg per mL. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use this solution on the day it is prepared.]

**System suitability solution**—Dissolve a quantity of USP Cefaclor, Delta-3 Isomer RS in the *Standard solution* to obtain a solution having a concentration of about 0.05 mg per mL.

**Test solutions**—Transfer about 50 mg of Cefaclor, accurately weighed, to each of two 10-mL volumetric flasks, dilute each with *Solvent* to volume, and mix. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use these *Test solutions* within 2 hours when stored at room temperature or within 20 hours when stored under refrigeration.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0–30	95→75	5→25	linear gradient
30–45	75→0	25→100	linear gradient
45–55	0	100	isocratic
55–60	0→95	100→5	reset composition
60–70	95	5	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the retention time for the cefaclor peak is between 23 and 29 minutes; the resolution, *R*, between cefaclor, delta-3 isomer and cefaclor is not less than 2.0; and the tailing factor for the cefaclor peak is not more than 1.2. Chromatograph the *Blank solution* as directed for *Procedure*. Examine the chromatogram for any extraneous peaks, and disregard any corresponding peaks observed in the chromatogram of the *Test solutions*. [NOTE—Ensure that any extraneous peaks observed do not represent carryover from previous injections.]

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solutions* into the chromatograph, record the chromatograms, and measure all of the peak areas. Calculate the percentage of each cefaclor related compound in the portion of Cefaclor taken by the formula:

$$(CP/W)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard solution*; *P* is the designated potency, in µg per mg, of USP Cefaclor RS; *W* is the weight, in mg, of the portion of Cefaclor taken to prepare the respective *Test solution*; *r<sub>i</sub>* is the peak response of an individual related compound in the chromatogram obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response for the cefaclor peak in the chromatogram of the *Standard solution*. Determine the mean values for each cefaclor related compound: not more than 0.5% of any individual cefaclor related compound is found, and not more than 2.0% of total cefaclor related compounds is found. In an acceptable determination, the difference between duplicate determinations of total cefaclor related compounds is not more than 0.2% absolute, or the variation from the mean of the two values is not more than 10%, whichever is greater.

**Assay**—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, add 220 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 15 mg of USP Cefaclor RS, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this *Standard preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

**Assay preparation**—Transfer about 15 mg of Cefaclor, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this *Assay preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

**Resolution solution**—Prepare a solution in *Mobile phase* containing about 0.3 mg of cefaclor and 0.3 mg of USP Cefaclor, Delta-3 Isomer RS per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 25-cm column containing 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times for cefaclor and cefaclor, delta-3 isomer are about 0.8 and 1.0, the resolution, *R*, between the cefaclor peak and the cefaclor, delta-3 isomer peak is not less than 2.5, the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay* into the chromat-

ograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in  $\mu\text{g}$  per mg, of cefaclor ( $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$ ) in each mg of the Cefaclor taken by the formula:

$$(W_S / W_U)(P)(r_U / r_S)$$

in which  $W_S$  and  $W_U$  are the weights, in mg, of USP Cefaclor RS and of Cefaclor taken to prepare the *Standard preparation* and the *Assay preparation*, respectively;  $P$  is the designated potency, in  $\mu\text{g}$  of cefaclor ( $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$ ) per mg, of USP Cefaclor RS; and  $r_U$  and  $r_S$  are the peak responses of the cefaclor peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefaclor Capsules

» Cefaclor Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of cefaclor ( $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$ ) dissolved from UV absorbances at the wavelength of maximum absorbances at about 264 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Cefaclor RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of cefaclor ( $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 8.0%.

**Related compounds**—

**Diluent**—Dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5.

**Blank solution**—Use the *Diluent*.

**Solution A**—Dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.0.

**Solution B**—Prepare a mixture of *Solution A* and acetonitrile (55:45), degassing for no longer than 2 minutes.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Reducing the acetonitrile content increases the retention time of cefaclor and increases the resolution between the delta-3 isomer and cefaclor.]

**Standard solution**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Diluent* to obtain a solution having a known concentration of about 0.05 mg per mL of cefaclor. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use this solution on the day it is prepared.]

**System suitability solution**—Dissolve a quantity of USP Cefaclor, Delta-3 Isomer RS in the *Standard solution* to obtain a solution having a known concentration of about 0.05 mg per mL of the delta-3 isomer.

**Test solution**—Remove as completely as possible the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the combined contents, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Diluent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Diluent* to volume, mix, and filter. This solution has a nominal concentration of 5 mg per mL based on the label claim. [NOTE—Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0–30	95→75	5→25	linear gradient
30–45	75→0	25→100	linear gradient
45–55	0	100	isocratic
55–60	0→95	100→5	reset composition
60–70	95	5	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: identify the peaks by their relative retention times, which are about 0.85 and 1.0 for the delta-3 isomer and cefaclor, respectively; the resolution,  $R$ , between the delta-3 isomer and cefaclor is not less than 2.0; and the tailing factor for the cefaclor peak is not more than 1.2. Chromatograph the *Blank solution* as directed for *Procedure*. Examine the chromatogram for any extraneous peaks, and disregard any corresponding peaks observed in the chromatogram of the *Test solution*.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the percentage of each related compound in the portion of Capsules taken by the formula:

$$100(P)(C_S / C_U)(r_i / r_S)$$

in which  $P$  is the potency, in mg of cefaclor, per mg of USP Cefaclor RS;  $C_S$  is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard solution*;  $C_U$  is the nominal concentration, in mg per mL, of cefaclor in the *Test solution*;  $r_i$  is the peak response of an individual related compound in the chromatogram obtained from the *Test solution*; and  $r_S$  is the peak response for the cefaclor peak in the chromatogram of the *Standard solution*. The reporting level for impurities is 0.1%. Not more than 0.5% of any individual related compound is found; and the sum of all related compounds is not more than 2.0%.

**Assay**—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ , add 220 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL of cefaclor. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this

*Standard preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 75 mg of cefaclor, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the cefaclor. Filter to obtain the clear *Assay preparation*. The nominal concentration of this solution is 0.3 mg per mL of cefaclor based on the label claim.

*System suitability solution*—Dissolve accurately weighed quantities of USP Cefaclor RS and USP Cefaclor Delta-3 Isomer RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg each of cefaclor and the delta-3 isomer per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*: identify the peaks by their relative retention times, which are about 0.8 and 1.0 for cefaclor and the delta-3 isomer, respectively; the resolution, *R*, between the cefaclor peak and the delta-3 isomer peak is not less than 2.5; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percent label claim of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Capsules taken by the formula:

$$(P)(C_S / C_U)(r_U / r_S)(100)$$

in which *P* is the potency, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) per mg, of USP Cefaclor RS; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard preparation*; *C<sub>U</sub>* is the nominal concentration, in mg per mL, of cefaclor in the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the cefaclor peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefaclor for Oral Suspension

» Cefaclor for Oral Suspension is a dry mixture of Cefaclor and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{15}H_{14}ClN_3O_4S$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak for cefaclor in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 2.5 and 5.0, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 2.0%.

**Related compounds**—

*Solvent, Blank solution, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, and Chromatographic system*—Proceed as directed for *Related compounds* under Cefaclor.

*Test solution*—Constitute Cefaclor for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of Cefaclor for Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Solvent* to volume, mix, and filter. Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the mg of each related compound in the portion of Cefaclor for Oral Suspension taken by the formula:

$$0.01CP(r_i / r_S)$$

in which the terms are as defined for *Related compounds* under Cefaclor. Not more than 1.0% of any individual cefaclor-related compound is found; and the sum of all cefaclor-related compounds found is not more than 3.0%, not including the contribution of any peak that gives a result of less than 0.1%.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under Cefaclor.

*Assay preparation*—Constitute Cefaclor for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the resulting suspension, freshly mixed and free from air bubbles, dilute quantitatively with *Mobile phase* to obtain a final solution containing about 0.3 mg of cefaclor per mL. Sonicate if necessary to ensure complete dissolution of the cefaclor. Filter to obtain the clear *Assay preparation*.

*Procedure*—Proceed as directed in the *Assay* under Cefaclor. Calculate the quantity, in mg, of  $C_{15}H_{14}ClN_3O_4S$  in the portion of the constituted Cefaclor for Oral Suspension taken by the formula:

$$V_U(W_S / 50)(P/1000)(r_U / r_S)$$

in which *V<sub>U</sub>* is the final volume, in mL, of the *Assay preparation*, and the other terms are as defined therein.

## Cefaclor Chewable Tablets

» Cefaclor Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—The product label and product labeling indicate that the Chewable Tablets must be chewed or crushed before administration.

**USP Reference standards** (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—*Medium*: water; 900 mL.*Apparatus 2*: 50 rpm.*Time*: 30 minutes.

**Procedure**—Determine the amount of cefaclor dissolved by employing UV absorption at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cefaclor RS in the same *Medium*. Calculate the amount of cefaclor dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times D \times LC}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the solution under test and the Standard solution, respectively;  $C_S$  is the concentration, in mg per mL, of the Standard solution; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage;  $D$  is the dilution factor of the solution under test; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of cefaclor is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water**, *Method I* (921): not more than 5.0%.

**Related compounds**—

*Solvent*, *Blank solution*, *Solution A*, *Solution B*, *Mobile phase*, *Standard solution*, *System suitability solution*, and *Chromatographic system*—Proceed as directed for *Related compounds* under Cefaclor.

**Test solution**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the composite, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to dissolve. Avoid heating. Dilute with *Solvent* to volume, mix, and filter. [NOTE—Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the quantity, in mg, of each related compound in the portion of Chewable Tablets taken by the formula:

$$0.01CP(r_i / r_s)$$

in which the terms are as defined for *Related compounds* under Cefaclor. Not more than 1.0% of any individual cefaclor related compound is found; and the sum of all cefaclor related compounds found is not more than 3.0%, not including the contribution of any peak that gives a result of less than 0.1%.

**Assay**—

*Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under Cefaclor.

**Assay preparation**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of cefaclor, to a 250-mL volumetric flask, dilute with *Mobile*

*phase* to volume, and mix. Sonicate, if necessary, to dissolve the cefaclor. Filter to obtain a clear solution.

**Procedure**—Proceed as directed in the *Assay* under Cefaclor. Calculate the quantity, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Chewable Tablets taken by the formula:

$$5W_s(P/1000)(r_U / r_S)$$

in which the terms are as defined therein.

## Cefaclor Extended-Release Tablets

» Cefaclor Extended-Release Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—*Medium*: 0.1 N hydrochloric acid; 900 mL.*Apparatus 1* (10-mesh basket): 100 rpm.*Times*: 30, 60, and 240 minutes.

**Procedure**—Quantitatively dilute filtered portions of the solution under test with 0.1 N hydrochloric acid to obtain a test solution having a concentration of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) estimated to be about 25  $\mu$ g per mL. Determine the amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm, in comparison with a Standard solution having a similar, known concentration of USP Cefaclor RS in the same *Medium*.

**Tolerances**—The percentages of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) dissolved at the times specified conform to *Acceptance Table 2*.

Time (minutes)	Amount dissolved
30	between 5% and 30%
60	between 20% and 50%
240	not less than 80%

**Uniformity of dosage units** (905): meet the requirements.

**Water**, *Method I* (921): not more than 7.0%.

**Related compounds**—

*Solvent*, *Blank solution*, *Solution A*, *Solution B*, *Mobile phase*, *Standard solution*, *System suitability solution*, and *Chromatographic system*—Proceed as directed for *Related compounds* under Cefaclor.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the composite, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Solvent* to volume, mix, and filter. Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the mg of each related compound in the portion of Tablets taken by the formula:

$$0.01CP(r_i / r_s)$$

in which the terms are as defined for *Related compounds* under *Cefaclor*. Not more than 0.6% of any individual cefaclor-related compound is found; and the sum of all cefaclor-related compounds found is not more than 2.0%, not including the contribution of any peak that gives a result of less than 0.1%.

#### Assay—

**Mobile phase, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the *Assay* under *Cefaclor*.

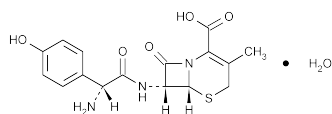
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of cefaclor, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate, if necessary, to dissolve the cefaclor. Filter to obtain a clear solution.

**Procedure**—Proceed as directed in the *Assay* under *Cefaclor*. Calculate the quantity, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Tablets taken by the formula:

$$5W_5(P/1000)(r_U / r_S)$$

in which the terms are as defined therein.

## Cefadroxil



$C_{16}H_{17}N_3O_5S \cdot H_2O$  381.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-, monohydrate [6R-[6 $\alpha$ ,7 $\beta$ (R\*)]]-

(6R,7R)-7-[(R)-2-Amino-2-(p-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [66592-87-8].

Hemihydrate 372.39 [119922-85-9].

Anhydrous 363.40 [50370-12-2].

» Cefadroxil has a potency equivalent to not less than 950  $\mu$ g and not more than 1050  $\mu$ g of  $C_{16}H_{17}N_3O_5S$  per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The hemihydrate form is so labeled.

**USP Reference standards** (11)—

USP Cefadroxil RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** Place a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of binder-free silica gel in a chamber containing a mixture of *n*-hexane and tetradecane (95:5) to a depth of about 1 cm, allow the solvent front to move the length of the plate, remove the plate from the chamber, and allow the solvent

to evaporate. Apply 20  $\mu$ L each of a solution of Cefadroxil in water containing 2 mg per mL and a similarly prepared *Standard solution* of USP Cefadroxil RS to this plate. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and a 1 in 15 solution of ninhydrin in acetone (60:40:1.5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with a 1 in 500 solution of ninhydrin in dehydrated alcohol [NOTE—Protect this solution from light], dry for 10 minutes at 110°, and examine the chromatogram: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Specific rotation** (781S): between +165.0° and +178.0°.

**Test solution:** 10 mg per mL, in water.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.0 and 6.0, in a suspension containing 50 mg per mL.

**Water, Method I** (921): between 4.2% and 6.0%, except that where it is labeled as being in the hemihydrate form it is between 2.4% and 4.5%.

**Chromatographic purity**—

**Adsorbent:** a 0.25-mm layer of chromatographic silica gel mixture.

**Solvent**—Prepare a mixture of alcohol, water, and 2.4 N hydrochloric acid (75:22:3).

**Test solution**—Prepare a solution of Cefadroxil in *Solvent* containing 25 mg per mL.

**Standard solution 1**—Dilute 1.0 mL of the *Test solution* with *Solvent* to 100 mL, and mix.

**Standard solution 2**—Prepare a solution in *Solvent* containing 0.25 mg each of 7-aminodesacetoxycephalosporanic acid and D- $\alpha$ -4-hydroxyphenylglycine per mL.

**Standard solution 3**—Prepare a solution in *Solvent* containing 0.25 mg of D- $\alpha$ -4-hydroxyphenylglycine per mL.

**Resolution solution**—Mix 1.0 mL of the *Test solution* and 1.0 mL of *Standard solution 2*.

**Developing solvent system:** a mixture of ethyl acetate, alcohol, water, and formic acid (14:5:5:1).

**Procedure**—Apply separate 2- $\mu$ L portions of the *Test solution*, *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*, and a 4- $\mu$ L portion of the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography* under *Chromatography* (621)), and develop the chromatograms until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the plate to dry, and examine the chromatograms under short-wavelength UV light: any secondary spot in the chromatogram obtained from the *Test solution* corresponding to 7-aminodesacetoxycephalosporanic acid or D- $\alpha$ -4-hydroxyphenylglycine is not more intense than the corresponding spot in the chromatogram obtained from *Standard solution 2* (1.0%); and any spot, other than the principal spot and any spot corresponding to 7-aminodesacetoxycephalosporanic acid or D- $\alpha$ -4-hydroxyphenylglycine, is not more intense than the principal spot in the chromatogram obtained from *Standard solution 1* (1.0%). In a valid test, the chromatogram obtained from the *Resolution solution* shows three clearly separated spots.

**Dimethylaniline** (223): meets the requirement.

**Assay**—

**pH 5.0 Buffer**—Dissolve 13.6 g of monobasic potassium phosphate in water to make 2000 mL of solution. Adjust with 10 N potassium hydroxide to a pH of 5.0, and mix.

**Mobile phase**—Prepare a suitable mixture of *pH 5.0 Buffer* and acetonitrile (960:40), and pass through a filter having a 0.5- $\mu$ m or finer porosity. Make adjustments if necessary (see

*System Suitability* under *Chromatography* (621)). Increasing the acetonitrile content of the *Mobile phase* decreases the retention time of cefadroxil, and decreasing the acetonitrile content increases the retention time.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefadroxil RS in *pH 5.0 Buffer* to obtain a solution having a known concentration of about 1.06 mg per mL. This solution contains the equivalent of about 1000 µg of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) per mL. Use this solution on the day prepared.

**Assay preparation**—Transfer about 212 mg of Cefadroxil, accurately weighed, to a 200-mL volumetric flask, dilute with *pH 5.0 Buffer* to volume, and stir by mechanical means for 5 minutes until dissolved. Use this solution on the day prepared.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the capacity factor,  $k'$ , is between 2.0 and 3.5; the column efficiency determined from the analyte peak is not less than 1800 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) in each mg of the Cefadroxil taken by the formula:

$$200(CE/W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cefadroxil RS taken to prepare the *Standard preparation*;  $E$  is the cefadroxil equivalent, in µg per mg, of USP Cefadroxil RS;  $W$  is weight, in mg, of the portion of Cefadroxil taken; and  $r_U$  and  $r_S$  are the cefadroxil peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefadroxil Capsules

» Cefadroxil Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{16}H_{17}N_3O_5S$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Capsules prepared using the hemihydrate form of Cefadroxil are so labeled.

**USP Reference standards** (11)—  
USP Cefadroxil RS

**Identification**—Mix the contents of 1 Capsule with water to obtain a concentration of about 2 mg of cefadroxil per mL, and filter: the filtrate so obtained responds to *Identification test B* under *Cefadroxil*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{17}N_3O_5S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 263 nm of filtered portions of the solution under test, suitably diluted with water if necessary, in

comparison with a Standard solution having a known concentration of USP Cefadroxil RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{17}N_3O_5S$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 7.0%.

**Assay**—

*pH 5.0 Buffer, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefadroxil*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 10 Capsules, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 200 mg of cefadroxil, to a 200-mL volumetric flask, dilute with *pH 5.0 Buffer* to volume, and stir by mechanical means for 5 minutes. Use this solution on the day prepared.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefadroxil*. Calculate the quantity, in mg, of  $C_{16}H_{17}N_3O_5S$  in the portion of Capsules contents taken by the formula:

$$0.2CE(r_U / r_S)$$

in which the terms are as defined therein.

## Cefadroxil for Oral Suspension

### DEFINITION

Cefadroxil for Oral Suspension is a dry mixture of Cefadroxil and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of  $C_{16}H_{17}N_3O_5S$ .

### IDENTIFICATION

#### • THIN-LAYER CHROMATOGRAPHY

**Standard solution**: 2 mg/mL of USP Cefadroxil RS

**Sample solution**: Constitute 1 container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with water to a concentration of 2 mg/mL. Pass through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode**: TLC

**Adsorbent**: 0.25-mm layer of binder-free silica gel

**Application volume**: 20 µL

**Pre-developing solvent system**: *n*-Hexane and tetradecane (95:5)

**Solution A**: 1 in 15 solution of ninhydrin in acetone

**Developing solvent system**: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Solution A* (60:40:15)

**Spray reagent**: 1 in 500 solution of ninhydrin in dehydrated alcohol. Protect this solution from light.

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Place the thin-layer chromatographic plate in a chamber containing the *Pre-developing solvent system* and allow the solvent front to move the length of the plate. Remove the plate from the chamber and allow the solvent to evaporate. Apply the *Sample solution* and *Standard solution* to the plate, allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with the *Spray reagent*, dry for 10 min at 110°, and examine the chromatogram.



**Acceptance criteria:** The  $R_F$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

## ASSAY

### • PROCEDURE

**Buffer:** 6.86 g/L of monobasic potassium phosphate. Adjust with 10 N potassium hydroxide to a pH of 5.0.

**Mobile phase:** Acetonitrile and *Buffer* (40:960)

**Standard solution:** 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. Use this solution on the day prepared. [NOTE—This solution contains the equivalent of 1 mg/mL of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ).]

**Sample solution:** Constitute a container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with *Buffer* to prepare a solution containing nominally 1 mg/mL. Pass through a suitable filter of 0.8- $\mu$ m or finer pore size, and use the filtrate. Use this solution on the day prepared.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Capacity factor,  $k'$ :** 2.0–3.5

**Column efficiency:** NLT 1800 theoretical plates

**Tailing factor:** NMT 2.2

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) in the portion of Cefadroxil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of cefadroxil from the *Sample solution*

$r_S$  = peak response of cefadroxil from the *Standard solution*

$C_S$  = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)

$P$  = potency of cefadroxil in USP Cefadroxil RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 25 rpm

**Time:** 30 min

**Standard solution:** USP Cefadroxil RS in *Medium* at a known concentration

**Sample solution:** Transfer 5.0 mL of the constituted Cefadroxil for Oral Suspension (weighed) to the dissolution vessel.

**Analysis:** Determine the amount of cefadroxil dissolved by employing UV absorption at the wavelength of 263 nm on the *Sample solution* in comparison with the *Standard solution*.

Calculate the amount of cefadroxil dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/D) \times V$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor

**Tolerances:** NLT 75% (Q) of the labeled amount of cefadroxil is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>

**For solid packaged in single-unit containers:** Meets the requirements

### • DELIVERABLE VOLUME <698>: Meets the requirements

## SPECIFIC TESTS

• **pH <791>:** 4.5–6.0, in the suspension constituted as directed in the labeling

• **WATER DETERMINATION, Method I <921>:** NMT 2.0%, except where it is labeled as containing 100 mg of cefadroxil per mL after constitution, in which case the limit is NMT 3.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS <11>**  
USP Cefadroxil RS

## Cefadroxil Tablets

» Cefadroxil Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{16}H_{17}N_3O_5S$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The Tablets prepared using the hemihydrate form of Cefadroxil are so labeled.

**USP Reference standards <11>**—  
USP Cefadroxil RS

**Identification**—Mix a quantity of powdered Tablets, equivalent to about 250 mg of cefadroxil, with water to obtain a concentration of about 2 mg of cefadroxil per mL, and filter: the filtrate so obtained responds to *Identification test B* under *Cefadroxil*.

### Dissolution <711>—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{17}N_3O_5S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 263 nm of filtered portions of the solution under test, suitably diluted with water if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefadroxil RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{17}N_3O_5S$  is dissolved in 30 minutes.

**Uniformity of dosage units <905>:** meet the requirements.

**Water, Method I <921>:** not more than 8.0%.

### Assay—

*pH 5.0 Buffer, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay under Cefadroxil*.

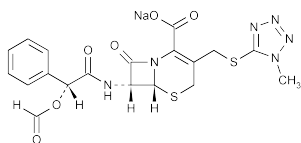
**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of cefadroxil, to a 200-mL volumetric flask, dilute with *pH 5.0 Buffer* to volume, and stir by mechanical means for 5 minutes. Use this solution on the day prepared.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefadroxil*. Calculate the quantity, in mg, of  $C_{16}H_{17}N_3O_5S$  in the portion of Tablets taken by the formula:

$$0.2CE(r_U / r_S)$$

in which the terms are as defined therein.

## Cefamandole Nafate



$C_{19}H_{17}N_6NaO_6S_2$  512.49  
 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(formyloxy)phenylacetyl]amino]-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (R\*)]]-;  
 Sodium (6R,7R)-7-(R)-mandelamido-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate formate (ester) [42540-40-9].

### DEFINITION

Cefamandole Nafate has a potency equivalent to NLT 810  $\mu$ g/mg and NMT 1000  $\mu$ g/mg of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5

**Mobile phase:** Acetonitrile and *Buffer* (25:75)

**Standard solution:** 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.

**System suitability solution:** 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.

**Sample solution:** 0.5 mg/mL of Cefamandole Nafate in *Mobile phase*. Use this solution immediately after it is prepared.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 7.0 between the two main peaks, *System suitability solution*

**Relative standard deviation:** NMT 0.8% for the cefamandole peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration, in  $\mu$ g/mg, of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ) in the portion of Cefamandole Nafate taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times (M_{r1} / M_{r2}) \times P \times F$$

- $r_U$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Sample solution*
  - $r_S$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*
  - $C_S$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)
  - $C_U$  = concentration of the *Sample solution* (mg/mL)
  - $M_{r1}$  = molecular weight of cefamandole, 462.50
  - $M_{r2}$  = molecular weight of cefamandole nafate, 512.49
  - $P$  = potency of cefamandole nafate in USP Cefamandole Nafate RS (mg/mg)
  - $F$  = conversion factor, 1000  $\mu$ g/mg
- Acceptance criteria:** 810–1000  $\mu$ g/mg on the anhydrous basis

### SPECIFIC TESTS

#### PH <791>

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 3.5–7.0

#### WATER DETERMINATION, Method I <921>

**Acceptance criteria:** NMT 2.0%

#### BACTERIAL ENDOTOXINS TEST (85)

NMT 0.15 USP Endotoxin Unit/mg of cefamandole, when the label states that Cefamandole Nafate is sterile or must be subjected to further processing during the preparation of injectable dosage forms

#### STERILITY TESTS <71>

Meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration* when the label states that Cefamandole Nafate is sterile

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- USP REFERENCE STANDARDS <11>**  
 USP Cefamandole Nafate RS  
 USP Endotoxin RS

## Cefamandole Nafate for Injection

### DEFINITION

Cefamandole Nafate for Injection is a sterile mixture of Cefamandole Nafate and one or more suitable buffers. It has a potency equivalent to NLT 810  $\mu$ g/mg and NMT 1000  $\mu$ g/mg of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ), calculated on the anhydrous and sodium carbonate-free basis. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5

**Mobile phase:** Acetonitrile and *Buffer* (25:75)

**Standard solution:** 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.

**System suitability solution:** 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.

**Sample solution:** Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection

in *Mobile phase*. Use this solution immediately after it is prepared.

**Sample solution 1** (where the article is represented as being in a single-dose container): Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute a container of Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase*.

**Sample solution 2** (where the label states the quantity of cefamandole in a given volume of constituted solution): Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Dilute a volume of the constituted solution with *Mobile phase*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 7.0 between the two main peaks, *System suitability solution*

**Relative standard deviation:** NMT 0.8% for the cefamandole peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of cefamandole (C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>) in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times 100$$

$r_U$  = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*

$r_S$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*

$C_S$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefamandole in *Sample solution 1* or in *Sample solution 2* (mg/mL)

$M_{r1}$  = molecular weight of cefamandole, 462.50

$M_{r2}$  = molecular weight of cefamandole nafate, 512.49

$P$  = potency of USP Cefamandole Nafate RS (mg/mg)

Calculate the potency, in μg/mg, of cefamandole (C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>) in the portion of Cefamandole Nafate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times F$$

$r_U$  = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*

$r_S$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*

$C_S$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefamandole in *Sample solution 1* or *Sample solution 2* (mg/mL)

$M_{r1}$  = molecular weight of cefamandole, 462.50

$M_{r2}$  = molecular weight of cefamandole nafate, 512.49

$P$  = potency of USP Cefamandole Nafate RS (mg/mg)

$F$  = conversion factor, 1000 μg/mg

**Acceptance criteria:** 810–1000 μg/mg of C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>, calculated on the anhydrous and sodium carbonate-free basis; 90.0%–115.0% of the labeled amount of C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

**Procedure for content uniformity:** Perform the *Assay* on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.

**Acceptance criteria:** Meets the requirements

#### SPECIFIC TESTS

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* <1>, *Constituted Solutions*.

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.15 USP Endotoxin Unit/mg of cefamandole

• **STERILITY TESTS (71):** It meets the requirements for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

• **PH (791)**

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 6.0–8.0, determined after 30 min

• **PARTICULATE MATTER (788):** Meets the requirements for small-volume injections

• **WATER DETERMINATION, Method I (921):** NMT 3.0%

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* <1>.

#### ADDITIONAL REQUIREMENTS

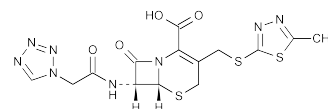
• **PACKAGING AND STORAGE:** Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

• **USP REFERENCE STANDARDS (11)**

USP Cefamandole Nafate RS

USP Endotoxin RS

## Cefazolin



C<sub>14</sub>H<sub>14</sub>N<sub>8</sub>O<sub>4</sub>S<sub>3</sub> 454.51

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[[1*H*-tetrazol-1-yl)acetyl]amino]-, (6*R*-*trans*).

(6*R*,7*R*)-3-[[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [25953-19-9].

» Cefazolin contains not less than 95.0 percent and not more than 103.0 percent of C<sub>14</sub>H<sub>14</sub>N<sub>8</sub>O<sub>4</sub>S<sub>3</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Cefazolin RS

**Identification**—The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, Method I (921): not more than 2.0%.

**Heavy metals**, Method II (231): 0.002%.

**Assay—**

*pH 3.6 Buffer*—Dissolve 0.900 g of anhydrous dibasic sodium phosphate and 1.298 g of citric acid monohydrate in water to make 1000 mL.

*pH 7.0 Buffer*—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL.

*Mobile phase*—Prepare a suitable mixture of *pH 3.6 Buffer* and acetonitrile (9:1). Pass through a membrane filter having a 10- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Transfer 750 mg of salicylic acid to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *pH 7.0 Buffer* to volume, and mix.

*Standard preparation*—Transfer about 25 mg of USP Cefazolin RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

*Assay preparation*—Proceed as directed for *Standard preparation*, except to use about 25 mg of Cefazolin, accurately weighed.

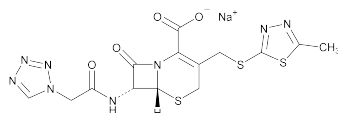
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for cefazolin; the resolution, *R*, between the analyte and internal standard peaks is not less than 4.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{13}N_8O_4S_3$  in the portion of Cefazolin taken by the formula:

$$500C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of cefazolin to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$  476.49  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[[[(1H-tetrazol-1-yl)acetyl]amino]-, monosodium salt (6*R-trans*);  
Monosodium (6*R,7R*)-3-[[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[2-(1H-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [27164-46-1].

### DEFINITION

Cefazolin Sodium has a potency equivalent to NLT 89.1% and NMT 110.1% of cefazolin sodium ( $C_{14}H_{13}NaN_8O_4S_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

**A. ULTRAVIOLET ABSORPTION (197U)**

**Sample solution:** 20  $\mu$ g/mL in 0.1 M sodium bicarbonate

**Acceptance criteria:** Meets the requirements

**B.** The retention time of the major peak for cefazolin in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**C. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements

### ASSAY

**PROCEDURE**

**Buffer A:** 0.9 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate in water

**Buffer B:** 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer A* (1:9). Pass through a membrane filter having a 10- $\mu$ m or finer pore size.

**Internal standard solution:** 7.5 mg/mL of salicylic acid in methanol and *Buffer B* (1:9). Dissolve first in methanol, using 10% of the final volume, and dilute with water to volume.

**Standard stock solution:** 1 mg/mL of USP Cefazolin RS in *Buffer B*

**Standard solution:** 50  $\mu$ g/mL of cefazolin from the *Standard stock solution* and 0.4 mg/mL of salicylic acid from the *Internal standard solution* in *Buffer B*

**Sample stock solution:** 1 mg/mL of Cefazolin Sodium in *Buffer B*

**Sample solution:** 50  $\mu$ g/mL of cefazolin sodium from the *Sample stock solution* and 0.4 mg/mL of salicylic acid from the *Internal standard solution* in *Buffer B*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.0-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for salicylic acid and cefazolin are about 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 4.0 between the analyte and the internal standard peaks

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cefazolin sodium ( $C_{14}H_{13}N_8NaO_4S_3$ ) in the portion of Cefazolin Sodium taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times (M_{r1} / M_{r2}) \times 100$$

*R<sub>U</sub>* = peak response ratio of cefazolin to the internal standard from the *Sample solution*

*R<sub>S</sub>* = peak response ratio of cefazolin to the internal standard from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of cefazolin sodium, 476.49  
 $M_{r2}$  = molecular weight of cefazolin, 454.51  
**Acceptance criteria:** 89.1%–110.1% on the anhydrous basis

**IMPURITIES****• ORGANIC IMPURITIES**

[NOTE—Use the *Sample solution* immediately after preparation.]

**Buffer A:** 6.8 g/L of monobasic potassium phosphate  
**Solution B:** 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution  
**Solution C:** Acetonitrile and *Buffer A* (1:1)  
**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2

**Blank:** Use *Solution B*.

**System suitability stock solution:** 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [NOTE—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]

**System suitability solution:** *System suitability stock solution* and *Buffer B* (1:24)

**Standard solution:** 25 µg/mL of USP Cefazolin RS in *Solution B*

**Sample solution:** 2.5 mg/mL of Cefazolin Sodium in *Solution B*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 8.0 between cefazolin and cefazolin epimer, 254 nm

**Analysis**

**Samples:** *Blank*, *Standard solution*, and *Sample solution*  
 Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(210)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(210)}$  = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the *Sample solution*

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the *Standard solution*

$C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Table 2**

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid <sup>a</sup>	210	0.07	0.40	1.0
Tetrazolylacetamide acetal <sup>b</sup>	210	0.08	0.33	1.0
<sup>c</sup> Cefazolin open-ring lactone <sup>d</sup> or Cefazolin 3-hydroxy-methyl <sup>e</sup>	254	0.20	1.0	0.5
Methylthiadiazole thiol <sup>f</sup>	254	0.23	0.91	1.0
7-Aminocephalosporanic acid <sup>g</sup>	254	0.42	1.1	1.0
Cefazolin 3-methyl analog <sup>h</sup>	254	0.44	0.87	1.0
Cefazolin lactone <sup>i</sup>	254	0.50	0.85	1.0
Cefazolin acetoxymethyl analog <sup>j</sup>	254	0.61	0.68	1.0
Cefazolin deacylated <sup>k</sup>	254	0.68	1.2	1.0
Cefazoloic acid isomers <sup>l</sup>	254	0.84	1.0	1.0

<sup>a</sup> 2-(1*H*-Tetrazol-1-yl)acetic acid.

<sup>b</sup> *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>c</sup> The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes <sup>d</sup> and <sup>e</sup>.

<sup>d</sup> (6*R*)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

<sup>g</sup> (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

<sup>h</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> *N*-(5*aR*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>j</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows:

Cefazolin open-ring delta-3: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

Cefazolin open-ring delta-2: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

Cefazolin open-ring delta-4: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>m</sup> (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Table 2 (Continued)

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefazolin	254	1.0	—	—
Cefazolin epimer <sup>m</sup>	254	1.2	0.98	1.0
Cefazolin pivaloyl <sup>n</sup>	254	1.4	0.92	1.0
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

<sup>a</sup> 2-(1*H*-Tetrazol-1-yl)acetic acid.

<sup>b</sup> *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>c</sup> The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes <sup>d</sup> and <sup>e</sup>.

<sup>d</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

<sup>g</sup> (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

<sup>h</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> *N*-[(5*aR*,6*R*)-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>j</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows:

Cefazolin open-ring delta-3: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

Cefazolin open-ring delta-2: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

Cefazolin open-ring delta-4: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>m</sup> (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(254)} / r_{S(254)}) \times (C_S / C_U) \times (1/F) \times 100$$

$r_{U(254)}$  = peak response of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal at 254 nm from the *Sample solution*

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the *Standard solution*

$C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard peaks corresponding to those in the *Blank*.

## SPECIFIC TESTS

### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 55 mg/mL, in 0.1 M sodium bicarbonate

**Acceptance criteria:**  $-10^\circ$  to  $-24^\circ$

### • pH (791): 4.0–6.0, in a solution containing 100 mg/mL of cefazolin

### • WATER DETERMINATION, *Method I* (921): NMT 6.0%

### • STERILITY TESTS (71): Where the label states that Cefazolin Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

### • BACTERIAL ENDOTOXINS TEST (85): Where the label states that Cefazolin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of cefazolin.

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight containers.

### • LABELING: Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

## • USP REFERENCE STANDARDS (11)

USP Cefazolin RS

USP Endotoxin RS

## Cefazolin Injection

» Cefazolin Injection is a sterile solution of Cefazolin and Sodium Bicarbonate in a diluent containing one or more suitable tonicity-adjusting agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

### USP Reference standards (11)—

USP Cefazolin RS

USP Endotoxin RS

**Identification**—The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to Be Examined*.

**pH** (791): between 4.5 and 7.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay—**

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Cefazolin*.

*Assay preparation*—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefazolin, to a 50-mL volumetric flask, dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Cefazolin*. Calculate the quantity, in mg, of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in each mL of the Injection taken by the formula:

$$(1000C / V)(R_U / R_S)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Cefazolin for Injection

» Cefazolin for Injection contains an amount of Cefazolin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cefazolin RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification—**

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 20 µg per mL.

*Medium:* 0.1 M sodium bicarbonate.

**B:** The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** It meets the requirements of the tests for *Sodium* (191).

**Specific rotation** (781S): between  $-10^\circ$  and  $-24^\circ$ .

*Test solution:* 55 mg per mL, in 0.1 M sodium bicarbonate.

**Bacterial endotoxins** (85)—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements.

*Procedure for content uniformity*—Perform the Assay on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

**pH** (791): between 4.0 and 6.0, in a solution containing 100 mg of cefazolin per mL.

**Water, Method I** (921): not more than 6.0%.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Labeling* under *Injections* (1).

**Assay—**

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Cefazolin*.

*Assay preparation 1* (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 buffer* to volume, and mix.

*Assay preparation 2* (where the label states the quantity of cefazolin in a given volume of constituted solution)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 buffer* to volume, and mix.

*Procedure*—Proceed as directed in the Assay under *Cefazolin*. Calculate the quantity, in mg, of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in the container, and in the volume of constituted solution taken by the formula:

$$(CL / D)(R_U / R_S)$$

in which *L* is the labeled quantity, in mg, of cefazolin in the container, or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of cefazolin in the stock solution used in preparing *Assay preparation 1* or *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the volume of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the Assay value.

## Cefazolin Ophthalmic Solution

**DEFINITION**

Cefazolin Ophthalmic Solution contains an amount of cefazolin sodium equivalent to NLT 29.7 mg and NMT 36.3 mg of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in 10.0 mL of Ophthalmic Solution.

Use Cefazolin Sodium or Cefazolin for Injection that contains the designated amount of cefazolin, and prepare the Ophthalmic Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Cefazolin Sodium	35 mg
Thimerosal	0.2 mg
Sodium Chloride Injection (0.9%), a sufficient quantity to make	10.0 mL

Dissolve *Cefazolin Sodium* and *Thimerosal* in *Sodium Chloride Injection* (0.9%), and dilute quantitatively, and stepwise if necessary, with *Sodium Chloride Injection* (0.9%) to obtain a solution containing 3.5 mg/mL of *Cefazolin Sodium* and 0.02 mg/mL of *Thimerosal*. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution. If Cefazolin for Injection is used, prepare the Ophthalmic Solution as follows. Dissolve *Thimerosal* in *Sodium Chloride Injection* (0.9%), and dilute

quantitatively, and stepwise if necessary, with *Sodium Chloride Injection* (0.9%) to obtain a solution containing 0.3 mg/mL of *Thimerosal*. Add 9.8 mL of the resulting solution to a vial of *Cefazolin for Injection*, containing 500 mg of cefazolin, and mix to obtain a stock solution. Transfer 3.3 mL of the stock solution to a 50-mL volumetric flask, dilute with *Sodium Chloride Injection* (0.9%) to volume, and mix. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution.

## ASSAY

### PROCEDURE

**Buffer A:** 0.900 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate; this solution should have a pH of 3.6.

**Buffer B:** 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L monobasic potassium phosphate; this solution should have a pH of 7.0.

**Solution A:** Acetonitrile and *Buffer A* (10:90). Pass through a filter having a pore size of 5- $\mu$ m or finer, and degas.

**Solution B:** Acetonitrile and *Buffer A* (80:20). Pass through a filter having a pore size of 5- $\mu$ m or finer, and degas.

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	0	100
25	100	0

**Standard solution:** 0.32 mg/mL of USP Cefazolin RS in *Buffer B*. Maintain at 4° before injection. Use low-actinic volumetric glassware.

**Sample solution:** Transfer 1.0 mL of Ophthalmic Solution to a 10-mL low-actinic volumetric flask, dilute with *Buffer B* to volume, and mix. Maintain at 4° before injection.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Column temperature:** 25°

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in mg, of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in 10 mL of Ophthalmic Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)  
 $D$  = dilution factor, 10  
 $V$  = final volume of Ophthalmic Solution, 10 mL

**Acceptance criteria:** 29.7–36.3 mg

## SPECIFIC TESTS

### STERILITY

(See *Pharmaceutical Compounding—Nonsterile Preparations* <795>, *General Guidelines for Assigning Beyond-Use Dates*.)

**Acceptance criteria:** Meets the requirements

**pH <791>:** 4.5–6.0

## ADDITIONAL REQUIREMENTS

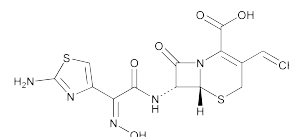
**PACKAGING AND STORAGE:** Package in tight, sterile ophthalmic containers. Store in a refrigerator.

**LABELING:** Label it to state that it is intended for use in the eye, and is not to be used if a precipitate is present.

**BEYOND-USE DATE:** NMT 5 days after the date on which it was compounded

**USP REFERENCE STANDARDS <11>**  
 USP Cefazolin RS

## Cefdinir



$C_{14}H_{13}N_5O_5S_2$  395.41

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-;

(-)-(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7 $\beta$ -(Z)-oxime [91832-40-5].

## DEFINITION

Cefdinir contains NLT 940  $\mu$ g/mg and NMT 1030  $\mu$ g/mg of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ), calculated on the anhydrous basis.

## IDENTIFICATION

**A. INFRARED ABSORPTION <197M>**

**B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

**Solution A:** 14.2 g/L of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 g/L of monobasic potassium phosphate

**Solution C:** Dilute tetramethylammonium hydroxide (10%) with water to obtain a 0.1% solution. Adjust with 10% phosphoric acid to a pH of 5.5.

**Solution D:** 37.2 mg/mL of edetate disodium

**Buffer:** Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of 7.0.

**Mobile phase:** Acetonitrile, methanol, *Solution C*, and *Solution D* (300:200:4500:2)

**System suitability solution:** 0.2 mg/mL of USP Cefdinir RS and 0.5 mg/mL of USP Cefdinir Related Compound A RS in *Buffer*

**Standard solution:** 0.2 mg/mL of USP Cefdinir RS in *Buffer*

**Sample solution:** 0.2 mg/mL of Cefdinir in *Buffer*



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 1 mL/min**Injection size:** 5 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*. USP Cefdinir Related Compound A RS should produce four peaks.**Tailing factor:** NMT 1.5 for cefdinir, *System suitability solution***Resolution:** NLT 1.2 between the second peak of cefdinir related compound A and cefdinir, *System suitability solution***Relative standard deviation:** NMT 1.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the quantity, in μg/mg, of cefdinir (C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>) in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL) $P$  = purity of USP Cefdinir RS (μg/mg)**Acceptance criteria:** 940–1030 μg/mg on the anhydrous basis**IMPURITIES**• **RESIDUE ON IGNITION** <281>: NMT 0.20%• **HEAVY METALS**, *Method II* <231>: 10 ppm• **ORGANIC IMPURITIES***Solution A*, *Solution B*, *Solution C*, *Solution D*, and*Buffer*: Prepare as directed in the *Assay*.*Solution E*: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.*Solution F*: Acetonitrile, methanol, *Solution C*, and *Solution D* (300: 200: 500: 0.4)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

**System suitability solution 1:** 15 μg/mL of cefdinir from the *Sample solution*, diluted with *Solution C***System suitability solution 2:** 1.5 μg/mL of cefdinir from *System suitability solution 1*, diluted with *Solution C***System suitability solution 3:** 1.5 mg/mL of USP Cefdinir RS and 0.1 mg/mL of USP Cefdinir RelatedCompound A RS, dissolved initially in *Buffer* corresponding to 15% of the final volume, and diluted with *Solution C* to volume**Sample stock solution:** 10 mg/mL of Cefdinir in *Buffer***Sample solution:** 1.5 mg/mL of cefdinir from the *Sample stock solution*, in *Solution C*. [NOTE—Prepare fresh immediately before use.]**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Samples:** *System suitability solution 1*, *System suitability solution 2*, and *System suitability solution 3*. USP Cefdinir Related Compound A RS should produce four peaks.**Suitability requirements****Response ratio:** The response of cefdinir from *System suitability solution 2* is between 7% and 13% of that from *System suitability solution 1*.**Resolution:** NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution 3***Relative standard deviation:** NMT 2.0% for cefdinir, *System suitability solution 3***Analysis****Sample:** *Sample solution*. Record the chromatogram for at least 1.8 times the retention time of the cefdinir peak.

Calculate the percentage of each impurity in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_T$  = sum of all the peak responses from the *Sample solution***Acceptance criteria:** See *Table 2*.**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* <781S>**Sample solution:** 10 mg/mL in *Buffer*, as obtained in the *Assay***Acceptance criteria:** −61° to −67° at 20°• **WATER DETERMINATION**, *Method I* <921>: NMT 2.0% for anhydrous; 4.0%–8.5% for hydrated forms. For this monograph, the term “hydrated forms” refers to several known forms of Cefdinir. Use a mixture of formamide and methanol (2:1) as the solvent.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **USP REFERENCE STANDARDS** <11>

USP Cefdinir RS

USP Cefdinir Related Compound A RS

(2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).

C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub> 413.43

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.12	0.5
3-Methyl cefdinir <sup>c</sup>	0.74	0.7
Cefdinir related compound A (cefdinir open ring lactone a) <sup>d,e</sup>	0.85	0.7
Cefdinir related compound A (cefdinir open ring lactone b) <sup>d,e</sup>	0.93	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>d,e</sup>	1.11	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>d,e</sup>	1.14	
Cefdinir lactone <sup>f</sup>	1.22	0.5
Cefdinir isoxazole analog <sup>g</sup>	1.36	0.5
<i>E</i> -Cefdinir <sup>h</sup>	1.51	0.7
Cefdinir decarboxy open ring lactone a <sup>i,j</sup>	1.61	0.5
Cefdinir decarboxy open ring lactone b <sup>i,j</sup>	1.64	
Any other individual, unidentified impurity	—	0.2
Total impurities	—	3.0

<sup>a</sup> 1N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> Cefdinir related compound A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the 4 isomers is 0.7%.

<sup>f</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3R,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.

<sup>g</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>h</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.

<sup>j</sup> Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactones a and b. The sum of the values is reported. The limit for sum of the 2 isomers is 0.5%.

## Cefdinir Capsules

### DEFINITION

Cefdinir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir (C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>).

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

**Buffer:** Prepare as directed in the *Assay*.

**Blank:** Use the *Buffer*.

**Standard solution:** 10 µg/mL of USP Cefdinir RS in *Buffer*.

**Sample solution:** Equivalent to 10 µg/mL of cefdinir from Capsules in *Buffer*. Filter before use.

**Cell size:** 1 cm

**Acceptance criteria:** *Sample solution* maxima and minima occur at the same wavelengths as those in the *Standard solution*.

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 10.7 g/L of dibasic sodium phosphate and 3.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.

**Solution A:** 7 g/L citric acid monohydrate. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.

**Mobile phase:** Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)

**System suitability solution:** 50 µg/mL of USP Cefdinir RS and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*

**Standard solution:** 50 µg/mL of USP Cefdinir RS in *Buffer*

**Sample solution:** Equivalent to 50 µg/mL of cefdinir, from Capsule contents (NLT 20) in *Buffer*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 1.4 mL/min

**Injection size:** 15 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** Greater than 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*

**Tailing factor:** NMT 2.0 for cefdinir, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for cefdinir, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefdinir (C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for cefdinir from the *Sample solution*

$r_S$  = peak response for cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–100.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** 50 mM phosphate buffer pH 6.8; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 290 nm

**Cell length:** 0.1-cm flow cell

**Standard solution:** 0.33 mg/mL of USP Cefdinir RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Dilute with *Medium* to a concentration of about 0.33 mg/mL of cefdinir.

**Blank:** Dissolve 1 empty Capsule in 100 mL of *Medium*, and dilute to 900 mL. Filter if necessary.

**Analysis:** Determine the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$D$  = dilution factor of the *Sample solution* (mL/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Capsule)

**Tolerances:** NLT 80% (Q) of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## IMPURITIES

### Change to read:

### • ORGANIC IMPURITIES

**Solution A:** 14.2 g/L of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 g/L of monobasic potassium phosphate

**Solution C:** Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of  $5.5 \pm 0.1$ .

**Solution D:** 37.2 mg/mL of edetate disodium

**Solution E:** To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

**Solution F:** Acetonitrile, methanol, *Solution C*, and *Solution D* (150: 100: 250: 0.2)

**Buffer:** Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of  $7.0 \pm 0.1$ .

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

**System suitability stock solution 1:** 40 μg/mL of USP Cefdinir Related Compound A RS in *Solution C*

**System suitability stock solution 2:** 40 μg/mL of USP Cefdinir Related Compound B RS in *Solution C*

**System suitability solution:** Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL of each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

**Standard stock solution:** 750 μg/mL of USP Cefdinir RS in *Buffer*

**Standard solution:** 15 μg/mL of USP Cefdinir RS, from the *Standard stock solution* in *Solution C*

**Sample solution:** Transfer an equivalent to 300 mg of cefdinir from Capsule contents (NLT 20) into a 200-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume to obtain a solution having a nominal concentration of about 1.5 mg/mL of cefdinir.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Autosampler temperature:** 4°

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.5 between cefdinir and the third peak of the USP Cefdinir Related Compound A RS, *System suitability solution*

**Tailing factor:** NMT 1.5 for cefdinir related compound B, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the cefdinir peak response, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (mg/mL) (ERR 1-Jul-2012)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight light-resistant containers, and store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Cefdinir RS

USP Cefdinir Related Compound A RS

(2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).

$C_{14}H_{15}N_5O_6S_2$  413.43

USP Cefdinir Related Compound B RS

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

$C_{14}H_{13}N_4O_4S_2$  365.41

Table 2

Name	Relative Retention Time	Relative Response Factor	Reporting Threshold (% Cefdinir)	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	1.1	0.1	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.13	1.1	0.1	0.5
Cefdinir sulfoxide <sup>c</sup>	0.36	1.0	0.05	0.2
Cefdinir thiazine analog <sup>d</sup>	0.46	1.5	0.05	0.7
3-Methyl cefdinir <sup>e</sup>	0.75	1.0	0.05	0.7
Cefdinir impurity 1 <sup>f</sup>	0.77	1.0	0.05	0.3
Cefdinir related compound A (cefdinir open ring lactone a) <sup>g,h</sup>	0.85	1.5	0.1	2.5
Cefdinir related compound A (cefdinir open ring lactone b) <sup>g,h</sup>	0.94	1.5	0.1	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>g,h</sup>	1.11	1.5	0.1	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>g,h</sup>	1.14	1.5	0.1	
7S-Cefdinir <sup>i</sup>	1.18	1.1	0.05	0.2
Cefdinir lactone <sup>j</sup>	1.23	1.2	0.05	1.0
Cefdinir related compound B <sup>k</sup>	1.28	1.1	0.05	0.2
Cefdinir isoxazole analog <sup>l</sup>	1.37	1.4	0.05	0.5
Cefdinir impurity 2 <sup>e</sup>	1.44	1.0	0.05	0.5
Cefdinir glyoxalic analog <sup>m</sup>	1.49	1.0	0.05	0.2
E-cefdinir <sup>n</sup>	1.51	1.1	0.05	0.7
Cefdinir decarboxy open ring lactone a <sup>o,p</sup>	1.62	1.3	0.05	1.0
Cefdinir decarboxy open ring lactone b <sup>o,p</sup>	1.64	1.3	0.05	
Cefdinir impurity 3 <sup>e</sup>	1.82	1.0	0.05	0.2
Individual unidentified impurities	—	1.0	0.05	0.2
Total impurities	—	—	—	5.0

<sup>a</sup> N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> (R,Z)-2-[(R)-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxy)methyl]-5-ethylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

<sup>e</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.

<sup>g</sup> Cefdinir related compound A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the 4 isomers is 2.5%.

<sup>h</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.

<sup>i</sup> (6R,7S)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>j</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3R,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.

<sup>k</sup> (6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>m</sup> (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported. The limit for sum of the 2 isomers is 1.0%.

<sup>p</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.

## Cefdinir for Oral Suspension

### DEFINITION

Cefdinir for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir (C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>). It may contain one or more suitable buffers, flavors, preservatives, stabilizing agents, sweeteners, and suspending agents.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 10.7 mg/mL of anhydrous dibasic sodium phosphate and 3.4 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.

**Solution A:** 7 mg/mL of citric acid monohydrate. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

**Mobile phase:** Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)

**System suitability solution:** 50 µg/mL of USP Cefdinir RS and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*

**Standard solution:** 50 µg/mL of USP Cefdinir RS in *Buffer*

**Sample solution:** Equivalent to 50 µg/mL of cefdinir, from constituted Cefdinir for Oral Suspension, in *Buffer*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  15-cm; 4-µm packing L1

**Flow rate:** 1.4 mL/min

**Injection size:** 15 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*

**Tailing factor:** NMT 2.0 for cefdinir, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for cefdinir, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cefdinir from the *Sample solution*

$r_S$  = peak response of cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Medium:** 0.05 M phosphate buffer pH 6.8; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 290 nm

**Standard solution:** 0.14 mg/mL of USP Cefdinir RS in *Medium*

**Sample solution:** Transfer 5 mL, by weight, of the reconstituted Cefdinir for Oral Suspension into the vessel. After the appropriate time, withdraw a portion of the solution under test, and pass through a suitable filter of 0.45-µm pore size. Dilute a portion of each filtered sample with *Medium* as necessary to obtain a solution having a concentration of about 0.14 mg/mL of cefdinir.

**Blank:** *Medium*

#### Analysis:

Determine the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times [(C_S \times d \times D \times V)/W \times L] \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$d$  = density of the Cefdinir for Oral Suspension (mg/mL)

$D$  = dilution factor of the *Sample solution* (mL/mL)

$V$  = volume of *Medium*, 900 mL

$W$  = weight of Cefdinir for Oral Suspension taken (mg)

$L$  = label claim (mg/mL)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{14}H_{13}N_5O_5S_2$  is dissolved.

• **UNIFORMITY OF DOSAGE UNITS <905>** (for solids packaged in single-unit containers): Meets the requirements

• **DELIVERABLE VOLUME <698>** (for solids packaged in single-unit containers): Meets the requirements

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**Solution A:** 14.2 mg/mL of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 mg/mL of monobasic potassium phosphate

**Buffer:** Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of  $7.0 \pm 0.1$ .

**Solution C:** Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of  $5.5 \pm 0.1$ .

**Solution D:** 37.2 mg/mL of edetate disodium

**Solution E:** To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

**Solution F:** Acetonitrile, methanol, *Solution C*, and *Solution D* (150:100:250:0.2)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

**System suitability stock solution 1:** 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*

**System suitability stock solution 2:** 40 µg/mL of USP Cefdinir Related Compound B RS in *Buffer*

**System suitability solution:** Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

**Standard stock solution:** 750 µg/mL of USP Cefdinir RS in *Buffer*

**Standard solution:** 15 µg/mL of USP Cefdinir RS, from the *Standard stock solution* in *Solution C*

**Sample solution:** Transfer a quantity equivalent to 150 mg of cefdinir from constituted Cefdinir for Oral Suspension to a 100-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5-µm packing L1

**Column temperature:** 40°

**Autosampler temperature:** 4°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cefdinir and the third peak for USP Cefdinir Related Compound A RS, *System suitability solution*

**Tailing factor:** NMT 1.5 for cefdinir related compound B, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the cefdinir peak response, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Reporting Threshold (% Cefdinir)	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	1.1	0.1	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.13	1.1	0.1	0.6
Cefdinir sulfoxide <sup>c</sup>	0.36	1.0	0.05	0.2
Cefdinir thiazine analog <sup>d</sup>	0.46	1.5	0.05	0.3
3-Methyl cefdinir <sup>e</sup>	0.75	1.0	0.05	0.7
Cefdinir impurity 1 <sup>f</sup>	0.77	1.0	0.05	0.2
Cefdinir related compound A (cefdinir open ring lactone a) <sup>g,h</sup>	0.85	1.5	0.1	3.3
Cefdinir related compound A (cefdinir open ring lactone b) <sup>g,h</sup>	0.94	1.5	0.1	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>g,h</sup>	1.11	1.5	0.1	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>g,h</sup>	1.14	1.5	0.1	
7S-Cefdinir <sup>i</sup>	1.18	1.1	0.05	0.2
Cefdinir lactone <sup>j</sup>	1.23	1.2	0.05	0.8
Cefdinir related compound B <sup>k</sup>	1.28	1.1	0.05	0.2
Cefdinir isoxazole analog <sup>l</sup>	1.37	1.4	0.05	0.5
Cefdinir impurity 2 <sup>e</sup>	1.44	1.0	0.05	0.2
Cefdinir glyoxalic analog <sup>m</sup>	1.49	1.0	0.05	0.2
E-Cefdinir <sup>n</sup>	1.51	1.1	0.05	1.2
Cefdinir decarboxy open ring lactone a <sup>o,p</sup>	1.62	1.3	0.05	1.1
Cefdinir decarboxy open ring lactone b <sup>o,p</sup>	1.64	1.3	0.05	
Cefdinir impurity 3 <sup>e</sup>	1.82	1.0	0.05	0.2
Individual unidentified impurities	—	1.0	0.05	0.2
Total impurities	—	—	—	6.2

<sup>a</sup> *N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

<sup>b</sup> (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

<sup>c</sup> (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> (*R*,*Z*)-2-[(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxymethyl)-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>e</sup> (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.

<sup>g</sup> Cefdinir related compound A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported; the limit for the sum of the 4 isomers is 3.3%.

<sup>h</sup> 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*R*,5*R*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

<sup>i</sup> (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>j</sup> (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-[(3*R*,5*aR*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]acetamide.

<sup>k</sup> (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> (6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>m</sup> (6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported; the limit for the sum of the 2 isomers is 1.1%.

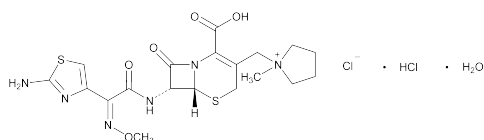
<sup>p</sup> (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-[(2*R*,5*R*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]methyl]acetamide.

**SPECIFIC TESTS**

- **PH** (791): 3.5–4.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** The label specifies the directions for the constitution of the powder and states the equivalent amount of  $C_{14}H_{13}N_5O_5S_2$  in a given volume of Cefdinir for Oral Suspension after constitution.
- **USP REFERENCE STANDARDS** (11)
  - USP Cefdinir RS
  - USP Cefdinir Related Compound A RS  
(2*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*R*,5*R*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).  
 $C_{14}H_{13}N_5O_6S_2$  413.43
  - USP Cefdinir Related Compound B RS  
(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
 $C_{14}H_{13}N_4O_4S_2$  365.41

**Cefepime Hydrochloride**

$C_{19}H_{25}ClN_6O_5S_2 \cdot HCl \cdot H_2O$  571.50  
 Pyrrolidinium, 1-[[[7-[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-, chloride, monohydrochloride, monohydrate, [6*R*-[6*α*,7*β*(*Z*)]-; 1-[[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7*2*-(*Z*)-(O-methyloxime), monohydrochloride, monohydrate [123171-59-5].

**DEFINITION**

Cefepime Hydrochloride contains the equivalent of NLT 825 µg and NMT 911 µg of cefepime ( $C_{19}H_{24}N_6O_5S_2$ )/mg, calculated on the anhydrous basis.

**IDENTIFICATION**

- **INFRARED ABSORPTION** (197M)  
**Sample:** Proceed as directed in the chapter, but do not dry.

**ASSAY**

- **PROCEDURE**
  - Solution A:** 0.68 mg/mL of monobasic potassium phosphate in water
  - Solution B:** Acetonitrile and *Solution A* (1:9). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.
  - Solution C:** Acetonitrile and *Solution A* (1:1). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.
  - Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0

Time (min)	Solution B (%)	Solution C (%)
30	50	50
35	50	50
36	100	0
45	100	0

**Standard solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]

**Sample solution:** 1.4 mg/mL of Cefepime Hydrochloride in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of  $C_{19}H_{24}N_6O_5S_2$  in each mg of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL)

$P$  = content of cefepime in USP Cefepime Hydrochloride RS (µg/mg)

**Acceptance criteria:** 825–911 µg/mg on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS, Method II** (231): NMT 20 ppm

**Organic Impurities**

- **PROCEDURE 1: LIMIT OF N-METHYLPYRROLIDINE**

**Mobile phase:** Acetonitrile and 0.01 N nitric acid (1:19)

**Standard solution:** 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid

**Sample solution:** Equivalent to 5 mg/mL of cefepime hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 4.0-mm × 25-cm; 5-µm packing L#1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 4.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE— Record the chromatogram of the *Sample solution* for about 6 times the retention time of the *N*-methylpyrrolidine peak.]

<sup>1</sup> Available as Metrosep C4-250.

Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of *N*-methylpyrrolidine from the *Sample solution*  
 $r_S$  = peak response of *N*-methylpyrrolidine from the *Standard solution*  
 $C_S$  = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%

• **PROCEDURE 2: OTHER ORGANIC IMPURITIES**

**Solution A, Solution B, Solution C, Mobile phase, Sample solution and Chromatographic system:** Prepare as directed in the *Assay*.

**System suitability solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS and 15 µg/mL each of USP Cefepime Related Compound D RS and USP Cefepime Related Compound E RS in *Solution B*

**System suitability**

**Samples:** *System suitability solution*

**Suitability requirements**

[NOTE— See *Impurity Table 1* for the relative retention times.]

**Resolution:** NLT 2.0 between cefepime related compound E and cefepime related compound D

**Tailing factor:** NMT 1.5, cefepime

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

- $r_U$  = peak response of each impurity  
 $r_T$  = sum of the peak responses for all the peaks in the chromatogram  
 $F$  = relative response factor from *Impurity Table 1*

**Acceptance criteria**

[NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%. [NOTE— Total impurities does not include *N*-methylpyrrolidine.]

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS <85>:** Where the label states that Cefepime Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.04 USP Endotoxin Unit/mg of cefepime hydrochloride.
- **STERILITY TESTS <71>:** Where the label states that Cefepime Hydrochloride is sterile, it meets the requirements when tested as directed in the *Test for Sterility of the Product to be Examined*, *Membrane Filtration*.
- **CRYSTALLINITY <695>:** Meets the requirements
- **WATER DETERMINATION, Method I <921>:** 3.0%–4.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or

must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS <11>**

USP Cefepime Hydrochloride RS

USP Cefepime Related Compound D RS

Thiazolylglyoxalic methyloxime; (Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

$C_6H_7N_3O_3S$  201.20

USP Cefepime Related Compound E RS

Cefepime amine derivative; 1-[[[(6*R*,7*R*)-7-amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidin-1-ium chloride.

$C_{13}H_{20}ClN_3O_3S$  333.83

USP Endotoxin RS

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative <sup>a</sup> (cefepime related compound E)	0.4	0.48	0.1
Thiazolylglyoxalic methyloxime <sup>b</sup> (cefepime related compound D)	0.5	1.0	0.1
Thiazolylloxime acetaldehyde <sup>c</sup> (cefepime related compound C)	0.6	0.63	0.3
Cefepime dimer <sup>d</sup> (cefepime related compound F)	0.8	1.0	0.2
Cefepime	1.0	—	—
E-Cefepime <sup>e</sup> (cefepime related compound A)	2.7	0.71	0.3
Cefepime dioxime <sup>f</sup> (cefepime related compound B)	4.3	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1

<sup>a</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.

<sup>d</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbonyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidin-1-ium chloride.

<sup>e</sup> 1-[[[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidin-1-ium chloride.

<sup>f</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidin-1-ium chloride.



## Cefepime for Injection

### DEFINITION

Cefepime for Injection is a sterile mixture of Cefepime Hydrochloride and Arginine. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefepime ( $C_{19}H_{24}N_6O_5S_2$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 20 mg/mL of arginine

**Sample solution:** 40 mg/mL of Cefepime for Injection

**Developing solvent system:** *n*-Propyl alcohol, ammonium hydroxide, and water (7:4:5)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in *Thin-Layer Chromatographic Identification Test* (201), except to spray the plate with ninhydrin TS.

**Acceptance criteria:** Arginine appears as a dark red spot. The intensity and the  $R_f$  value of the spot from the *Sample solution* correspond to those from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Solution A:** 0.68 mg/mL of monobasic potassium phosphate in water

**Solution B:** Acetonitrile and *Solution A* (1:9), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0

**Solution C:** Acetonitrile and *Solution A* (1:1), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

**Standard solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]

**Sample solution:** Constitute one container of Cefepime for Injection as directed on the label, and dilute using *Solution B* to 1 mg/mL of cefepime. [NOTE—For products that are designed for administration with a syringe, withdraw the entire withdrawable contents of the vial and transfer to a suitable volumetric flask. Dilute with *Solution B* to volume. For all other types, transfer the contents of the reconstituted vial quantitatively to a suitable volumetric flask, and dilute with *Solution B* to volume.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{19}H_{24}N_6O_5S_2$  in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefepime for Injection in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE 1: LIMIT OF N-METHYLPYRROLIDINE

**Mobile phase:** Acetonitrile and 0.01 N nitric acid (1:19)

**Standard solution:** 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid

**Sample solution:** Equivalent to 5 mg/mL of cefepime hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 4.0-mm × 25-cm; 5-μm packing L#1

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 4.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Record the chromatogram of the *Sample solution* for about 6 times the retention time of the *N*-methylpyrrolidine peak.]

Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of *N*-methylpyrrolidine from the *Sample solution*

$r_S$  = peak response of *N*-methylpyrrolidine from the *Standard solution*

$C_S$  = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefepime in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 1.0%

##### • PROCEDURE 2: OTHER ORGANIC IMPURITIES

**Solution A, Solution B, Solution C, Mobile phase, Chromatographic system, and Sample solution:** Prepare as directed for Assay.

**System suitability solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS and 15 μg/mL each of USP Cefepime Related Compound D RS and USP Cefepime Related Compound E RS in *Solution B*

<sup>1</sup> Available as Metrosep C4-250.

**System suitability****Sample:** System suitability solution**Suitability requirements**[NOTE— See *Impurity Table 1* for the relative retention times.]**Resolution:** NLT 2.0 between cefepime related compound E and cefepime related compound D**Tailing factor:** NMT 1.5, cefepime**Analysis****Sample:** Sample solution

Calculate the percentage of each impurity in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

 $r_U$  = peak response for each impurity $r_T$  = sum of the peak responses from the chromatogram $F$  = relative response factor from *Impurity Table 1***Acceptance criteria**

[NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]

**Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 2.2%. [NOTE— Total impurities include *N*-methylpyrrolidine.]**Impurity Table 1**

Name	Relative Retention Time (%)	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative <sup>a,b</sup> (cefepime related compound E)	0.4	—	—
Thiazolylglyoxalic methyloxime <sup>a,c</sup> (cefepime related compound D)	0.5	—	—
Thiazolylloxime acetaldehyde <sup>d</sup> (cefepime related compound C)	0.6	0.63	0.5
Cefepime dimer <sup>a,e</sup> (cefepime related compound F)	0.8	—	—
Cefepime	1.0	—	—
<i>E</i> -Cefepime <sup>f</sup> (cefepime related compound A)	2.7	0.71	0.5

<sup>a</sup> These impurities are synthetic process impurities that are controlled in the drug substance. They are listed here for reference only.<sup>b</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.<sup>d</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.<sup>e</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbonyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>f</sup> 1-[[[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>g</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl]-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.**Impurity Table 1** (Continued)

Name	Relative Retention Time (%)	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime dioxime <sup>a,g</sup> (cefepime related compound B)	4.3	—	—
Any individual unspecified impurity	—	1.0	0.5

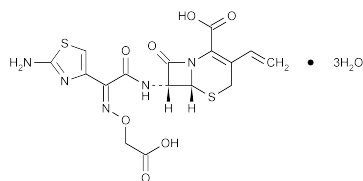
<sup>a</sup> These impurities are synthetic process impurities that are controlled in the drug substance. They are listed here for reference only.<sup>b</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.<sup>d</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.<sup>e</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbonyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>f</sup> 1-[[[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>g</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl]-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.**SPECIFIC TESTS**

- **INJECTIONS, Constituted Solutions** (1): At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.06 USP Endotoxin Unit/mg of cefepime
- **STERILITY TESTS** (71): Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PH** (791): 4.0–6.0, in a solution containing 100 mg/mL of cefepime
- **WATER DETERMINATION, Method I** (921): NMT 4.0%
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections* (1), *Labels and Labeling*

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers as described under *Injections* (1), *Containers for Sterile Solids*, and store in a refrigerator or at controlled room temperature. Store reconstituted solution in a refrigerator for NMT 7 days.
- **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.
- **USP REFERENCE STANDARDS** (11)
  - USP Cefepime Hydrochloride RS
  - USP Cefepime Related Compound D RS
  - Thiazolylglyoxalic methyloxime; (Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
  - C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub>S 201.20
  - USP Cefepime Related Compound E RS
  - Cefepime amine derivative; 1-[[[(6*R*,7*R*)-7-amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.
  - C<sub>13</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>3</sub>S 333.83
  - USP Endotoxin RS

## Cefixime



$C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$  507.50  
 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,  
 7-[[[(2-amino-4-thiazolyl)  
 [(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-,  
 trihydrate, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-.  
 (6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-  
 3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic  
 acid, 7 $\alpha$ -(Z)-[O-(carboxymethyl)oxime]trihydrate  
 [79350-37-1].  
 Anhydrous 453.46

» Cefixime contains the equivalent of not less than 950  $\mu$ g and not more than 1030  $\mu$ g of Cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label to indicate that it is the trihydrate form. Where the quantity of Cefixime is indicated in the labeling of any preparation containing Cefixime, this shall be understood to be in terms of anhydrous cefixime ( $C_{16}H_{15}N_5O_7S_2$ ).

### USP Reference standards (11)—

USP Cefixime RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Dissolve about 5 mg of it by trituration in 2 mL of methanol and evaporate with the aid of gentle heat to dryness.

**Specific rotation** (781S): between  $-75^\circ$  and  $-88^\circ$ .

*Test solution*: 10 mg per mL, in sodium bicarbonate solution (2 in 100).

**Crystallinity** (69S): meets the requirements.

**pH** (791): between 2.6 and 4.1, in a solution containing the equivalent of 0.7 mg of cefixime per mL.

**Water, Method I** (921): between 9.0% and 12.0%.

### Chromatographic purity—

*Tetrabutylammonium hydroxide solution, Mobile phase, Monobasic potassium phosphate solution, pH 7.0 Phosphate Buffer, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Use the *Standard preparation* prepared as directed in the Assay.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of each impurity in the portion of Cefixime taken by the formula:

$$0.1P(r_i / r_s)$$

in which  $P$  is the potency, in  $\mu$ g per mg, of cefixime calculated in the Assay;  $r_i$  is the peak area for each impurity; and  $r_s$  is the cefixime peak area: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

### Assay—

*Tetrabutylammonium hydroxide solution*—Dilute 25 mL of 0.4 M tetrabutylammonium hydroxide solution with water

to obtain 1000 mL of solution, and adjust with 1.5 M phosphoric acid to a pH of 6.5.

*Mobile phase*—Prepare a suitable filtered and degassed mixture of *Tetrabutylammonium hydroxide solution* and acetonitrile (3:1). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Monobasic potassium phosphate solution*—Dissolve 6.8 g of monobasic potassium phosphate in water to make 500 mL of solution.

*pH 7.0 Phosphate Buffer*—Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Adjust a volume of this solution with a sufficient volume of *Monobasic potassium phosphate solution* to a pH of 7.0.

*Resolution solution*—Dissolve USP Cefixime RS in water to obtain a solution having a concentration of about 1 mg per mL. Heat this solution at  $95^\circ$  in an oil bath for 45 minutes, cool, and use promptly.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cefixime RS in *pH 7.0 Phosphate buffer* to obtain a solution having a known concentration of about 0.2 mg of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) per mL. Use this solution promptly.

*Assay preparation*—Transfer about 110 mg of Cefixime, accurately weighed, to a 100-mL volumetric flask, dilute with *pH 7.0 Phosphate buffer* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *pH 7.0 Phosphate buffer* to volume, and mix. Use this solution promptly.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  12.5-cm column containing 4- $\mu$ m packing L1. The flow rate is adjusted so that the retention time of cefixime is about 10 minutes. The column is maintained at a constant temperature of about  $40^\circ$ . Chromatograph the *Resolution solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.9 for cefixime (*E*-isomer) and 1.0 for cefixime; and the resolution,  $R$ , between cefixime and cefixime (*E*-isomer) is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 4000 theoretical plates when calculated by the formula:

$$5.545(t / W_{h/2})^2$$

the tailing factor for the analyte peak is not less than 0.9 and not more than 2.0, when calculated by the formula:

$$W_{0.1} / 2f$$

in which  $W_{0.1}$  is width of peak of 10% height; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu$ g, of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) in each mg of Cefixime taken by the formula:

$$500,000(C / W)(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of cefixime in the *Standard preparation*;  $W$  is the quantity, in mg, of Cefixime taken to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the cefixime peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefixime for Oral Suspension

### DEFINITION

Cefixime for Oral Suspension is a dry mixture of Cefixime and one or more suitable diluents, flavors, preservatives, and suspending agents. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ )/mL when constituted as directed in the labeling.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 0.4 M tetrabutylammonium hydroxide solution and water (1:39). Adjust with 1.5 M phosphoric acid to a pH of 6.5.

**Solution B:** 13.6 mg/mL of monobasic potassium phosphate

**Solution C:** 14.2 mg/mL of anhydrous dibasic sodium phosphate. Adjust a volume of this solution with a sufficient volume of *Solution B* to a pH of 7.0.

**Mobile phase:** Acetonitrile and *Solution A* (1:3)

**System suitability solution:** 1 mg/mL of USP Cefixime RS. [NOTE—Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.]

**Standard solution:** 0.2 mg/mL of USP Cefixime RS in *Solution C*. [NOTE—Use this solution promptly.]

**Sample solution:** Constitute Cefixime for Oral Suspension as directed in the labeling. Quantitatively dilute a suitable aliquot of the suspension, freshly mixed and free from air bubbles, with *Solution C* to obtain a solution having a nominal concentration of 0.2 mg of cefixime/mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 12.5-cm; 4-μm packing L1

**Temperature:** 40°

**Flow rate:** Adjust flow rate so that the retention time of cefixime is about 10 min.

**Injection size:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefixime (*E*)-isomer and cefixime are about 0.9 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cefixime and cefixime (*E*)-isomer, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*. Use the following formula to calculate column efficiency:

$$\text{Result} = 5.545(t/W_{h/2})^2$$

**Tailing factor:** NLT 0.9 and NMT 2.0 for the analyte peak, *Standard solution*. Use the following formula to calculate tailing factor:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = peak width at 10% peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{15}N_5O_7S_2$  in the constituted suspension prepared from the Cefixime for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cefixime from the *Sample solution*

$r_S$  = peak response of cefixime from the *Standard solution*

$C_S$  = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefixime in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS** (905) FOR SOLIDS PACKAGED IN SINGLE-UNIT CONTAINERS: Meets the requirements
- DELIVERABLE VOLUME** (698): Meets the requirements

### SPECIFIC TESTS

- pH** (791): 2.5–4.5, in the suspension constituted as directed in the labeling

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Label it to indicate that the cefixime contained therein is in the trihydrate form.
- USP REFERENCE STANDARDS** (11)  
USP Cefixime RS

## Cefixime Tablets

» Cefixime Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Tablets to indicate that the cefixime contained therein is in the trihydrate form.

**USP Reference standards** (11)—

USP Cefixime RS

**Identification**—The retention time of the major peak for cefixime in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

**Medium:** 0.05 M potassium phosphate buffer, pH 7.2, prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjusting with 1 N sodium hydroxide to a pH of 7.2; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 288 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium* if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefixime RS in the same medium. [NOTE—An amount of methanol not to exceed 0.1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution prior to dilution with *Dissolution Medium*, and the solution may be sonicated to assure complete dissolution of the Reference Standard.]

**Tolerances**—Not less than 75% (Q) of the labeled amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 10.0%.

#### Assay—

*Tetrabutylammonium hydroxide solution, Mobile phase, pH 7.0 Phosphate buffer, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Cefixime.

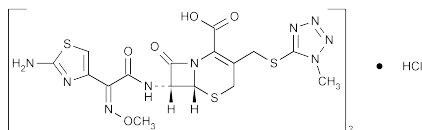
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 400 mg of cefixime, to a 100-mL volumetric flask, add 75 mL of pH 7.0 Phosphate buffer, and sonicate. Dilute with pH 7.0 Phosphate buffer to volume, mix, and centrifuge. Transfer 5.0 mL of the clear supernatant to a second 100-mL volumetric flask, dilute with pH 7.0 Phosphate buffer to volume, and mix.

**Procedure**—Proceed as directed in the Assay under Cefixime. Calculate the quantity, in mg, of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) in the portion of Tablets taken by the formula:

$$2000C(r_u / r_s)$$

in which the terms are as defined therein.

## Cefmenoxime Hydrochloride



( $C_{16}H_{17}N_9O_5S_3$ )<sub>2</sub> · HCl 1059.58

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, hydrochloride (2:1), [6R-[6α,7β(Z)]]-

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 7<sup>2</sup>-(Z)-(O-methyloxime), hydrochloride (2:1) [75738-58-8].

» Cefmenoxime Hydrochloride contains the equivalent of not less than 869 μg and not more than 1015 μg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—  
USP Cefmenoxime Hydrochloride RS

#### Identification—

**A: Ultraviolet Absorption** (197U)—

**Solution:** 25 μg per mL.

**Medium:** pH 6.8 buffer (prepared as directed in the Assay).

**B:** The retention time of the cefmenoxime peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, both relative to the internal standard, as obtained in the Assay.

**Crystallinity** (695): meets the requirements.

**Pyrogen** (151)—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements, the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 14.0 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of sterile water for injection) containing 60 mg per mL.

**Sterility** (71)—Where the label states that it is sterile, it meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined, except to use Fluid A to each 100 mL of which has been added 2.0 g of sodium carbonate previously sterilized by heating at 180° for 2 hours.

**Water, Method I** (921): not more than 1.5%, the Test Preparation being prepared as directed for a hygroscopic specimen, except to use 20 mL of a mixture of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, to use two 5-mL portions of the same formamide and methanol mixture to rinse the container, and to determine the water content of the formamide and methanol mixture.

#### Assay—

**pH 6.8 buffer**—Dissolve 6.4 g of monobasic potassium phosphate and 18.9 g of dibasic sodium phosphate in 750 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.8 ± 0.1, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, and glacial acetic acid (50:10:1). Filter through a suitable filter of 0.5 μm or finer porosity, and degas. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Internal standard solution**—Prepare a solution of phthalimide in methanol containing 1.5 mg per mL.

**Standard preparation**—Transfer about 50 mg of USP Cefmenoxime Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of pH 6.8 buffer, and dissolve by swirling. Dilute with Mobile phase to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix. This solution contains the equivalent of about 80 μg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mL.

**Assay preparation**—Transfer about 50 mg of Cefmenoxime Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add 10 mL of pH 6.8 buffer, and dissolve by swirling. Dilute with Mobile phase to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the resolution, *R*, between the phthalimide and the cefmenoxime peaks is not less than 2.3; the column efficiency, determined from the cefmenoxime peak, is not less than 1200 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

the tailing factor for the cefmenoxime peak is not more than 1.6; and the relative standard deviation of replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) in each mg of the Cefmenoxime Hydrochloride taken by the formula:

$$(W_S P_S / W_U)(R_U / R_S)$$

in which  $W_S$  is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the *Standard preparation*;  $P_S$  is the designated cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) content, in  $\mu\text{g}$  per mg, of USP Cefmenoxime Hydrochloride RS;  $W_U$  is the weight, in mg, of Cefmenoxime Hydrochloride taken to prepare the *Assay preparation*, and  $R_U$  and  $R_S$  are the peak response ratios of the cefmenoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmenoxime for Injection

» Cefmenoxime for Injection contains an amount of Cefmenoxime Hydrochloride equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ). It may contain Sodium Carbonate.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—  
USP Cefmenoxime Hydrochloride RS

### Identification—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 25  $\mu\text{g}$  per mL.

*Medium:* pH 6.8 buffer prepared as directed in the *Assay under Cefmenoxime Hydrochloride*.

**B:** The retention time of the cefmenoxime peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Pyrogen** (151)—It meets the requirements, the test dose being 1.0 mL per kg of a solution of Cefmenoxime for Injection in sterile water for injection having a concentration of 60 mg of cefmenoxime per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.4 and 7.9, in a solution containing the equivalent of 100 mg of cefmenoxime per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.5% of its weight.

**Particulate matter** (788): meets the requirements for small-volume injections.

### Assay—

pH 6.8 buffer, *Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay under Cefmenoxime Hydrochloride*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with water to obtain a solution containing the equivalent of about 1 mg of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask,

add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80  $\mu\text{g}$  of cefmenoxime per mL.

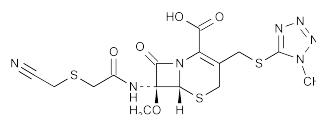
*Assay preparation 2* (where the label states the quantity of cefmenoxime in a given volume of constituted solution)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with water to obtain a solution containing about 1 mg of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80  $\mu\text{g}$  of cefmenoxime per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Cefmenoxime Hydrochloride*. Calculate the quantity, in mg, of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) withdrawn from the container or in the portion of constituted solution taken by the formula:

$$1.6(L / D)(W_S P_S / 1000)(R_U / R_S)$$

in which  $L$  is the labeled quantity, in mg, of cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) in the container or in the volume of constituted solution taken;  $D$  is the concentration, in  $\mu\text{g}$  of cefmenoxime per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution;  $W_S$  is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the *Standard preparation*;  $P_S$  is the designated cefmenoxime ( $\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3$ ) content, in  $\mu\text{g}$  per mg, of USP Cefmenoxime Hydrochloride RS; and  $R_U$  and  $R_S$  are the response ratios of the cefmenoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmetazole



$\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$  471.53

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, (6*R*-*cis*)-, (6*R*,7*S*)-7-[2-[(Cyanomethyl)thio]acetamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [56796-20-4].

» Cefmetazole contains not less than 970  $\mu\text{g}$  and not more than 1030  $\mu\text{g}$  of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefmetazole RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water, Method I** (921): not more than 0.5%.

#### Assay—

**Mobile phase**—Dissolve 5.75 g of monobasic ammonium phosphate in 700 mL of water, add 3.2 mL of a 40% solution of tetrabutylammonium hydroxide, 280 mL of methanol, and 25 mL of tetrahydrofuran, and mix. Adjust with phosphoric acid to a pH of  $4.5 \pm 0.1$ , pass through a filter having a 0.5- $\mu\text{m}$  or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Cefmetazole RS in *Mobile phase* to obtain a solution having a known concentration of about 200  $\mu\text{g}$  of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) per mL. [NOTE—Use this solution within 10 minutes.]

**Resolution solution**—Prepare a solution of USP Cefmetazole RS in 0.01 N sodium hydroxide containing about 1 mg per mL. Heat at  $95^\circ$  for 10 minutes. To 1 mL of this solution add 2 mL of *Standard preparation*, and dilute with *Mobile phase* to obtain 20 mL of solution. This solution contains cefmetazole and cefmetazole lactone (resolution compound).

**Assay preparation**—Transfer about 20 mg of Cefmetazole, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cefmetazole and cefmetazole lactone is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1250 theoretical plates; the tailing factor is not less than 0.94 and not more than 1.6; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) in each mg of Cefmetazole taken by the formula:

$$100(C/M)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) in the *Standard preparation*;  $M$  is the quantity, in mg, of Cefmetazole taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cefmetazole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmetazole Injection

» Cefmetazole Injection is a sterile isoosmotic solution of Cefmetazole and Sodium Citrate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

#### USP Reference standards (11)—

USP Cefmetazole RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that observed in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use water instead of *Fluid A*.

**pH** (791): between 4.2 and 6.2.

**Particulate matter** (788): meets the requirements for small-volume injections.

#### Assay—

**Mobile phase, Resolution solution, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Cefmetazole*.

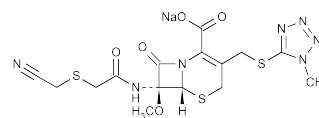
**Assay preparation**—Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer an accurately measured volume of this solution, equivalent to about 40 mg of cefmetazole, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefmetazole*. Calculate the quantity, in mg, of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) in each mL of the Injection by the formula:

$$0.2(C/VM)(r_U/r_S)$$

in which  $V$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and the other terms are as defined therein.

## Cefmetazole Sodium



$\text{C}_{15}\text{H}_{16}\text{N}_7\text{NaO}_5\text{S}_3$  493.52

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, (6*R*-*cis*)-.

Sodium (6*R*,7*S*)-7-[2-[(cyanomethyl)thio]acetamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [56796-39-5].

» Cefmetazole Sodium contains the equivalent of not less than 860  $\mu\text{g}$  and not more than 1003  $\mu\text{g}$  of cefmetazole ( $\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must

be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefmetazole RS  
USP Endotoxin RS

**Identification—**

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** (791): between 4.2 and 6.2, in a solution (1 in 10).

**Water, Method I** (921): not more than 0.5%.

**Other requirements**—Where the label states that Cefmetazole Sodium is sterile, it meets the requirements in the tests for *Sterility* (71) and for *Bacterial endotoxins* under *Cefmetazole for Injection*. Where the label states that Cefmetazole Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements in the test for *Bacterial endotoxins* under *Cefmetazole for Injection*.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole*.

*Assay preparation*—Transfer about 21 mg of Cefmetazole Sodium, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

*Procedure*—Proceed as directed in the *Assay* under *Cefmetazole*. Calculate the quantity, in µg, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mg of Cefmetazole Sodium taken by the formula:

$$100(C/M)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in the *Standard preparation*; *M* is the quantity, in mg, of Cefmetazole Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cefmetazole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmetazole for Injection

» Cefmetazole for Injection contains an amount of Cefmetazole Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cefmetazole RS  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements in the tests for *Identification*, *pH*, and *Water* under *Cefmetazole Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]

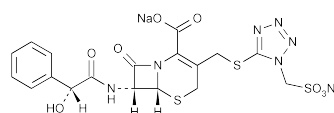
*Assay preparation 2* (where the label states the quantity of cefmetazole in a given volume of constituted solution)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]

*Procedure*—Proceed as directed in the *Assay* under *Cefmetazole*. Calculate the quantity, in mg, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C/1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of cefmetazole in the container, or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; *C* is the concentration, in µg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cefmetazole peak responses obtained from the relevant *Assay preparation* and the *Standard preparation*, respectively.

## Cefonicid Sodium



$C_{18}H_{16}N_6Na_2O_8S_3$  586.53

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(hydroxyphenylacetyl)amino]-8-oxo-3-[[[1-(sulfomethyl)-1H-tetrazol-5-yl]thio]methyl]disodium salt, [6R-[6α, 7β(R\*)]]-

(6R,7R)-[7-[(R)-Mandelamido]-8-oxo-3-[[[1-(sulfomethyl)-1H-tetrazol-5-yl]thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [61270-78-8].

» Cefonicid Sodium contains the equivalent of not less than 832 µg and not more than 970 µg of cefonicid ( $C_{18}H_{16}N_6O_8S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefonicid Sodium RS  
USP Endotoxin RS



**Identification—**

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefonicid, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** It responds to the tests for *Sodium* (191).

**Specific rotation** (781S): between  $-37^{\circ}$  and  $-47^{\circ}$ .

*Test solution:* 10 mg per mL, in methanol.

**pH** (791): between 3.5 and 6.5, in a solution (1 in 20).

**Water, Method I** (921): not more than 5.0%.

**Other requirements—**Where the label states that Cefonicid Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefonicid for Injection*. Where the label states that Cefonicid Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefonicid for Injection*.

**Assay—**

**Mobile phase—**Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:2). Pass through a filter having a 0.5- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation—**Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 200  $\mu$ g of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mL.

**Assay preparation—**Transfer about 40 mg of Cefonicid Sodium, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Resolution solution—**Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution *R*, between the cefonicid and the desacetyl cefonicid peaks is not less than 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.

**Procedure—**Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mg of the Cefonicid Sodium taken by the formula:

$$200(C / M)(r_U / r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) in the *Standard preparation*; *M* is the quantity, in mg, of Cefonicid Sodium taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

90.0 percent and not more than 120.0 percent of the labeled amount of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ).

**Packaging and storage—**Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards (11)—**

USP Cefonicid Sodium RS

USP Endotoxin RS

**Constituted solution—**At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Bacterial endotoxins** (85)—It contains not more than 0.35 USP Endotoxin Unit per mg of cefonicid.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements—**It responds to the *Identification* tests and meets the requirements for *Specific rotation*, *pH*, and *Water* under *Cefonicid Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay—**

**Mobile phase—**Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:3). Pass through a filter having a 0.5- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation—**Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 200  $\mu$ g of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mL.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 200  $\mu$ g of cefonicid per mL.

**Assay preparation 2** (where the label states the quantity of cefonicid in a given volume of constituted solution)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 200  $\mu$ g of cefonicid per mL.

**Resolution solution—**Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution *R*, between the cefonicid peak and the desacetyl cefonicid peak is not less than 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.

**Procedure—**Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) withdrawn from the

**Cefonicid for Injection**

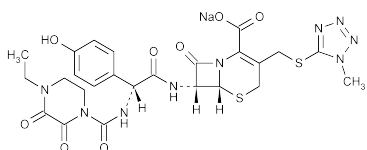
» Cefonicid for Injection contains an amount of Cefonicid Sodium equivalent to not less than

container, or in the portion of constituted solution taken by the formula:

$$(L / D)(C)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of cefonicid in the container, or in the volume of constituted solution taken; *D* is the concentration, in µg per mL, of cefonicid in Assay preparation 1 or Assay preparation 2, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; *C* is the concentration, in µg per mL, of cefonicid in the Standard preparation; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the relevant Assay preparation and the Standard preparation, respectively.

## Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$  667.65

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]-(4-hydroxyphenyl)acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, [6R-[6α, 7β(R\*)]]-

Sodium (6R,7R)-7-[(R)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(p-hydroxyphenyl)acetamido-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [62893-20-3].

» Cefoperazone Sodium contains the equivalent of not less than 870 µg and not more than 1015 µg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefoperazone Dihydrate RS

USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak for cefoperazone in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**B:** It responds to the tests for Sodium (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.5 and 6.5, in a solution (1 in 4).

**Water, Method I** (921): not more than 5.0%.

**Other requirements**—Where the label states that Cefoperazone Sodium is sterile, it meets the requirements for Sterility and Bacterial endotoxins under Cefoperazone for Injection. Where the label states that Cefoperazone Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for Bacterial endotoxins under Cefoperazone for Injection.

**Assay**—

**Mobile phase**—Place 14 mL of triethylamine and 5.7 mL of glacial acetic acid in a 100-mL volumetric flask, dilute

with water to volume, and mix. Prepare a suitable mixture of water, acetonitrile, 1 N acetic acid, and this solution (876:120:2.8:1.2). Filter through a membrane filter (1-µm or finer porosity), and degas.

**Standard preparation**—Dissolve a suitable quantity of USP Cefoperazone Dihydrate RS, accurately weighed, in Mobile phase to obtain a solution having a known concentration of about 0.16 mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL.

**Assay preparation**—Using a suitable quantity of Cefoperazone Sodium, accurately weighed, proceed as directed under Standard preparation.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed under Procedure: the relative standard deviation for replicate injections is not more than 2.0%, and the tailing factor is not more than 1.5.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg of cefoperazone per mg, of the Cefoperazone Sodium taken by the formula:

$$1000(C / M)(r_U / r_S)$$

in which *C* is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of the Standard preparation; *M* is the concentration, in mg per mL, of the Assay preparation based on the weight of Cefoperazone Sodium taken and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses from the Assay preparation and the Standard preparation, respectively.

## Cefoperazone Injection

» Cefoperazone Injection is a sterile solution of Cefoperazone Sodium and a suitable osmolality adjusting substance in Water for Injection. It may contain a suitable buffer. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ).

**Packaging and storage**—Preserve in Containers for Injections as described under Injections (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for Labeling under Injections (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefoperazone Dihydrate RS

USP Endotoxin RS

**Identification**—The retention time of the major peak for cefoperazone in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefoperazone.

**Sterility** (71)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**pH** <791>: between 4.5 and 6.5.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Cefoperazone Sodium*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Cefoperazone Sodium*. Calculate the quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), in the volume of Injection taken by the formula:

$$(L / D)(C)(r_u / r_s)$$

in which *L* is the labeled quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), in the volume of Injection taken; *D* is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of the *Assay preparation*, based on the labeled quantity in the portion of Injection taken and the extent of dilution; and the other terms are as defined therein.

## Cefoperazone for Injection

» Cefoperazone for Injection contains an amount of Cefoperazone Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

**USP Reference standards** <11>—

USP Cefoperazone Dihydrate RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Bacterial endotoxins** <85>—It contains not more than 0.20 USP Endotoxin Unit per mg of cefoperazone.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** <791>: between 4.5 and 6.5, in a solution (1 in 4).

**Water, Method I** <921>: not more than 5.0%, except that where it is in the freeze-dried form, the limit is not more than 2.0%.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for the *Identification* tests under *Cefoperazone Sodium* and meets the requirements for *Uniformity of Dosage Units* <905> and for *Labeling* under *Injections* <1>.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Cefoperazone Sodium*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Assay preparation 2* (where the label states the quantity of cefoperazone in a given volume of constituted solution)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g of cefoperazone per mg, of the Cefoperazone for Injection taken by the formula:

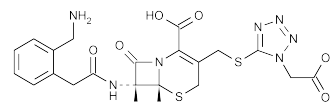
$$1000(C / M)(r_u / r_s)$$

in which *C* is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of the *Standard preparation*; *M* is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Cefoperazone for Injection taken and the extent of dilution; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L / D)(C)(r_u / r_s)$$

in which *L* is the labeled quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), in the container, or in the volume of constituted solution taken; and *D* is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

## Ceforanide



$C_{20}H_{21}N_7O_6S_2$  519.55

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[2-(aminomethyl)phenyl]acetyl]amino]-3-[[[1-(carboxymethyl)1H-tetrazol-5-yl]thio]methyl]-8-oxo-, (6*R*-trans)-, (6*R*,7*R*)-7-[2-( $\alpha$ -Amino-*o*-tolyl)acetamido]-3-[[[1-(carboxymethyl)-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. 7-[*o*-(Aminomethyl)phenylacetamido]-3-[[[1-(carboxymethyl)-1H-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid [60925-61-3].

» Ceforanide contains not less than 900  $\mu$ g and not more than 1050  $\mu$ g of ceforanide ( $C_{20}H_{21}N_7O_6S_2$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** <11>—

USP Ceforanide RS

USP Endotoxin RS

**Identification—**

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak for ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—Where the label states that Ceforanide is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

**Sterility** (71)—Where the label states that Ceforanide is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to dissolve 6 g of Ceforanide in *Fluid A* to each 1000 mL of which has been added 10 g of sterile L-lysine, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A*.

**pH** (791): between 2.5 and 4.5, in a suspension containing 50 mg per mL.

**Water, Method I** (921): not more than 5.0%.

**Assay—**

**Mobile phase**—Mix 18 mL of tetrabutylammonium hydroxide solution (1 in 10) and 8.6 mL of 11 N potassium hydroxide, and add the mixture to 700 mL of water. Add 200 mL of methanol, adjust with phosphoric acid to a pH of 7.0, and add water to obtain 1000 mL of solution, making adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Filter, using a filter having a porosity of 1  $\mu$ m or finer, and degas.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ceforanide RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Use this solution within 5 minutes.

**Assay preparation**—Using a suitable quantity of Ceforanide, accurately weighed, proceed as directed under *Standard preparation*. Use this solution within 5 minutes.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1900 theoretical plates; the tailing factor for the analyte peak is not more than 1.2; the capacity factor,  $k'$ , is not less than 1.8 and not more than 5.0; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of  $C_{20}H_{21}N_7O_6S_2$  in each mg of the Ceforanide taken by the formula:

$$(CP / M)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ceforanide RS in the *Standard preparation*; P is the potency, in  $\mu$ g per mg, of the USP Ceforanide RS; M is the concentration, in mg per mL, of the *Assay preparation*, based on the amount of Ceforanide taken and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Ceforanide for Injection**

» Ceforanide for Injection is a sterile mixture of Ceforanide and L-lysine. It contains not less than 900  $\mu$ g and not more than 1050  $\mu$ g of ceforanide ( $C_{20}H_{21}N_7O_6S_2$ ) per mg on the L-lysine-free basis, and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{20}H_{21}N_7O_6S_2$ .

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Ceforanide RS

USP Endotoxin RS

**Identification—**

**A:** The retention time of the major peak for L-lysine in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the test for *Content of L-lysine*.

**B:** The retention time of the major peak in ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*; as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to constitute each container with 3 mL of *Fluid A* for each g of ceforanide contained therein, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A*.

**pH** (791): between 5.5 and 8.5, when constituted as directed in the labeling.

**Water, Method I** (921): not more than 3.0%.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Content of L-lysine—**

**Mobile phase**—Mix 62 volumes of methanol and 38 volumes of water, and adjust with glacial acetic acid to a pH of 3.0, making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Stock standard solution**—Transfer about 36 mg of L-lysine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Transfer 2.0 mL of *Stock standard solution* to a glass-stoppered, 10-mL volumetric flask, add 2.0 mL of a 1.4% solution of tris(hydroxymethyl)aminomethane and 3.0 mL of a 1.5% solution of 1-fluoro-2,4-dinitrobenzene in dehydrated alcohol, insert the stopper tightly, and mix. Heat at 50° in a water bath for 30 minutes. Remove the flask from the water bath, allow to cool, dilute with methanol to volume, and mix.

**Test preparation**—Transfer about 150 mg of Ceforanide for Injection, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 2.0 mL of the resulting solution to a glass-stoppered, 10-mL volumetric flask, and proceed as directed under *Standard preparation*, beginning with "add 2.0 mL of a 1.4% solution of tris(hydroxymethyl)aminomethane."

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the derivatized L-lysine peak is not less than 1500 theoretical plates; the tailing factor for the same



**Other requirements**—Where the label states that Cefotaxime Sodium is sterile, it meets the requirements for *Sterility* (71) when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*, and for *Bacterial endotoxins under Cefotaxime for Injection*. Where the label states that Cefotaxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins under Cefotaxime for Injection*.

**Assay**—

**0.05 M Phosphate Buffer**—Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 6.25.

**Solution A**—Prepare a mixture of 0.05 M Phosphate Buffer and methanol (86:14). Pass through a filter having a porosity of 0.5 µm or less, and degas before use.

**Solution B**—Prepare a mixture of 0.05 M Phosphate Buffer and methanol (60:40). Pass through a filter having a porosity of 0.5 µm or less, and degas before use.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

**Standard preparation**—Transfer about 40 mg of USP Cefotaxime Sodium RS, accurately weighed, to a 50-mL volumetric flask, add about 40 mL of *Solution A*, swirl to dissolve, dilute with *Solution A* to volume, and mix. [NOTE—Use this solution promptly. It may be used within 24 hours if stored in the refrigerator.]

**Sensitivity solution**—Transfer 2.0 mL of *Standard preparation* to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix. Transfer 2.0 mL of this solution to a 20-mL volumetric flask, dilute with *Solution A* to volume, and mix.

**Resolution solution**—Mix 1 mL of *Standard preparation*, 7.0 mL of water, and 2.0 mL of methanol. Add 25 mg of sodium carbonate, mix, and allow to stand at room temperature for 10 minutes, with occasional swirling. Add 3 drops of glacial acetic acid and 1 mL of *Standard preparation*, and mix.

**Assay preparation**—Transfer about 40 mg of Cefotaxime Sodium, accurately weighed, to a 50-mL volumetric flask, add *Solution A* to dissolve it, dilute with *Solution A* to volume, and mix. [NOTE—Use this solution promptly. It may be used within 24 hours if stored in a refrigerator.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1 mL per minute. The system is equilibrated with 100% *Solution A*. Seven minutes after injection of the solution under test, the proportion of *Solution B* is increased linearly from 0% to 20% at a rate of 10% per minute and is maintained at that composition for 7 minutes. The proportion of *Solution B* is then increased linearly at a rate of 2.7% per minute until the proportion of *Solution B* is 100%, and is held at that composition for 5 minutes, after which the proportion of *Solution A* is increased linearly to 100% at a rate of 20% per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the retention times are about 3.5 minutes for desacetylcefotaxime and 14 minutes for cefotaxime, and the resolution,  $R$ , between the two peaks is not less than 20. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the retention time for the main cefotaxime peak is between 12 and 15 minutes, the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 1.5%. Chromatograph the *Sensitivity solution*, and record the peak responses as directed under *Procedure*: the response of the cefotaxime peak is between 0.18% and 0.22% of the response of the cefotaxime peak in the chromatogram obtained from the *Standard preparation*.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg per mg, of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) in the portion of Cefotaxime Sodium taken by the formula:

$$50(CP / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cefotaxime Sodium RS in the *Standard preparation*;  $P$  is the designated content, in µg per mg, of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) in USP Cefotaxime Sodium RS;  $W$  is the weight, in mg, of Cefotaxime Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cefotaxime peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefotaxime Injection

» Cefotaxime Injection is a sterile solution of Cefotaxime Sodium in Water for Injection. It contains one or more suitable buffers, and it may contain Dextrose or Sodium Chloride as a tonic-ity-adjusting agent. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ).

**Packaging and storage**—Preserve in single-dose containers, as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefotaxime Sodium RS

USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefotaxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of cefotaxime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Chromatographic purity**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of each impurity by the formula:

$$100r_i / (r_{is} + r_c)$$

in which  $r_i$  is the peak area response of a given impurity;  $r_{is}$  is the sum of all of the impurity peak area responses; and  $r_c$  is the peak area response for the main cefotaxime peak. [NOTE—Disregard any impurity peak that is less than 0.1%.] Not more than 6.0% of any individual impurity is found, and the sum of all impurities found is not more than 10.0%.

**Assay—**

0.05 M Phosphate buffer, Solution A, Solution B, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Prepare as directed in the Assay under Cefotaxime Sodium.

**Assay preparation**—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 80 mg of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ), to a 100-mL volumetric flask, dilute with Solution A to volume, and mix.

**Procedure**—Proceed as directed for Procedure in the Assay under Cefotaxime Sodium. Calculate the quantity, in mg, of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) in each mL of the Injection taken by the formula:

$$0.1(CP / V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Injection taken, to prepare the Assay preparation, and the other terms are as defined therein.

## Cefotaxime for Injection

» Cefotaxime for Injection contains an amount of Cefotaxime Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ).

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under Injections (1).

**USP Reference standards (11)—**

USP Cefotaxime Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for Constituted Solutions under Injections (1).

**Identification—**

WHERE THE LABEL INDICATES THAT THERE ARE NO ADDED SUBSTANCES—

A: Infrared Absorption (197K).

B: It responds to the tests for Sodium (191).

WHERE THE LABEL INDICATES THAT THERE ARE ADDED SUBSTANCES—

C: It responds to Identification test B under Cefotaxime Sodium.

**Bacterial endotoxins (85)**—It contains not more than 0.20 USP Endotoxin Unit per mg of cefotaxime.

**Sterility (71)**—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**Uniformity of dosage units (905)**—meets the requirements.

**Particulate matter (788)**: meets the requirements for small-volume injections.

**Chromatographic purity**—Using the chromatogram of the Assay preparation obtained in the Assay, calculate the percentage of each impurity by the formula:

$$100r_i / (r_s + r_c)$$

in which  $r_i$  is the peak area response of a given impurity;  $r_s$  is the sum of all the impurity peak area responses; and  $r_c$  is the peak area response for the main cefotaxime peak. [NOTE—Disregard any impurity peak that is less than 0.1%.] Not more than 6.0% of any individual impurity is found, and the sum of all impurities found is not more than 10.0%.

**Other requirements**—It meets the requirements for pH and Loss on drying under Cefotaxime Sodium and for Labeling under Injections (1).

**Assay—**

0.05 M Phosphate buffer, Solution A, Solution B, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Prepare as directed in the Assay under Cefotaxime Sodium.

**Assay preparation 1** (for use where the Weight Variation test is to be performed)—Transfer about 40 mg of Cefotaxime for Injection, accurately weighed, to a 50-mL volumetric flask, add about 40 mL of Solution A, swirl to dissolve, dilute with Solution A to volume, and mix. [NOTE—Use this solution promptly. It may be used within 24 hours if stored in the refrigerator.]

**Assay preparation 2** (for use in assaying vials and infusion bottles packaged for dispensing)—Constitute 1 container of Cefotaxime for Injection with the smallest volume of diluent specified in the labeling. Invert the container, and withdraw all of the withdrawable contents of the container with a hypodermic needle and syringe. Transfer the contents of the syringe to a 100-mL volumetric flask, dilute with Solution A to volume, and mix. [NOTE—Do not rinse the syringe or container.] Dilute an accurately measured volume of this solution quantitatively with Solution A to obtain a solution having a concentration of about 0.8 mg of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) per mL. [NOTE—Use this solution promptly. It may be used within 24 hours if stored in the refrigerator.]

**Assay preparation 3** (for use in assaying piggyback infusion bottles)—Constitute 1 container of Cefotaxime for Injection with the smallest volume of diluent recommended in the labeling, using the directions specified in the labeling. Proceed as directed for Assay preparation beginning with "Invert the container. . ."

**Assay preparation 4** (for use in assaying pharmacy bulk packages where the label states the quantity of cefotaxime in a given volume of constituted solution)—Constitute 1 container of Cefotaxime for Injection with the volume of diluent, accurately measured, specified in the labeling. With a hypodermic needle and syringe, withdraw an accurately measured portion of the resultant solution, equivalent to about 1000 mg of cefotaxime, to a 100-mL volumetric flask, dilute with Solution A to volume, and mix. [NOTE—Do not rinse the syringe or container.] Dilute an accurately measured volume of this solution quantitatively with Solution A to obtain a solution having a concentration of about 0.8 mg of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) per mL. [NOTE—Use this solution promptly. It may be used within 24 hours if stored in the refrigerator.]

**Procedure**—Proceed as directed for Procedure in the Assay under Cefotaxime Sodium. Calculate the quantity, in  $\mu$ g, of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) in each mg of the Cefotaxime for Injection taken by the formula:

$$50(CP / W)(r_U / r_S)$$

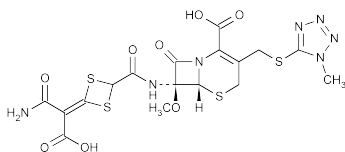
in which  $W$  is the weight, in mg, of the Cefotaxime for Injection taken, and the other terms are as defined therein. Calculate the quantity, in mg, of cefotaxime in the container, and in the portion of constituted solution taken by the formula:

$$(CP)(L/1000D)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of cefotaxime in the container, or in the volume of constituted solution taken; and  $D$  is the concentration, in mg per mL, of cefotaxime in Assay preparation 2 or in Assay preparation 3, or in Assay preparation 4, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively; and the extent of dilution, and the other terms are as defined therein. Where the test for Uniformity of dosage units has been performed using the Procedure for

content uniformity, use the average of these determinations as the Assay value.

## Cefotetan



$C_{17}H_{17}N_7O_8S_4$  575.62

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, [6R-(6 $\alpha$ ,7 $\alpha$ )]-, (6R,7S)-4-[[2-Carboxy-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbamoyl]-1,3-dithietane- $\Delta^{2,\alpha}$ -malonic acid, (6R,7S)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [69712-56-7].

» Cefotetan contains not less than 950  $\mu$ g and not more than 1030  $\mu$ g of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefotetan RS

USP Endotoxin RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that observed in the chromatogram of the Standard preparation obtained as directed in the Assay.

**Water**, Method I (921): not more than 2.5%.

**Sterility** (71) (where it is labeled as sterile)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined, except to use Fluid A to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.

**Other requirements**—Where the label states that Cefotetan is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for Bacterial endotoxins under Cefotetan for Injection.

**Assay**—[NOTE—Protect the Standard preparation, the Resolution solution, and the Assay preparation from light, and use within 2 hours.]

**Mobile phase**—Prepare a filtered and degassed mixture of 0.1 M phosphoric acid, methanol, acetonitrile, and glacial acetic acid (1700:105:105:100). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Transfer about 20 mg of USP Cefotetan RS, accurately weighed, to a 100-mL volumetric flask, add 5 mL of methanol, swirl for several minutes, add 5 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume, and mix.

**Resolution solution**—Place 10 mL of Standard preparation in a glass-stoppered flask containing a few mg of magnesium carbonate, and sonicate for 10 minutes. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Filter the turbid solution through a filter of 0.5  $\mu$ m or finer porosity. Collect the clear filtrate, and use as the Resolution solution.

**Assay preparation**—Using a suitable quantity of Cefotetan, accurately weighed, proceed as directed for Standard preparation.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph 20  $\mu$ L of the Resolution solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.75 for cefotetan and 1.0 for cefotetan tautomer; and the resolution between the cefotetan peak and the cefotetan tautomer peak is not less than 2.0. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ) in each mg of the Cefotetan taken by the formula:

$$200(CP / M)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefotetan RS in the Standard preparation; P is the designated potency, in  $\mu$ g of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ) per mg, of USP Cefotetan RS; M is the weight, in mg, of Cefotetan taken to prepare the Assay preparation; and  $r_U$  and  $r_S$  are the cefotetan peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Cefotetan Injection

» Cefotetan Injection is a sterile isoosmotic solution of Cefotetan and Sodium Bicarbonate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ).

**Packaging and storage**—Preserve in Containers for Injections as described under Injections (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for Labeling under Injections (1). The label states that it is to be thawed just prior to use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefotetan RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that observed in the chromatogram of the Standard preparation obtained as directed in the Assay.

**Bacterial endotoxins** (85)—It contains not more than 0.17 USP Endotoxin Unit per mg of cefotetan.



**Sterility**  $\langle 71 \rangle$ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH**  $\langle 791 \rangle$ : between 4.0 and 6.5.

**Particulate matter**  $\langle 788 \rangle$ : meets the requirements for small-volume injections.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cefotetan*.

*Assay preparation*—Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer an accurately measured volume of this solution, equivalent to about 40 mg of cefotetan, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Cefotetan*. Calculate the quantity, in mg, of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ) in each mL of the Injection taken by the formula:

$$0.2(CP / V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and the other terms are as defined therein.

## Cefotetan for Injection

### DEFINITION

Cefotetan for Injection contains an amount of Cefotetan Disodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the appropriate *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B. IDENTIFICATION TESTS—GENERAL, Sodium**  $\langle 191 \rangle$ : Meets the requirements

### ASSAY

#### PROCEDURE

[NOTE—Protect the *Standard solution*, the *System suitability solution*, *Sample solution A*, and *Sample solution B* from light, and use within 2 h.]

**Solution A:** Acetonitrile, methanol, and water (1:1:18)

**Mobile phase:** Acetonitrile, methanol, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

**Standard solution:** 20 mg of USP Cefotetan RS in a 100-mL volumetric flask. Add 5 mL of methanol, swirl for several min, add 5 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume.

**System suitability solution:** 10 mL of *Standard solution* in a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Filter the turbid solution through a filter of 0.5- $\mu$ m or finer pore size. Use the clear filtrate.

**Sample solution A** (where the package is represented as being in a single-dose container): Constitute Cefotetan for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and quantitatively dilute with *Solution A* to obtain a solution containing the equivalent of 200  $\mu$ g/mL of cefotetan.

**Sample solution B** (where the label states the quantity of cefotetan in a given volume of constituted solu-

tion): Constitute Cefotetan for Injection as directed in the labeling. Dilute an aliquot of the constituted solution with *Solution A* to obtain a solution containing the equivalent of 200  $\mu$ g/mL of cefotetan.

#### Chromatographic system

(See *Chromatography*  $\langle 621 \rangle$ , *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively, *System suitability solution*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution*, and *Sample solution A* or *Sample solution B*

Calculate the percentage of  $C_{17}H_{17}N_7O_8S_4$  withdrawn from the container, or in the portion of solution taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from *Sample solution A* or *Sample solution B*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefotetan RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of cefotetan in *Sample solution A* or *Sample solution B* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS**  $\langle 905 \rangle$ : Meets the requirements

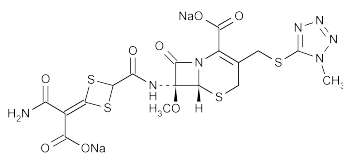
### SPECIFIC TESTS

- INJECTIONS, Constituted Solutions**  $\langle 1 \rangle$ : Meets the requirements at the time of use
- BACTERIAL ENDOTOXINS TEST**  $\langle 85 \rangle$ : NMT 0.17 USP Endotoxin Unit/mg of cefotetan
- STERILITY TESTS**  $\langle 71 \rangle$ : Meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*
- PARTICULATE MATTER IN INJECTIONS**  $\langle 788 \rangle$ : Meets the requirements for small-volume injections
- pH**  $\langle 791 \rangle$ : 4.0–6.5, in a solution 100 mg/mL
- WATER DETERMINATION, Method Ic**  $\langle 921 \rangle$ : NMT 2.8%
- OTHER REQUIREMENTS:** It meets the requirements under *Injections*  $\langle 1 \rangle$ , *Labels and Labeling*.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve as described under *Injections*  $\langle 1 \rangle$ , *Containers for Sterile Solids*.
- USP REFERENCE STANDARDS**  $\langle 11 \rangle$   
USP Cefotetan RS  
USP Endotoxin RS

## Cefotetan Disodium



$C_{17}H_{15}N_7Na_2O_8S_4$  619.58

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-methoxy-3-[[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, disodium salt, [6*R*-(6 $\alpha$ ,7 $\alpha$ )]-. (6*R*,7*S*)-4-[[[2-Carboxy-7-methoxy-3-[[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbamoyl]-1,3-dithietane- $\Delta^{2,\alpha}$ -malonic acid, disodium salt. (6*R*,7*S*)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [74356-00-6].

» Cefotetan Disodium contains the equivalent of not less than 830  $\mu$ g and not more than 970  $\mu$ g of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefotetan RS  
USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**B:** It responds to the tests for *Sodium* (191).

**pH** (791): between 4.0 and 6.5, in a solution (1 in 10).

**Water, Method 1c** (921): not more than 2.5%.

**Other requirements**—Where the label states that Cefotetan Disodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefotetan for Injection*. Where the label states that Cefotetan Disodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefotetan for Injection*.

**Assay**—[NOTE—Protect the *Standard preparation*, the *Resolution solution*, and the *Assay preparations* from light, and use within 2 hours.]

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefotetan*.

*Assay preparation*—Transfer about 40 mg of Cefotetan Disodium, accurately weighed, to a 200-mL volumetric flask, add 10 mL of methanol, swirl for several minutes, add 10 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume, and mix.

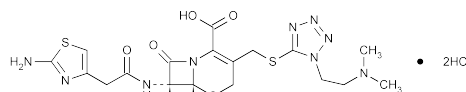
*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefotetan*. Calculate the quantity, in  $\mu$ g, of cefotetan

( $C_{17}H_{17}N_7O_8S_4$ ) per mg in the portion of Cefotetan Disodium taken by the formula:

$$200(CP / M)(r_U / r_S)$$

in which the terms are as defined therein.

## Cefotiam Hydrochloride



$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$  598.55

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(2-amino-4-thiazolyl)acetyl]-amino]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]-thio]methyl]-8-oxo, hydrochloride, (6*R*-*trans*)-. (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrochloride. 7(*R*)-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid dihydrochloride [66309-69-1].

» Cefotiam Hydrochloride contains the equivalent of not less than 790  $\mu$ g and not more than 925  $\mu$ g of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefotiam Hydrochloride RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 20  $\mu$ g per mL.

*Medium:* water.

**B:** The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Crystallinity** (695): meets the requirements.

**Pyrogen**—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements of the *Pyrogen Test* (151), the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 25.6 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of Sterile Water for Injection) containing 40 mg per mL.

**Sterility** (71)—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Water, Method 1** (921): not more than 7.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen, except to use a mixture of 20 mL of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, and to determine the water content of the formamide and methanol mixture.

**Assay—**

**Mobile phase**—Dissolve 13.1 g of ammonium sulfate in 850 mL of water, adjust with 2 N ammonium hydroxide to a pH of  $6.5 \pm 0.1$ , add 150 mL of acetonitrile, and mix. Filter through a suitable filter of 0.5  $\mu\text{m}$  or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefotiam Hydrochloride RS, quantitatively in water to obtain a solution having a known concentration of about 1000  $\mu\text{g}$  of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50  $\mu\text{g}$  of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) per mL. Use this solution without delay.

**Assay preparation**—Transfer about 60 mg of Cefotiam Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution without delay.

**System suitability solution**—Prepare a solution of USP Cefotiam Hydrochloride RS in water containing about 1 mg per mL. Heat this solution at  $95^\circ$  for 3 minutes, and cool. Transfer 1 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, determined from the cefotiam peak, is not less than 1985 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

the tailing factor for the cefotiam peak is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for de-tetrazol-cefotiam and 1.0 for cefotiam; and the resolution,  $R$ , between the de-tetrazol-cefotiam peak and the cefotiam peak is not less than 4.0.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) in each mg of the Cefotiam Hydrochloride taken by the formula:

$$1000(C/W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) in the *Standard preparation*, based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare the *Standard preparation*, the designated cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) content, in  $\mu\text{g}$  per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution;  $W$  is the weight, in mg, of Cefotiam Hydrochloride taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefotiam for Injection

» Cefotiam for Injection contains an amount of Cefotiam Hydrochloride equivalent to not less than 90.0 percent and not more than 120.0 per-

cent of the labeled amount of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ). It may contain Sodium Carbonate.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cefotiam Hydrochloride RS

**Identification—**

**A: Ultraviolet Absorption** (197U)—

*Solution:* 20  $\mu\text{g}$  per mL.

*Medium:* water.

**B:** The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*; as obtained in the *Assay*.

**Pyrogen**—It meets the requirements of the *Pyrogen Test* (151), the test dose being 1.0 mL per kg of a solution prepared by diluting Cefotiam for Injection with Sterile Water for Injection to a concentration of 40 mg of cefotiam per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.7 and 7.2, in a solution containing the equivalent of 100 mg of cefotiam per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 hours: it loses not more than 6.0% of its weight.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay—**

**Mobile phase, Standard preparation, System suitability solution, and Chromatographic system**—Prepare as directed in the *Assay* under *Cefotiam Hydrochloride*.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing the equivalent of about 1 mg of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50  $\mu\text{g}$  of cefotiam per mL. Use this solution without delay.

**Assay preparation 2** (where the label states the quantity of cefotiam in a given volume of constituted solution)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50  $\mu\text{g}$  of cefotiam per mL. Use this solution without delay.

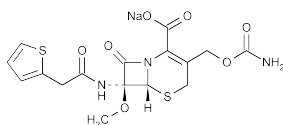
**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefotiam Hydrochloride*. Calculate the quantity, in mg, of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$C(L / D)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of cefotiam ( $\text{C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3$ ) in the *Standard preparation*, based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare

the *Standard preparation*, the designated cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) content, in  $\mu\text{g}$  per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution;  $L$  is the labeled quantity, in mg, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) in the container, or in the volume of constituted solution taken;  $D$  is the concentration, in  $\mu\text{g}$  of cefotiam per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution; and  $r_U$  and  $r_S$  are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefoxitin Sodium



$C_{16}H_{16}N_3NaO_7S_2$  449.43

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(aminocarbonyl)oxy]methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl)amino]-, sodium salt (6*R*-*cis*)-.

Sodium (6*R*,7*S*)-3-(hydroxymethyl)-7-methoxy-8-oxo-7-[2-(2-thienyl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate carbamate (ester) [33564-30-6; 35607-66-0].

» Cefoxitin Sodium contains the equivalent of not less than 927  $\mu\text{g}$  and not more than 970  $\mu\text{g}$  of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) per mg, corresponding to not less than 97.5 percent and not more than 102.0 percent of cefoxitin sodium ( $C_{16}H_{16}N_3NaO_7S_2$ ), calculated on the anhydrous and acetone- and methanol-free basis.

**Packaging and storage**—Preserve in tight containers, and store in a cold place.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefoxitin RS

USP Endotoxin RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefoxitin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**B: Ultraviolet Absorption** (197U)—

*Solution:* 20  $\mu\text{g}$  per mL.

*Medium:* phosphate buffer (prepared by dissolving 1.0 g monobasic potassium phosphate and 1.8 g of anhydrous dibasic sodium phosphate in water to make 1000 mL).

**C:** A solution (1 in 20) responds to the tests for *Sodium* (191).

**Specific rotation** (781S): between  $+206^\circ$  and  $+214^\circ$ , calculated on the anhydrous and acetone- and methanol-free basis.

*Test solution:* 10 mg per mL, in methanol.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.2 and 7.0, in a solution containing 100 mg per mL.

**Water, Method I** (921): not more than 1.0%, a mixture of ethylene glycol and pyridine (3:1) being used in place of methanol in the titration vessel.

**Heavy metals, Method II** (231): 0.002%.

**Limit of acetone and methanol**—

*Standard preparation*—Transfer 5.0 mL of acetone to a 1000-mL volumetric flask, dilute with water to volume, and mix (*Solution A*). Transfer 5.0 mL of methanol to a 1000-mL volumetric flask, dilute with water to volume, and mix (*Solution B*). Transfer 50.0 mL of *Solution A* and 5.0 mL of *Solution B* to a 500-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having concentrations of acetone and methanol of 0.050% and 0.005% (v/v), respectively.

*Test preparation*—Transfer 5.0 g of Cefoxitin Sodium to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 3.0 mL of the resulting solution to a 15-mL centrifuge tube, cool in an ice-water bath for 2 minutes, and add 3.0 mL of 0.24 N hydrochloric acid while swirling vigorously. Centrifuge to obtain a clear solution (*Test preparation*).

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector, and contains a 1.8-m  $\times$  6.3-mm glass column containing support S2, and a pre-column packed with 60- to 80-mesh silane-treated glass beads. The injection port is maintained at  $100^\circ$ , the columns are maintained at  $110^\circ$ , the detector is maintained at  $200^\circ$ , and nitrogen is used as the carrier gas at a flow rate of about 50 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the acetone and methanol peaks is not less than 160 and 200 theoretical plates, respectively; the tailing factors for the acetone and methanol peaks are not more than 1.3 and 2.3, respectively; and the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 2  $\mu\text{L}$ ) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the acetone and methanol peak responses. Calculate the percentages of acetone and methanol in the Cefoxitin Sodium taken by the same formula:

$$DP/C(r_U / r_S)$$

in which  $D$  is the density of acetone and methanol at  $20^\circ$  in g per mL;  $P$  is the percentage (v/v) of acetone or methanol in the *Standard preparation*;  $C$  is the concentration, in g per mL, of Cefoxitin Sodium in the *Test preparation*; and  $r_U$  and  $r_S$  are the acetone or methanol peak responses of the *Test preparation* and the *Standard preparation*, respectively: not more than 0.7% of acetone and 0.1% of methanol are found.

**Other requirements**—Where the label states that Cefoxitin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefoxitin for Injection*. Where the label states that Cefoxitin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefoxitin for Injection*.

**Assay**—

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, and glacial acetic acid (840:160:10), filter through a membrane filter (1  $\mu\text{m}$  or finer porosity), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Phosphate buffer**—Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water, adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $7.1 \pm 0.1$ , dilute with water to

make 1000 mL, and mix. Filter through a membrane filter of 1  $\mu$ m or finer porosity.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefoxitin RS in *Phosphate buffer* to obtain a solution having a known concentration of about 0.3 mg per mL. [NOTE—Sonicate, if necessary, to dissolve the specimen.] Use this solution within 5 hours.

**Assay preparation**—Transfer about 150 mg of Cefoxitin Sodium, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix. Use this solution within 5 hours.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2800 theoretical plates, the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) per mg of the Cefoxitin Sodium taken by the formula:

$$500(CP / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefoxitin RS in the *Standard preparation*; P is the potency, in  $\mu$ g per mg, of USP Cefoxitin RS; W is the quantity, in mg, of Cefoxitin Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefoxitin Injection

» Cefoxitin Injection is a sterile solution of Cefoxitin Sodium and one or more suitable buffer substances in Water for Injection. It contains Dextrose or Sodium Chloride as a tonicity-adjusting agent. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* <1>. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* <1>. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** <11>—

USP Cefoxitin RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefoxitin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 0.13 USP Endotoxin Unit per mg of cefoxitin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** <791>: between 4.5 and 8.0.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Assay**—

*Mobile phase*, *Phosphate buffer*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefoxitin Sodium*.

**Assay preparation**—Allow 1 container of Injection to thaw, and mix. Dilute an accurately measured volume of Injection quantitatively with *Phosphate buffer* to obtain a solution containing about 0.3 mg of cefoxitin per mL. Use this solution within 5 hours.

**Procedure**—Proceed as directed in the *Assay* under *Cefoxitin Sodium*. Calculate the quantity, in mg, of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) in each mL of the Injection taken by the formula:

$$(CP / 1000)(L / D)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) in each mL of Injection taken; D is the concentration, in mg per mL, of the *Assay preparation*, based on the volume of Injection taken and the extent of dilution; and the other terms are as defined therein.

## Cefoxitin for Injection

» Cefoxitin for Injection contains Cefoxitin Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

**USP Reference standards** <11>—

USP Cefoxitin RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Bacterial endotoxins** <85>—It contains not more than 0.13 USP Endotoxin Unit per mg of cefoxitin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification* tests and meets the requirements for *pH* and *Water* under *Cefoxitin Sodium*. It meets also the requirements for *Uniformity of Dosage Units* <905> and for *Labeling* under *Injections* <1>.

**Assay**—

*Mobile phase*, *Phosphate buffer*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefoxitin Sodium*.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Cefoxitin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution having a concentration of about 0.3 mg of cefoxitin per mL. Use this solution within 5 hours.

**Assay preparation 2** (where the label states the quantity of cefoxitin in a given volume of constituted solution)—Constitute Cefoxitin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured vol-

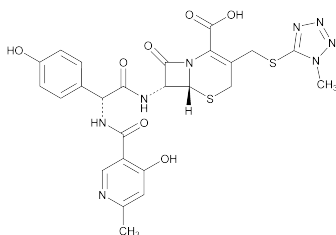
ume of the constituted solution quantitatively with water to obtain a solution containing about 0.3 mg of cefoxitin per mL. Use this solution within 5 hours.

**Procedure**—Proceed as directed in the Assay under *Cefoxitin Sodium*. Calculate the quantity, in mg, of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) in the portion of constituted solution taken by the formula:

$$0.001(CP)(L/D)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, in the portion of constituted solution taken; *D* is the concentration, in mg per mL, of Assay preparation 1 or Assay preparation 2, based on the volume of constituted solution taken and the extent of dilution; and the other terms are as defined therein.

## Cefpiramide



$C_{25}H_{24}N_8O_7S_2$  612.64

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(4-hydroxy-6-methyl-3-pyridinyl)carbonyl]amino]-(4-hydroxyphenyl)acetyl]amino]-3-[[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, [6*R*-[6*α*,7*β* (*R'*)]]-; (6*R*,7*R*)-7-[(*R*)-2-(4-Hydroxy-6-methylnicotinamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [70797-11-4].

### DEFINITION

Cefpiramide contains NLT 974 µg/mg and NMT 1026 µg/mg of  $C_{25}H_{24}N_8O_7S_2$ , calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of  $6.8 \pm 0.1$  prior to final dilution

**Mobile phase:** Tetrahydrofuran, acetonitrile, methanol, and *Buffer* (40:40:40:880)

**System suitability solution:** 1 mg/mL of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at 95° for 10 min. Mix 1 mL of this solution with 19 mL of *Mobile phase*. This solution contains a mixture of cefpiramide and cefpiramide lactone.

**Standard solution:** 0.25 mg/mL of USP Cefpiramide RS in *Mobile phase*

**Sample solution:** 0.25 mg/mL of Cefpiramide in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.0-mm  $\times$  15- to 30-cm; 5- to 10-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefpiramide and cefpiramide lactone are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5 between cefpiramide lactone and cefpiramide, *System suitability solution*

**Tailing factor:** 0.95–1.4, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of cefpiramide ( $C_{25}H_{24}N_8O_7S_2$ ) in each mg of the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefpiramide taken to prepare the *Sample solution* (mg/mL)

*P* = potency of cefpiramide in USP Cefpiramide RS (mg/mg)

*F* = conversion factor, 1000 µg/mg

**Acceptance criteria:** 974–1026 µg/mg on the anhydrous basis

### IMPURITIES

#### ORGANIC IMPURITIES

**System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Buffer:** 4.08 g/L of monobasic potassium phosphate in water, adjusted with 1 N sodium hydroxide to a pH of  $7.5 \pm 0.1$  prior to final dilution

**Mobile phase:** Methanol and *Buffer* (250:750)

**Standard stock solution:** 0.15 mg/mL of sodium 5-mercapto-1-methyltetrazole and 0.25 mg/mL of USP Cefpiramide RS in *Buffer*

**Standard solution:** 3 µg/mL of sodium 5-mercapto-1-methyltetrazole and 5 µg/mL of USP Cefpiramide RS from the *Standard stock solution* in *Mobile phase*

**Sample solution:** 0.5 mg/mL of Cefpiramide in *Mobile phase*

***N*-Ethylmaleimide solution:** 40 mg/mL of *N*-ethylmaleimide in methanol

**Test preparation:** 10 mg/mL of sodium 5-mercapto-1-methyltetrazole in *N*-Ethylmaleimide solution in a stoppered centrifuge tube. Sonicate for 15 min.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the water content of sodium 5-mercapto-1-methyltetrazole by the titrimetric method (see *Water Determination* (921)), using 5.0 mL of the *Test preparation*.

Calculate the percentage of 5-mercapto-1-methyltetrazole in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (M_{r1}/M_{r2}) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response of 5-mercapto-1-methyltetrazole from the *Sample solution*

$r_S$  = peak response of 5-mercapto-1-methyltetrazole from the *Standard solution*

$M_{r1}$  = molecular weight of 5-mercapto-1-methyltetrazole, 115.14

$M_{r2}$  = molecular weight of anhydrous sodium 5-mercapto-1-methyltetrazole, 138.13

- $C_S$  = concentration of sodium 5-mercapto-1-methyltetrazole, corrected for water, in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Cefpiramide in the *Sample solution* ( $\text{mg/mL}$ )  
 $F$  = conversion factor, 0.001  $\text{mg}/\mu\text{g}$   
 Calculate the percentage of each other impurity in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

- $r_U$  = peak response of each other impurity from the *Sample solution*  
 $r_S$  = peak response of cefpiramide from the *Standard solution*  
 $C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Cefpiramide in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $P$  = potency of cefpiramide in USP Cefpiramide RS ( $\mu\text{g}/\text{mg}$ )  
**Acceptance criteria:** See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Mercapto-1-methyltetrazole	0.20	0.7
Cefpiramide	1.0	—
Any other individual impurity	—	0.7
Total impurities	—	2.0

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation (781S)**  
**Sample solution:** 10  $\text{mg/mL}$ , in dimethylformamide  
**Acceptance criteria:**  $-100^\circ$  to  $-112^\circ$
- **CRYSTALLINITY (695):** Meets the requirements
- **PH (791)**  
**Sample solution:** 5- $\text{mg/mL}$  suspension in water  
**Acceptance criteria:** 3.0–5.0
- **WATER DETERMINATION, Method I (921):** NMT 9.0%
- **PYROGEN TEST (151)**  
**Sample solution:** 50  $\text{mg/mL}$  of cefpiramide in Sterile Water for Injection  
**Test dose:** 1.0  $\text{mL/kg}$  of the *Sample solution*  
**Acceptance criteria:** Where the label states that Cefpiramide is sterile, or it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements.
- **STERILITY TESTS (71):** Where the label states that Cefpiramide is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**  
 USP Cefpiramide RS

**Cefpiramide for Injection****DEFINITION**

Cefpiramide for Injection contains NLT 90.0% and NMT 120.0% of the labeled amount of cefpiramide ( $\text{C}_{25}\text{H}_{24}\text{N}_8\text{O}_7\text{S}_2$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 1.36  $\text{g/L}$  of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of  $6.8 \pm 0.1$  before final dilution

**Mobile phase:** Tetrahydrofuran, acetonitrile, methanol, and *Buffer* (40:40:40:880)

**System suitability solution:** 1  $\text{mg/mL}$  of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at  $95^\circ$  for 10 min. Dilute 1  $\text{mL}$  of this solution with *Mobile phase* to 20  $\text{mL}$ . This solution contains a mixture of cefpiramide and cefpiramide lactone.

**Standard solution:** 0.25  $\text{mg/mL}$  of USP Cefpiramide RS in *Mobile phase*

**Sample solution 1** (where it is represented as being in a single-dose container): Constitute a container of Cefpiramide for Injection in a volume of water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase* to obtain a solution containing the nominal equivalent of 0.25  $\text{mg/mL}$  of cefpiramide.

**Sample solution 2** (where the label states the quantity of cefpiramide in a given volume of constituted solution): Constitute a container of Cefpiramide for Injection in a volume of water equivalent to the volume of diluent specified in the labeling. Dilute the constituted solution with water to obtain a solution nominally containing 0.25  $\text{mg/mL}$  of cefpiramide.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254  $\text{nm}$

**Column:** 4.0- $\text{mm} \times 15\text{- to }30\text{-cm}$ ; 5- to 10- $\mu\text{m}$  packing L7

**Flow rate:** 1.5  $\text{mL/min}$

**Injection size:** 20  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefpiramide and cefpiramide lactone are 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 5 between cefpiramide lactone and cefpiramide, *System suitability solution*

**Tailing factor:** 0.95–1.4, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of cefpiramide ( $\text{C}_{25}\text{H}_{24}\text{N}_8\text{O}_7\text{S}_2$ ) withdrawn from the container, or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of the *Sample solution*  
 $r_S$  = peak response of the *Standard solution*  
 $C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* ( $\text{mg/mL}$ )  
 $C_U$  = nominal concentration of cefpiramide in *Sample solution 1* or *Sample solution 2* ( $\text{mg/mL}$ )

**Acceptance criteria:** 90.0%–120.0%

**SPECIFIC TESTS**• **PYROGEN TEST** (151)

**Sample solution:** 50 mg/mL of cefpiramide from Cefpiramide for Injection in Sterile Water for Injection

**Test dose:** 1.0 mL/kg of the *Sample solution*

**Acceptance criteria:** Meets the requirements

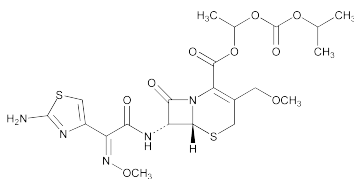
• **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.• **PH** (791)

**Sample solution:** Equivalent to 100 mg/mL of cefpiramide from Cefpiramide for Injection

**Acceptance criteria:** 6.0–8.0 in water

• **WATER DETERMINATION, Method I** (921): NMT 3.0%• **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve as described in *Injections* (1), *Containers for Sterile Solids*.• **USP REFERENCE STANDARDS** (11)

USP Cefpiramide RS

**Cefpodoxime Proxetil**

$C_{21}H_{27}N_5O_9S_2$  557.60

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-(methoxymethyl)-8-oxo-1-[[[(1-methylethoxy)carbonyl]oxy]ethyl ester, [6R-[6 $\alpha$ , 7 $\beta$ (Z)]]]-, (±)-1-Hydroxyethyl(+)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7 $^2$ -(Z)-(O-methyloxime), isopropyl carbonate (ester) [87239-81-4].

» Cefpodoxime Proxetil contains the equivalent of not less than 690  $\mu$ g and not more than 804  $\mu$ g of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 25°.

**USP Reference standards** (11)—

USP Cefpodoxime Proxetil RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 15  $\mu$ g per mL.

*Medium:* acetonitrile.

**C:** Dissolve 1 mg of it in 4 mL of water, add 1 mL of 1 N sulfuric acid while cooling in an ice bath, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), allow to stand for 2 minutes, then add 1 mL of ammonium sulfate solution (1 in 100). Allow to stand for 1 minute, and add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride TS: a red-purple color develops.

**Specific rotation** (781S): between +35.0° and +48.0°.

*Test solution:* 10 mg per mL, in methanol.

**Water, Method I** (921): not more than 3.0%.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

**Isomer ratio**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the ratio of the cefpodoxime proxetil *R*-epimer peak response to the sum of the peak responses of the cefpodoxime proxetil *S*-epimer peak and the cefpodoxime proxetil *R*-epimer peak: the ratio is between 0.5 and 0.6.

**Chromatographic purity**—

*Solution A*—Prepare filtered and degassed 0.02 M ammonium acetate.

*Solution B*—Use filtered and degassed acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a degassed mixture of water and acetonitrile (2:1).

*System suitability solution*—Dissolve a quantity of USP Cefpodoxime Proxetil RS in *Diluent* to obtain a solution containing about 10  $\mu$ g per mL. [NOTE—A volume of methanol not exceeding 10% of the total volume in the final solution may be used to facilitate dissolution.]

*Test solution*—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, using sonication if necessary, dilute with *Diluent* to volume, and mix. This solution should be injected promptly, but may be analyzed within 24 hours when stored at 8°.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The column temperature is maintained at a constant temperature of about 30°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration (10 minutes)
0–10	90→68	10→32	linear gradient
10–40	68	32	isocratic
40–80	68→50	32→50	linear gradient
80–85	50	50	isocratic
85–90	50→25	50→75	linear gradient
90–95	25	75	isocratic
95–100	25→90	75→10	linear gradient

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the retention time for cefpodoxime proxetil *R*-epimer is between 37 and 42 minutes; the relative retention times are about 0.9 for cefpodoxime proxetil *S*-epimer and 1.0 for cefpodoxime proxetil *R*-epimer; the resolution, *R*, between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 4.0; the column efficiency is not less than 19,000 theoretical plates determined from the cefpodoxime proxetil *R*-epimer peak; and the relative standard deviation for replicate injections determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks is not more than 2.0%.

*Procedure*—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage



of each impurity in the portion of Cefpodoxime Proxetil taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area for each impurity; and  $r_s$  is the sum of the areas of all the peaks: not more than 3.0% of any peak at a relative retention time of about 0.86 is found; not more than 1.0% for any peak at relative retention times of about 1.27, 1.39, and other individual peaks having relative retention times higher than 2.0 is found; not more than 0.5% of any other individual impurity is found; and not more than 6.0% of total impurities is found, impurity peaks of less than 0.05% being disregarded.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.02 M ammonium acetate and acetonitrile (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluent**—Prepare a degassed mixture of water and acetonitrile (6:4).

**Standard preparation**—Transfer about 25 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Assay preparation**—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 2 mL per minute. The column temperature is maintained at a constant temperature of about 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for cefpodoxime proxetil S-epimer and 1.0 for cefpodoxime proxetil R-epimer; the resolution,  $R$ , between cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer is not less than 2.5; the tailing factor for cefpodoxime proxetil R-epimer is not more than 1.5; and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer peaks for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity in  $\mu$ g of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in each mg of Cefpodoxime Proxetil taken by the formula:

$$2000(CP/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; W is the weight, in mg, of Cefpodoxime Proxetil taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the sums of the peak responses for cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefpodoxime Proxetil for Oral Suspension

» Cefpodoxime Proxetil for Oral Suspension contains Cefpodoxime Proxetil and one or more buffers, suspending agents, sweeteners, flavorings, and preservatives. When constituted as directed in the labeling, it contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ).

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 30°. Store the constituted Oral Suspension in a refrigerator.

**USP Reference standards** <11>—  
USP Cefpodoxime Proxetil RS

**Identification**—The retention times of the cefpodoxime proxetil R-epimer peak and the cefpodoxime proxetil S-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** <905>—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>: meets the requirements.

**pH** <791>: between 4.0 and 5.5, in the suspension constituted as directed in the labeling.

**Water** <921>: not more than 1.5%.

#### Assay—

**Mobile phase, Diluent, and Chromatographic system**—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil*.

**Standard preparation**—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Assay preparation**—Constitute a container of Cefpodoxime Proxetil for Oral Suspension as directed in the labeling. Shake the resulting suspension thoroughly, and determine its density. Transfer an accurately weighed quantity of the suspension, equivalent to about 50 mg of cefpodoxime, to a 100-mL volumetric flask. Add 10 mL of water, and shake to disperse. Add 20 mL of acetonitrile, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in the portion of Oral Suspension taken by the formula:

$$2CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; and  $r_U$  and  $r_S$  are the sums of the peak responses for cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefpodoxime Proxetil Tablets

» Cefpodoxime Proxetil Tablets contain an amount of Cefpodoxime Proxetil equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ).

**Packaging and storage**—Preserve in tight containers, at controlled room temperature.

**USP Reference standards** (11)—

USP Cefpodoxime Proxetil RS

**Identification**—The retention times of the cefpodoxime proxetil *R*-epimer peak and the cefpodoxime proxetil *S*-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

**Medium**—Dissolve 54.5 g of glycine and 42.6 g of sodium chloride in about 500 mL of water in a 1000-mL volumetric flask. Cautiously add, with swirling, 14.2 mL of hydrochloric acid, and allow to cool. Dilute with water to volume, and mix. Transfer 50 mL of this stock solution to a flask, and dilute with water to 900 mL to obtain a solution having a pH of  $3.0 \pm 0.1$ . [NOTE—If necessary, adjust the pH of the stock solution with 10 N sodium hydroxide so that when 50 mL is diluted with water to 900 mL the pH of the *Dissolution Medium* is  $3.0 \pm 0.1$ .]

**Apparatus 2**: 75 rpm.

**Time**: 30 minutes.

**Procedure**—Determine the amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) dissolved by employing UV absorption at about 259 nm on filtered portions of the solution under test in comparison with a *Standard solution* having a known concentration of USP Cefpodoxime Proxetil RS prepared by dissolving an accurately weighed portion in a small volume of methanol and diluting quantitatively with *Dissolution Medium*.

**Tolerances**—Not less than 70% (*Q*) of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water** (921): not more than 5.0%.

**Assay**—

**Mobile phase, Diluent, and Chromatographic system**—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil*.

**Standard preparation**—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of cefpodoxime to a 100-mL volumetric flask. Dissolve in 40 mL of *Diluent*, sonicate for 5 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

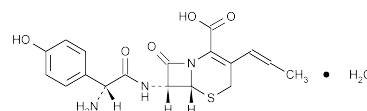
**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in the portion of Tablets taken by the formula:

$$2CP(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; *P* is the designated potency, in  $\mu$ g per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; and  $r_U$  and  $r_S$  are the sums of the peak responses for cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefprozil



$C_{18}H_{19}N_3O_5S \cdot H_2O$  407.44

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-(1-propenyl)-, monohydrate, [6*R*-(6 $\alpha$ ,7 $\beta$ (*R*<sup>\*</sup>))- (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [121123-17-9]. Anhydrous 389.43 [92665-29-7].

» Cefprozil contains not less than 900  $\mu$ g and not more than 1050  $\mu$ g of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefprozil (*Z*)-Isomer RS

USP Cefprozil (*E*)-Isomer RS

**Identification**—

**A: Infrared Absorption** (197K)—

**Standard specimen**: USP Cefprozil (*Z*)-Isomer RS.

**B**: The retention times of the cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparations*, as obtained in the *Assay*.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 3.5 and 6.5, in a solution containing 5 mg per mL.

**Water, Method I** (921): not less than 3.5% and not more than 6.5%.

**Cefprozil (*E*)-isomer ratio**—Calculate the ratio of the cefprozil (*E*)-isomer to the total cefprozil taken by the formula:

$$E / (E + Z)$$

in which *E* is the content of cefprozil (*E*)-isomer, in  $\mu$ g per mg, as determined in the *Assay*, and *Z* is the content of cefprozil (*Z*)-isomer, in  $\mu$ g per mg, as determined in the *Assay*; the ratio is between 0.06 and 0.11.

**Assay**—

**Mobile phase**—Dissolve 20.7 g of monobasic ammonium phosphate in 1800 mL of water, and adjust, if necessary, with phosphoric acid to a pH of 4.4. Add 200 mL of acetonitrile, and mix. Filter this solution through a filter having a porosity of 0.5  $\mu$ m or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621). [NOTE—Decreasing the proportion of acetonitrile in-

creases retention times and improves the separation of the cefprozil isomer peaks.]

**Standard cefprozil (Z)-isomer preparation**—Dissolve an accurately weighed quantity of USP Cefprozil (Z)-Isomer RS. quantitatively in water to obtain a solution having a known concentration of about 0.25 mg per mL. [NOTE—Use this solution within 6 hours.]

**Standard cefprozil (E)-isomer preparation**—Dissolve an accurately weighed quantity of USP Cefprozil (E)-Isomer RS. quantitatively in water to obtain a stock solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Use this solution within 6 hours.]

**Resolution solution**—Prepare a mixture of equal volumes of the Cefprozil (Z)-isomer standard preparation and of the stock solution used to prepare the Cefprozil (E)-isomer standard preparation. [NOTE—Use this solution within 6 hours.]

**Assay preparation**—Transfer about 15 mg of Cefprozil, accurately weighed, to a 50-mL volumetric flask, dilute with water to volume, and shake to assure dissolution. [NOTE—Use this solution within 6 hours.]

**Chromatographic system** (see Chromatography <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the Resolution solution, and record the responses as directed under Procedure: the relative retention times are about 0.7 for cefprozil (Z)-isomer and 1.0 for cefprozil (E)-isomer, and the resolution,  $R$ , between the cefprozil (Z)-isomer peak and the cefprozil (E)-isomer peak is not less than 2.5. Chromatograph the Cefprozil (Z)-isomer standard preparation, and record the responses as directed under Procedure: the column efficiency, determined from the cefprozil (Z)-isomer peak, is not less than 2500 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

the tailing factor, determined from the cefprozil (Z)-isomer peak, is not less than 0.9 and not more than 1.1, when calculated by the formula:

$$W_{0.1} / 2f$$

in which  $W_{0.1}$  is the width of the peak at 10% height, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the Cefprozil (Z)-isomer standard preparation, the Cefprozil (E)-isomer standard preparation, and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in μg, of cefprozil (Z)-isomer and cefprozil (E)-isomer in each mg of the Cefprozil taken by the formula:

$$50(CP / M)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cefprozil (Z)-Isomer RS in the Cefprozil (Z)-isomer standard preparation or of the USP Cefprozil (E)-Isomer RS in the Cefprozil (E)-isomer standard preparation, as appropriate;  $P$  is the assigned potency, in μg per mg, of the appropriate USP Reference Standard;  $M$  is the quantity, in mg, of Cefprozil taken to prepare the Assay preparation; and  $r_U$  and  $r_S$  are the peak responses of the cefprozil (Z)-isomer or the cefprozil (E)-isomer, as appropriate, obtained from the Assay preparation and the relevant Standard preparation, respectively. Calculate the quantity, in μg, of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) in each mg of the Cefprozil taken by adding the values, in μg per mg, obtained for the cefprozil (Z)-isomer and for cefprozil (E)-isomer.

## Cefprozil for Oral Suspension

### DEFINITION

Cefprozil for Oral Suspension is a dry mixture of Cefprozil and one or more suitable buffers, flavors, preservatives, suspending agents, and sweeteners. It contains NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Acetone and 0.1 N hydrochloric acid (4:1)

**Standard solution:** 5 mg/mL of USP Cefprozil (Z)-Isomer RS in Diluent

**Sample solution:** Nominally 5 mg/mL of cefprozil in Diluent from Cefprozil for Oral Suspension. Shake for 5 min, and allow to settle. Use the supernatant.

#### Chromatographic system

(See Chromatography <621>, Thin-Layer Chromatography.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 μL

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:20:20)

#### Analysis

**Samples:** Standard solution and Sample solution

Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the Developing solvent system until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the Sample solution corresponds to that of the Standard solution.

- **B.** The retention times of the cefprozil (Z)-isomer and cefprozil (E)-isomer peaks of the Sample solution correspond to those of Standard solution 1 and Standard solution 2, respectively, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.

**Mobile phase:** Acetonitrile and Buffer (100:900).

[NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]

**Standard solution 1:** 0.25 mg/mL of USP Cefprozil (Z)-Isomer RS. Use this solution within 6 h.

**Standard stock solution:** 0.25 mg/mL of USP Cefprozil (E)-Isomer RS

**Standard solution 2:** 0.025 mg/mL of USP Cefprozil (E)-Isomer RS in water from the Standard stock solution. Use this solution within 6 h.

**System suitability solution:** A mixture of equal volumes of Standard solution 1 and the Standard stock solution. Use this solution within 6 h.

**Sample stock solution:** Nominally 1 mg/mL of cefprozil in water from Cefprozil for Oral Suspension. Prepare as follows. Constitute one container of Cefprozil for Oral Suspension as directed in the labeling. Transfer a suitable aliquot, freshly mixed and free from air bubbles, to a volumetric flask, dilute with water to volume, and mix, sonicating briefly.

**Sample solution:** Nominally 0.3 mg/mL of cefprozil in water from the Sample stock solution. Pass a portion of this solution through a filter of 0.5-μm or finer pore size. Use this solution within 6 h.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *Standard solution 1* and *System suitability solution*

[NOTE—The relative retention times for the cefprozil (Z)-isomer and the cefprozil (E)-isomer are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between cefprozil (Z)-isomer and cefprozil (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates, cefprozil (Z)-isomer, *Standard solution 1*  
Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

$t_R$  = retention time of cefprozil (Z)-isomer

$W_{h/2}$  = peak width at half-height

**Tailing factor:** 0.9–1.1, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = width of the peak at 10% height

$f$  = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution 1*

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the concentration, in mg/mL, of the cefprozil (Z)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)

$P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-Isomer RS (μg/mg)

$F$  = correction factor, 0.001 mg/μg

Calculate the concentration, in mg/mL, of the cefprozil (E)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (E)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (E)-isomer from *Standard solution 2*

$C_S$  = concentration of USP Cefprozil (E)-Isomer RS in *Standard solution 2* (mg/mL)

$P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-Isomer RS (μg/mg)

$F$  = correction factor, 0.001 mg/μg

Calculate the percentage of the labeled amount of cefprozil (C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S) in the portion of Cefprozil for Oral Suspension taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

$C_Z$  = concentration of the cefprozil (Z)-isomer in the *Sample solution* (mg/mL)

$C_E$  = concentration of the cefprozil (E)-isomer in the *Sample solution* (mg/mL)

$C_U$  = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90%–120.0%

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

For solids packaged in single-unit containers: Meets the requirements

##### • DELIVERABLE VOLUME (698)

For solids packaged in multiple-unit containers: Meets the requirements

#### SPECIFIC TESTS

##### • PH (791)

**Sample solution:** Constitute Cefprozil for Oral Suspension as directed in the labeling.

**Acceptance criteria:** 4.0–6.0

##### • WATER DETERMINATION, Method I (921): NMT 3.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight containers.

##### • USP REFERENCE STANDARDS (11)

USP Cefprozil (E)-Isomer RS

USP Cefprozil (Z)-Isomer RS

## Cefprozil Tablets

#### DEFINITION

Cefprozil Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil (C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Acetone and 0.1 N hydrochloric acid (4:1)

**Standard solution:** 5 mg/mL of USP Cefprozil (Z)-Isomer RS in *Diluent*

**Sample solution:** Nominally 2.5 mg/mL of cefprozil from 1 Tablet in *Diluent*. Shake for 5 min, and allow the mixture to settle. Use the supernatant.

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 μL

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:20:20)

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

##### • B. The retention times of the cefprozil (Z)-isomer and cefprozil (E)-isomer peaks of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, respectively, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.

**Mobile phase:** Acetonitrile and *Buffer* (100:900).

[NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]

**Standard solution 1:** 0.25 mg/mL of USP Cefprozil (Z)-Isomer RS. Use this solution within 6 h.

**Standard stock solution:** 0.25 mg/mL of USP Cefprozil (E)-Isomer RS

**Standard solution 2:** 0.025 mg/mL of USP Cefprozil (E)-Isomer RS in water from the *Standard stock solution*. Use this solution within 6 h.

**System suitability solution:** A mixture of equal volumes of *Standard solution 1* and the *Standard stock solution*. Use this solution within 6 h.

**Sample stock solution:** Nominally 6 mg/mL of cefprozil in water from Tablets, prepared as follows. Transfer a suitable number of Tablets to a volumetric flask containing water. Allow the Tablets to disintegrate with the aid of swirling and sonication. Dilute with water to volume.

**Sample solution:** Nominally 0.3 mg/mL of cefprozil in water from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size. Use this solution within 6 h.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *Standard solution 1* and *System suitability solution*

[NOTE—The relative retention times for cefprozil (Z)-isomer and cefprozil (E)-isomer are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between cefprozil (Z)-isomer and cefprozil (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

$t_R$  = retention time of cefprozil (Z)-isomer

$W_{h/2}$  = peak width at half-height

**Tailing factor:** 0.9–1.1, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = width of the peak at 10% height

$f$  = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution 1*

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the concentration, in mg/mL, of the cefprozil (Z)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)

$P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the concentration, in mg/mL, of the cefprozil (E)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (E)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (E)-isomer from *Standard solution 2*

$C_S$  = concentration of USP Cefprozil (E)-Isomer RS in *Standard solution 2* (mg/mL)

$P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the percentage of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) in the portion of Tablets taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

$C_Z$  = concentration of the cefprozil (Z)-isomer in the *Sample solution* (mg/mL)

$C_E$  = concentration of the cefprozil (E)-isomer in the *Sample solution* (mg/mL)

$C_U$  = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Mobile phase, Standard solution 1, Standard solution 2, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Pass the solution under test through a suitable filter. Dilute, if necessary, to 0.3 mg/mL of cefprozil.

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the quantity, in mg, of cefprozil (Z)-isomer dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times P \times F$$

$r_U$  = peak response of cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the quantity, in mg, of cefprozil (E)-isomer dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times P \times F$$

$r_U$  = peak response of cefprozil (E)-isomer from the *Sample solution*

$r_S$  = peak response of cefprozil (E)-isomer from *Standard solution 2*

$C_S$  = concentration of USP Cefprozil (E)-Isomer RS in *Standard solution 2* (mg/mL)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the percentage of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) dissolved:

$$\text{Result} = (M_Z + M_E) \times 100/L$$

$M_Z$  = quantity of cefprozil (Z)-isomer dissolved (mg)  
 $M_E$  = quantity of cefprozil (E)-isomer dissolved (mg)  
 $L$  = label claim of cefprozil (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

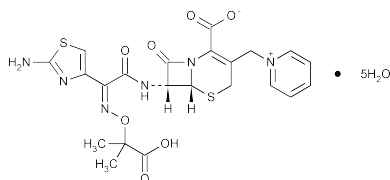
#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 7.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Cefprozil (E)-Isomer RS  
 USP Cefprozil (Z)-Isomer RS

## Ceftazidime



$C_{22}H_{22}N_6O_7S_2 \cdot 5H_2O$  636.65

Pyridinium, 1-[[7-[[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-hydroxide, inner salt, pentahydrate, [6R[6 $\alpha$ ,7 $\beta$ (Z)]]-1-[[[(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]pyridinium hydroxide, inner salt, 7 $^2$ -(Z)-[O(1-carboxy-1-methylethyl)oxime], pentahydrate [78439-06-2].

Anhydrous 546.59

» Ceftazidime contains not less than 95.0 percent and not more than 102.0 percent of  $C_{22}H_{22}N_6O_7S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

#### USP Reference standards (11)—

USP Ceftazidime Delta-3-Isomer RS  
 USP Ceftazidime Pentahydrate RS  
 USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Crystallinity (695):** meets the requirements.

**Sterility (71)**—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid A* to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.

**pH (791):** between 3.0 and 4.0, in a solution containing 5 mg per mL.

**Loss on drying (731)**—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses between 13.0% and 15.0% of its weight.

**Other requirements**—Where the label states that Ceftazidime is sterile or that it must be subjected to further processing during the preparation of injectable or other sterile dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ceftazidime for Injection*.

#### Assay—

**pH 7 Buffer**—Dissolve 42.59 g of anhydrous dibasic sodium phosphate and 27.22 g of monobasic potassium phosphate in water to make 1000 mL of solution.

**Mobile phase**—Mix 40 mL of acetonitrile and 200 mL of pH 7 Buffer, and dilute with water to obtain 2000 mL of solution. Filter, using a filter having a porosity of 1  $\mu$ m or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 29 mg of USP Ceftazidime Pentahydrate RS, accurately weighed, to a 25-mL volumetric flask containing 2.5 mL of pH 7 Buffer, and shake until dissolved. Dilute with water to volume, and mix. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 100  $\mu$ g of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) per mL.

**Assay preparation**—Transfer about 115 mg of Ceftazidime, accurately weighed, to a 100-mL volumetric flask containing 10.0 mL of pH 7 Buffer, and shake until dissolved. Dilute with water to volume, and mix. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Resolution solution**—Prepare a solution of USP Ceftazidime, Delta-3-Isomer RS in pH 7 Buffer containing about 0.1 mg per mL. Immediately prior to chromatography, mix 1 mL of this solution with 8 mL of water and 1 mL of the stock solution used to prepare the *Standard preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between ceftazidime and ceftazidime, delta-3-isomer is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not less than 0.75 and not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{22}N_6O_7S_2$  in the portion of Ceftazidime taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefotazidime Injection

» Cefotazidime Injection is a sterile isoosmotic solution of Cefotazidime in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{22}N_6O_7S_2$ .

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—  
USP Cefotazidime Delta-3-Isomer RS  
USP Cefotazidime Pentahydrate RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefotazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.1 USP Endotoxin Unit per mg of cefotazidime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

### Assay—

*pH 7 Buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefotazidime*.

*Assay preparation*—Allow a container of the Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefotazidime, to a 50-mL volumetric flask, dilute with *pH 7 buffer* to volume, and mix. Transfer 5.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefotazidime*. Calculate the quantity, in mg, of  $C_{22}H_{22}N_6O_7S_2$  in each mL of the Injection taken by the formula:

$$0.5(C / V)(r_U / r_S)$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of cefotazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the cefotazidime peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefotazidime for Injection

» Cefotazidime for Injection is a sterile mixture of Sterile Cefotazidime and Sodium Carbonate or Arginine. It contains not less than 90.0 percent and not more than 105.0 percent of cefotazidime ( $C_{22}H_{22}N_6O_7S_2$ ) on the dried and sodium carbon-

ate- or arginine-free basis, and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefotazidime ( $C_{22}H_{22}N_6O_7S_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), protected from light.

**USP Reference standards** (11)—  
USP L-Arginine RS  
USP Cefotazidime Delta-3-Isomer RS  
USP Cefotazidime Pentahydrate RS  
USP Endotoxin RS

### Identification—

**A:** The chromatograms of the *Assay preparations* exhibit a major peak for cefotazidime, the retention time of which corresponds to that in the chromatogram of the *Standard preparation*.

**B:** It dissolves in 1 N hydrochloric acid with effervescence, evolving a colorless gas, which when passed into *calcium hydroxide TS* produces a white precipitate immediately.

**Bacterial endotoxins** (85)—It contains not more than 0.1 USP Endotoxin Unit per mg of cefotazidime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5, in a solution constituted in the sealed container, taking care to relieve the pressure inside the container during constitution, containing 100 mg of cefotazidime per mL.

**Loss on drying** (731)—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 25° for 4 hours: where it contains arginine, it loses not more than 12.5% of its weight. Where it contains sodium carbonate, it loses not more than 13.5% of its weight. Where it contains arginine, use the percentage loss obtained, *m*, to calculate, on the dried and arginine-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*. Where it contains sodium carbonate, heat the residue in vacuum at a pressure not exceeding 5 mm of mercury at 100° an additional 3 hours, and calculate the total percentage of weight loss. Use this percentage, *m*, to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*.

**Particulate matter** (788): meets the requirements for small-volume injections.

### Sodium carbonate (where present)—

*Potassium chloride solution*—Dissolve 19.07 g of potassium chloride in water to make 1000 mL of solution.

*Standard preparation*—Dissolve a suitable quantity of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, in water to obtain a solution having a known concentration of about 14  $\mu\text{g}$  per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

*Test preparation*—Use the stock solution used to prepare *Assay preparation 1* in the *Assay*, diluting it quantitatively, and stepwise if necessary, with water to obtain a solution containing about 12.5  $\mu\text{g}$  of sodium carbonate per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

*Blank solution*—Transfer 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Test preparation* at the sodium emission line of 589.0 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a sodium hollow-cath-

ode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Calculate the percentage of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) in the portion of Ceftazidime for Injection taken by the formula:

$$(105.99/116.88)(0.1C/M)(A_U / A_S)$$

in which 105.99 is the molecular weight of sodium carbonate; 116.88 is twice the molecular weight of sodium chloride;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of sodium chloride in the *Standard preparation*;  $M$  is the quantity, in mg, of Ceftazidime for Injection in each mL of the *Test preparation*, based on the quantity taken to prepare the stock solution and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*.

#### Limit of pyridine—

**Mobile phase**—Mix 300 mL of acetonitrile and 100 mL of 0.25 M monobasic ammonium phosphate, dilute with water to obtain 1000 mL of solution, and adjust with ammonium hydroxide to a pH of  $7.0 \pm 0.1$ . Pass this solution through a filter having a 1- $\mu\text{m}$  or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**pH 7 Buffer**—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL of solution.

**Standard solution**—Transfer about 250 mg of pyridine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Immediately prior to chromatography, transfer 2.0 mL of this solution to a 200-mL volumetric flask, dilute with pH 7 Buffer to volume, and mix. This solution contains about 25  $\mu\text{g}$  of pyridine per mL.

**Test solution**—Transfer about 660 mg of Ceftazidime for Injection, just removed from its container and accurately weighed, to a 100-mL volumetric flask, promptly add pH 7 buffer to volume, and mix. Store this solution in a cool place, and use it within 1 hour.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 1.6 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 3%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the pyridine peaks. Calculate the percentage of pyridine in the portion of Ceftazidime for Injection taken by the formula:

$$10(C/W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of pyridine in the *Standard solution*;  $W$  is the weight, in mg, of Ceftazidime for Injection taken; and  $r_U$  and  $r_S$  are the pyridine peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.4% of pyridine is found where it contains sodium carbonate; and not more than 0.3% where it contains arginine.

#### Content of arginine (where present)—

**Mobile phase**—Dissolve 1.15 g of monobasic ammonium phosphate in about 800 mL of water. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ , dilute with water to 1000 mL, and mix. Prepare a filtered and degassed mixture of acetonitrile and this solution (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Ceftazidime Pentahydrate RS and USP L-Arginine RS in water to obtain a solution containing known concentrations of about 0.2 mg of each per mL.

**Test preparation**—Quantitatively dissolve an accurately weighed portion of Ceftazidime for Injection in water to obtain a solution having a concentration of about 0.2 mg of ceftazidime per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 206-nm detector, a 4.6-mm  $\times$  50-cm saturator pre-column containing packing L27, and a 4-mm  $\times$  25-cm analytical column containing packing L20. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the resolution,  $R$ , between the ceftazidime and the arginine peaks is not less than 6.0; and the tailing factor for the arginine peak is not more than 4.0.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of arginine ( $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$ ) in the Ceftazidime for Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP L-Arginine RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Ceftazidime for Injection in the *Test preparation*, based on the weight, in mg, of Ceftazidime for Injection taken and the extent of dilution; and  $r_U$  and  $r_S$  are the arginine peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on the anhydrous and arginine-free basis, the assay result from *Assay preparation 1* obtained as directed in the *Assay*.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

#### Assay—

**pH 7 buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the *Assay* under *Ceftazidime*.

**Assay preparation 1**—Transfer an accurately weighed quantity of Ceftazidime for Injection, equivalent to about 250 mg of ceftazidime ( $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2$ ), to a 250-mL volumetric flask, dilute with water to volume, and mix to obtain a stock solution. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation 2** (where it is represented as being in a single-dose container)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ( $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2$ ) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation 3** (where the label states the quantity of ceftazidime in a given volume of constituted solution)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ( $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2$ ) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography,



transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Ceftazidime*. Calculate the percentage of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) on the dried and sodium carbonate-free or arginine-free basis in the portion of Ceftazidime for Injection taken by the formula:

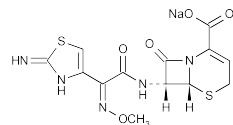
$$250,000[C/W(100 - m - s)](r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the *Standard preparation*;  $W$  is the quantity, in mg, of Ceftazidime for Injection taken to prepare *Assay preparation 1*;  $m$  is the total percentage of loss on drying;  $s$  is the percentage of sodium carbonate or arginine in the Ceftazidime for Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the container, or in the volume of constituted solution taken; and  $D$  is the concentration, in  $\mu\text{g}$ , of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) per mL, of *Assay preparation 2* or *Assay preparation 3*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution.

## Ceftizoxime Sodium



$C_{13}H_{12}N_5NaO_5S_2$  405.38

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2,3-dihydro-2-imino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxomonosodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-

Sodium (6R,7R)-7-[2-(2-imino-4-thiazolin-4-yl)glyoxylamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 7 $^2$ -(Z)-(O-methyloxime) [68401-82-1].

» Ceftizoxime Sodium contains the equivalent of not less than 850  $\mu\text{g}$  and not more than 995  $\mu\text{g}$  of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** {11}—

USP Ceftizoxime RS

USP Endotoxin RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftizoxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**B:** It responds to the tests for *Sodium* {191}.

**Crystallinity** {695}: meets the requirements.

**pH** {791}: between 6.0 and 8.0, in a solution (1 in 10).

**Water, Method I** {921}: not more than 8.5%.

**Other requirements**—Where the label states that Ceftizoxime Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Ceftizoxime for Injection*. Where the label states that Ceftizoxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ceftizoxime for Injection*.

**Assay**—

**pH 3.6 Buffer**—Dissolve 1.42 g of citric acid monohydrate and 1.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.

**pH 7.0 Buffer**—Dissolve 3.63 g of monobasic potassium phosphate and 10.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.

**Mobile phase**—Prepare a mixture of *pH 3.6 Buffer* and acetonitrile (about 9:1). Filter through a filter (1  $\mu\text{m}$  or finer porosity), and degas. Adjust the composition, if necessary, to meet the performance requirements under *Chromatographic system*.

**Internal standard solution**—Dissolve 1.2 g of salicylic acid in 10 mL of methanol, and dilute with *pH 7.0 Buffer* to obtain 200 mL of solution.

**Standard preparation**—Dissolve a suitable quantity of USP Ceftizoxime RS, accurately weighed, in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 1 mg of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix. This *Standard preparation* contains about 0.02 mg of ceftizoxime per mL.

**Assay preparation**—Using a suitable quantity of Ceftizoxime Sodium, accurately weighed, proceed as directed under *Standard preparation*.

**Chromatographic system** (see *Chromatography* {621})—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm  $\times$  30-cm column that contains 5- to 10- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2000 theoretical plates; the tailing factor for the analyte peak is not more than 2, the resolution;  $R$ , between the analyte and internal standard peaks is not less than 4; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for ceftizoxime and 1.0 for salicylic acid. Calculate the quantity, in  $\mu\text{g}$ , of ceftizoxime per mg of the Ceftizoxime Sodium taken by the formula:

$$1000(C / M)(R_U / R_S)$$

in which  $C$  is the concentration, in mg of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of the *Standard preparation*;  $M$  is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Ceftizoxime Sodium taken and the extent of dilution; and  $R_U$  and  $R_S$  are the peak response ratios of the ceftizoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefprozime Injection

» Cefprozime Injection is a sterile solution of Cefprozime Sodium in a diluent containing one or more tonicity-adjusting agents in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefprozime RS

USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefprozime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefprozime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.5 and 8.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay**—

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Cefprozime Sodium*.

*Assay preparation*—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 40 mg of cefprozime, to a 100-mL volumetric flask, dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefprozime Sodium*. Calculate the quantity, in mg, of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ) in each mL of the Injection taken by the formula:

$$2000(C / V)(R_U / R_S)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Cefprozime for Injection

» Cefprozime for Injection contains an amount of Cefprozime Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cefprozime RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Labeling* under *Injections* (1).

**Bacterial endotoxins** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefprozime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification* tests and meets the requirements for *Crystallinity, pH, and Water* under *Cefprozime Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay**—

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Cefprozime Sodium*.

*Standard preparation*—Dissolve a suitable quantity of USP Cefprozime RS, accurately weighed, in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 1 mg of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix. This *Standard preparation* contains about 0.02 mg of cefprozime per mL.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefprozime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of cefprozime per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

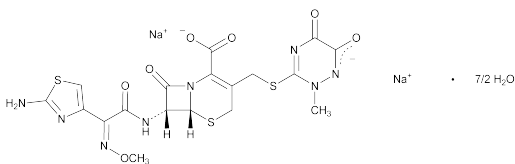
*Assay preparation 2* (where the label states the quantity of cefprozime in a given volume of constituted solution)—Constitute Cefprozime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of cefprozime per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

*Procedure*—Proceed with Cefprozime for Injection as directed for *Procedure* in the *Assay* under *Cefprozime Sodium*. Calculate the quantity, in mg, of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L / D)(C)(R_U / R_S)$$

in which *L* is the labeled quantity, in mg of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ), in the container, or in the volume of constituted solution taken, and *D* is the concentration, in mg of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; and the extent of dilution, *C* is the concentration, in mg of cefprozime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the cefprozime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftriaxone Sodium



$C_{18}H_{16}N_8Na_2O_7S_3 \cdot 3\frac{1}{2}H_2O$  661.60

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5-, 6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-, disodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-, hydrate, (2:7).

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7 $\alpha$ -(Z)-(O-methyloxime), disodium salt, hemiseptahydrate [14376-79-6].

Anhydrous 598.56

» Ceftriaxone Sodium contains the equivalent of not less than 795  $\mu$ g of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Ceftriaxone Sodium RS

USP Ceftriaxone Sodium E-Isomer RS

USP Endotoxin RS

**Identification**—

A: *Infrared Absorption* (197K).

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftriaxone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

C: It responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 6.0 and 8.0 in a solution (1 in 10).

**Water, Method I** (921): between 8.0% and 11.0%.

**Other requirements**—Where the label states that Ceftriaxone Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Ceftriaxone for Injection*. Where the label states that Ceftriaxone Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ceftriaxone for Injection*.

**Assay**—

**pH 7.0 Buffer**—Dissolve 13.6 g of dibasic potassium phosphate and 4.0 g of monobasic potassium phosphate in water to obtain 1000 mL of solution. Adjust this solution with phosphoric acid or 10 N potassium hydroxide to a pH of  $7.0 \pm 0.1$ .

**pH 5.0 Buffer**—Dissolve 25.8 g of sodium citrate in 500 mL of water, adjust with citric acid solution (1 in 5) to a pH of  $5.0 \pm 0.1$ , and dilute with water to a volume of 1000 mL.

**Mobile phase**—Dissolve 3.2 g of tetraheptylammonium bromide in 400 mL of acetonitrile, add 44 mL of pH 7.0 Buffer and 4 mL of pH 5.0 Buffer, and add water to make

1000 mL. Filter through a membrane filter of 0.5  $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ceftriaxone Sodium RS in *Mobile phase*, to obtain a solution having a known concentration of about 0.2 mg per mL. Use this solution promptly after preparation.

**Resolution solution**—Dissolve a suitable quantity of USP Ceftriaxone Sodium E-Isomer RS in *Standard preparation*, and dilute with *Mobile phase* to obtain a solution containing about 160  $\mu$ g of USP Ceftriaxone Sodium E-Isomer RS per mL and 160  $\mu$ g of USP Ceftriaxone Sodium RS per mL. Use this solution promptly after preparation.

**Assay preparation**—Transfer about 40 mg of Ceftriaxone Sodium, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Use this solution promptly after preparation.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a 4.0-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the ceftriaxone E-isomer and ceftriaxone peaks is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) per mg of the Ceftriaxone Sodium taken by the formula:

$$200(CP / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ceftriaxone Sodium RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g of ceftriaxone per mg, of USP Ceftriaxone Sodium RS; W is the quantity, in mg, of the Ceftriaxone Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the ceftriaxone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftriaxone Injection

» Ceftriaxone Injection is a sterile solution of Ceftriaxone Sodium in a diluent containing one or more tonicity-adjusting agents in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Ceftriaxone Sodium RS  
USP Ceftriaxone Sodium E-Isomer RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftriaxone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of ceftriaxone.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.0 and 8.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay**—

*pH 7.0 Buffer, pH 5.0 Buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Ceftriaxone Sodium*.

*Assay preparation*—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 40 mg of ceftriaxone, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution promptly after preparation.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Ceftriaxone Sodium*. Calculate the quantity, in mg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) in each mL of the Injection taken by the formula:

$$200(C / V)(r_U / r_S)$$

in which *V* is the volume, in mL, of Injection taken; and the other terms are as defined therein.

**Ceftriaxone for Injection**

» Ceftriaxone for Injection contains an amount of Ceftriaxone Sodium equivalent to not less than 776 µg of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) per mg, calculated on the anhydrous basis, and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Ceftriaxone Sodium RS  
USP Ceftriaxone Sodium E-Isomer RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of ceftriaxone.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification* tests and meets the requirements for *Crystallinity*, *pH*, and *Water* under *Ceftriaxone Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay**—

*pH 7.0 Buffer, pH 5.0 Buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Ceftriaxone Sodium*.

*Assay preparation 1*—Transfer about 40 mg of Ceftriaxone for Injection, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Use this solution promptly after preparation.

*Assay preparation 2* (where it is represented as being in a single-dose container)—Constitute Ceftriaxone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution containing about 180 µg of ceftriaxone per mL. Use this solution promptly after preparation.

*Assay preparation 3* (where the label states the quantity of ceftriaxone in a given volume of constituted solution)—Constitute Ceftriaxone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Mobile phase* to obtain a solution containing about 180 µg of ceftriaxone per mL. Use this solution promptly after preparation.

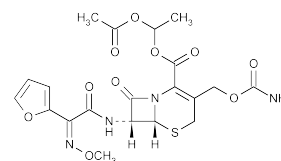
*Procedure*—Proceed as directed in the *Assay* under *Ceftriaxone Sodium*. Calculate the quantity, in µg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) per mg of the Ceftriaxone for Injection taken by the formula:

$$200(CP / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ceftriaxone Sodium RS in the *Standard preparation*; *P* is the designated potency, in µg, of ceftriaxone per mg of USP Ceftriaxone Sodium RS; *W* is the quantity, in mg, of Ceftriaxone for Injection taken to prepare *Assay preparation 1*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the ceftriaxone peak responses obtained from *Assay preparation 1* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L / D)(CP)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) in the container, or in the volume of constituted solution taken; *D* is the concentration, in µg per mL, of ceftriaxone in *Assay preparation 2* or *Assay preparation 3*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; *C* is the concentration, in mg per mL, of USP Ceftriaxone Sodium RS in the *Standard preparation*; *P* is the designated potency, in µg, of ceftriaxone per mg of USP Ceftriaxone Sodium RS; and *r<sub>U</sub>* and *r<sub>S</sub>* are the ceftriaxone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Cefuroxime Axetil**

$C_{20}H_{22}N_4O_{10}S$  510.47

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[aminocarbonyl]oxy]methyl]-7-[[2-furanyl(methoxyimino)acetyl]amino]-8-oxo-, 1-(acetyloxy)ethyl ester, [6*R*-[6 $\alpha$ 7 $\beta$ (*Z*)]]-  
(*RS*)-1-Hydroxyethyl (6*R*,7*R*)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 2-carboxylate, 7<sup>2</sup>-(*Z*)-(O-methyloxime), 1-acetate 3-carbamate [64544-07-6].

» Cefuroxime Axetil is a mixture of the diastereoisomers of cefuroxime axetil (C<sub>20</sub>H<sub>22</sub>N<sub>4</sub>O<sub>10</sub>S). It contains the equivalent of not less than 745  $\mu$ g and not more than 875  $\mu$ g of cefuroxime (C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>8</sub>S) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether it is amorphous or crystalline.

**USP Reference standards** (11)—

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

**Identification**, *Infrared Absorption* (197K).

**Crystallinity** (695)—Particles that do not show birefringence or exhibit extinction positions are amorphous, and particles that show birefringence and exhibit extinction positions are crystalline.

**Water**, *Method I* (921): not more than 1.5%.

**Diastereoisomer ratio**—

0.2 *M* Monobasic ammonium phosphate, *Mobile phase*, *Internal standard solution*, *Resolution solution*, *Standard preparation*, *Assay preparation*, and *Chromatographic system*—Prepare as directed in the Assay.

**Procedure**—Proceed as directed for Procedure in the Assay. Calculate the ratio of cefuroxime axetil diastereoisomer A to the sum of the cefuroxime axetil diastereoisomers A and B taken by the formula:

$$r_A / (r_A + r_B)$$

in which  $r_A$  and  $r_B$  are the peak responses of the cefuroxime axetil diastereoisomers A and B, respectively: between 0.48 and 0.55 is obtained.

**Assay**—

0.2 *M* Monobasic ammonium phosphate—Dissolve 23.0 g of monobasic ammonium phosphate in water to obtain 1000 mL of solution.

*Mobile phase*—Prepare a suitable filtered and degassed mixture of 0.2 *M* Monobasic ammonium phosphate and methanol (620: 380). Make adjustments if necessary (see System Suitability under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of acetanilide in methanol containing 5.4 mg per mL.

*Resolution solution*—In a 50-mL volumetric flask, mix 10.0 mL of a solution of USP Cefuroxime Axetil RS in methanol containing 1.2 mg per mL, 5.0 mL of Internal standard solution, and 3.8 mL of a solution of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol containing 0.16 mg per mL. Dilute with 0.2 *M* Monobasic ammonium phosphate to volume, and mix.

*Standard preparation*—Transfer about 30 mg of USP Cefuroxime Axetil RS, accurately weighed, to a 25-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Promptly transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution* and 3.8 mL of methanol, dilute with 0.2 *M* Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this *Standard preparation* promptly, or refrigerate and use on the day prepared.]

*Assay preparation*—Transfer about 30 mg of Cefuroxime Axetil to a 25-mL volumetric flask, dissolve in methanol, di-

lute with methanol to volume, and mix. Promptly transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution* and 3.8 mL of methanol, dilute with 0.2 *M* Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this *Assay preparation* promptly, or refrigerate and use on the day prepared.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm  $\times$  25-cm column containing 5- $\mu$ m packing L13. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for acetanilide, 0.8 for cefuroxime axetil diastereoisomer B, 0.9 for cefuroxime axetil diastereoisomer A, and 1.0 for cefuroxime axetil delta-3 isomers; the resolution,  $R$ , between cefuroxime axetil diastereoisomer A and B is not less than 1.5; and the resolution,  $R$ , between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of cefuroxime (C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>8</sub>S) in each mg of Cefuroxime Axetil taken by the formula:

$$(W_S / W_U)(P_S / 100)(100 - K)(R_U / R_S)$$

in which  $W_S$  is the weight, in mg, of USP Cefuroxime Axetil RS taken to prepare the *Standard preparation*;  $W_U$  is the weight, in mg, of Cefuroxime Axetil taken to prepare the *Assay preparation*;  $P_S$  is the designated cefuroxime (C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>8</sub>S) content, in  $\mu$ g per mg, of anhydrous USP Cefuroxime Axetil RS;  $K$  is the percentage water content of USP Cefuroxime Axetil RS; and  $R_U$  and  $R_S$  are the ratios of the sum of the peak responses of the cefuroxime axetil diastereoisomers A and B to the peak response of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefuroxime Axetil for Oral Suspension

### DEFINITION

Cefuroxime Axetil for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime (C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>8</sub>S).

### IDENTIFICATION

- The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 23 mg/mL of monobasic ammonium phosphate in water

**Mobile phase:** Methanol and *Solution A* (19:31)

**System suitability stock solution A:** 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol

**System suitability stock solution B:** 0.16 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

**System suitability solution:** Transfer 10.0 mL of *System suitability stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of methanol and 3.8 mL of *System suitability stock solution B*. Dilute with *Buffer* to volume.

**Standard stock solution:** 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol. [NOTE—Use this solution promptly.]

**Standard solution:** Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 8.8 mL of methanol, and dilute with *Buffer* to volume. [NOTE—Use this *Standard solution* promptly, or refrigerate and use on the day prepared.]

**Sample stock solution:** Equivalent to 2.5 mg/mL of cefuroxime, from constituted Oral Suspension, in methanol. Pass through a suitable filter. [NOTE—Constitute as directed on the label. To a suitable aliquot, freshly prepared and free of bubbles, add a suitable volume of methanol, shake by mechanical means for 10 min, dilute to volume with methanol, and mix.]

**Sample solution:** Transfer 5.0 mL of the filtered *Sample stock solution* to a 50-mL volumetric flask. Add 13.8 mL of methanol, and dilute with *Buffer* to volume. [NOTE—Protect the *Sample solution* from light and use promptly, or refrigerate and use on the day prepared.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L13

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for acetanilide, cefuroxime axetil diastereoisomer B, cefuroxime axetil diastereoisomer A, and cefuroxime axetil delta-3 isomers are 0.4, 0.8, 0.9, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

**Column efficiency:** NLT 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{16}H_{16}N_4O_8S$  in the Cefuroxime Axetil for Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times [1 - (K/100)] \times 100$$

$R_U$  = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

$R_S$  = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefuroxime axetil in the *Sample solution* (mg/mL)

$P$  = potency of cefuroxime in anhydrous USP Cefuroxime Axetil RS (μg/mg)

$F$  = unit conversion factor, 0.001 mg/μg

$K$  = water content of USP Cefuroxime Axetil RS (%)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.07 M of pH 7.0 phosphate buffer (dissolve 3.7 mg/mL of monobasic sodium phosphate and 5.7 mg/mL of anhydrous dibasic sodium phosphate in water); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Analysis:** Test 5.0 mL of constituted Cefuroxime Axetil for Oral Suspension equivalent to 125 or 250 mg of cefuroxime. Determine the amount of cefuroxime equivalent dissolved by using UV absorption at the wavelength of maximum absorbance at 280 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefuroxime Axetil RS in the same *Medium*.

**Tolerances:** NLT 60% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS <905>

**For solid packaged in single-unit containers:**

Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. Mix, and allow the container to drain into a beaker for 5 s. Withdraw and assay 5.0 mL of the Oral Suspension from the beaker, or the total amount if it is less than 5 mL. It meets the requirements.

##### • DELIVERABLE VOLUME <698>

**For solid packaged in multiple-unit containers:**

Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. It meets the requirements.

#### SPECIFIC TESTS

• **pH <791>:** 3.5–7.0, in the solution constituted as directed in the labeling

• **WATER DETERMINATION, Method I <921>:** NMT 6.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS <11>**

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

## Cefuroxime Axetil Tablets

» Cefuroxime Axetil Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The labeling indicates whether the Tablets contain amorphous or crystalline Cefuroxime Axetil. If Tablets contain a mixture of amorphous and crystalline Cefuroxime Axetil, label to indicate the percentage of each contained therein. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### USP Reference standards <11>—

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

**Identification**—The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

#### Dissolution <711>—

TEST 1—

**Medium:** 0.07 N hydrochloric acid; 900 mL.

**Apparatus 2:** 55 rpm.

**Times:** 15 and 45 minutes.

**Procedure**—Determine the amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) dissolved by employing UV absorption at the

wavelength of maximum absorbance at about 278 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cefuroxime Axetil RS, equivalent to about 0.01 to 0.02 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, in the same *Medium*.

**Tolerances**—Not less than 60% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than 75% (Q) is dissolved in 45 minutes; except that where Tablets are labeled to contain the equivalent of 500 mg of cefuroxime, not less than 50% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than 70% (Q) is dissolved in 45 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Apparatus 2:** 100 rpm.

**Medium, Times, and Procedure**—Proceed as directed under *Test 1*.

**Tolerances**—Not less than 60% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than 75% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 6.0%.

#### Assay—

0.2 M Monobasic ammonium phosphate, Mobile phase, Internal standard solution, Resolution solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Cefuroxime Axetil.

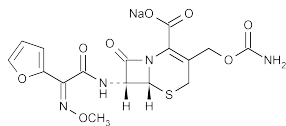
**Assay preparation**—Finely powder not fewer than 10 Tablets, accurately counted. Transfer the powder, with the aid of methanol, to a volumetric flask of such capacity that when filled to volume, the solution will contain the equivalent of about 2 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL. Add methanol to fill the volumetric flask to about half of its capacity, and shake by mechanical means for about 10 minutes. Dilute with methanol to volume, and mix. Filter a portion of this stock mixture, and transfer 5.0 mL of the filtrate to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution* and 8.8 mL of methanol, dilute with 0.2 M Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this Assay preparation promptly, or refrigerate and use on the day prepared.]

**Procedure**—Proceed as directed in the Assay under Cefuroxime Axetil. Calculate the quantity, in mg, of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) in each Tablet taken by the formula:

$$(V/12,500N)(P_5W_5/100)(100 - K)(R_U / R_S)$$

in which *V* is the volume, in mL, of the volumetric flask used to prepare the stock mixture; *N* is the number of Tablets taken; and the other terms are as defined therein.

## Cefuroxime Sodium



$C_{16}H_{15}N_4NaO_8S$  446.37

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(aminocarbonyl)oxy]methyl]-7-[[2-furyl(methoxyimino)acetyl]amino]-8-oxo-, monosodium salt [6 $\alpha$ ,7 $\beta$ (Z)]-.

Sodium (6 $R$ ,7 $R$ )-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7 $^2$ -(Z)-(O-methyloxime), carbamate (ester) [56238-63-2].

» Cefuroxime Sodium contains the equivalent of not less than 855  $\mu$ g and not more than 1000  $\mu$ g of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ), calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### USP Reference standards (11)—

USP Cefuroxime Sodium RS

USP Endotoxin RS

#### Identification—

**A:** The chromatogram of the Assay preparation obtained as directed in the Assay exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the Standard preparation obtained as directed in the Assay.

**B:** It responds to the tests for Sodium (191).

**pH** (791): between 6.0 and 8.5, in a solution (1 in 10).

**Water, Method I** (921): not more than 3.5%.

**Other requirements**—Where the label states that Cefuroxime Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under Cefuroxime for Injection. Where the label states that Cefuroxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under Cefuroxime for Injection.

#### Assay—

**pH 3.4 acetate buffer**—Transfer 50 mL of 0.1 M sodium acetate to a 1000-mL volumetric flask, dilute with 0.1 N acetic acid to volume, and mix.

**Mobile phase**—Prepare a suitable mixture of pH 3.4 acetate buffer and acetonitrile (about 10:1). Filter through a membrane filter (1  $\mu$ m or finer porosity), and degas.

**Internal standard solution**—Prepare a solution of orcinol in water containing 1.5 mg per mL.

**Standard preparation**—Dissolve a suitable quantity of USP Cefuroxime Sodium RS, accurately weighed, in water to obtain a solution having a known concentration of about 1 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix. This Standard preparation contains about 0.05 mg of cefuroxime per mL.

**Assay preparation**—Using a suitable quantity of Cefuroxime Sodium, accurately weighed, proceed as directed in the first sentence under *Standard preparation*. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L15. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1300 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for cefuroxime and 1.0 for orcinol. Calculate the quantity, in  $\mu\text{g}$ , of cefuroxime per mg of the Cefuroxime Sodium taken by the formula:

$$1000(C/M)(R_U / R_S)$$

in which C is the concentration, in mg of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ) per mL, in the *Standard preparation*; M is the concentration, in mg per mL, in the *Assay preparation* based on the weight of Cefuroxime Sodium taken and the extent of dilution; and  $R_U$  and  $R_S$  are the peak response ratios of the cefuroxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefuroxime Injection

» Cefuroxime Injection is a sterile isoosmotic solution of Cefuroxime Sodium in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefuroxime Sodium RS

USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial Endotoxins** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905).

**Assay**—

*pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Cefuroxime Sodium*.

*Assay preparation*—Allow a container of Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefuroxime, to a 50-mL volumetric flask, dilute with water to volume, and mix. Immediately transfer 5.0 mL of this solution to a second 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefuroxime Sodium*. Calculate the quantity, in mg, of

cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ) in each mL of the Injection taken by the formula:

$$1000(C / V)(R_U / R_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Cefuroxime for Injection

» Cefuroxime for Injection contains an amount of Cefuroxime Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cefuroxime Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, the constituted solution for intravenous administration prepared from Cefuroxime for Injection meets the requirements for *Constituted Solutions* under *Labeling* under *Injections* (1).

**Bacterial endotoxins** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements.

*Procedure for content uniformity*—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements of the tests for *Identification*, *pH*, and *Water* under *Cefuroxime Sodium*. It meets also the requirements for *Labeling* under *Injections* (1).

**Assay**—

*pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cefuroxime Sodium*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Assay preparation 2* (where the label states the quantity of cefuroxime in a given volume of constituted solution or suspension)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution or suspension quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefuroxime Sodium*. Calculate the quantity, in mg, of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ) withdrawn from the container, or



in the portion of constituted solution or suspension taken by the formula:

$$(L / D)(C / R_U / R_S)$$

in which *L* is the labeled quantity, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ), in the container, or in the volume of constituted solution or suspension taken; *D* is the concentration, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution or suspension taken, respectively, and the extent of dilution; *C* is the concentration, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, of the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of cefuroxime to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

## Celecoxib

$C_{17}H_{14}F_3N_3O_2S$  381.4  
4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide;  
*p*-[5-*p*-Tolyl-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide [169590-42-5].

### DEFINITION

Celecoxib contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{14}F_3N_3O_2S$ , calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197): [NOTE—Methods (197A), (197K), or (197M) under *Infrared Absorption* may be used.]  
[NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the Reference Standard separately in isopropyl alcohol, evaporate to dryness, and record the new spectra.]
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 2.7 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of  $3.0 \pm 0.2$   
**Mobile phase:** Methanol, acetonitrile, and *Buffer* (3:1:6)  
**Diluent:** Methanol and water (3:1)  
**System suitability solution:** 0.5 mg/mL of USP Celecoxib RS and 2.4 µg/mL each of USP Celecoxib Related Compound A RS and USP Celecoxib Related Compound B RS in *Diluent*  
**Standard solution:** 0.5 mg/mL of USP Celecoxib RS in *Diluent*  
**Sample solution:** 0.5 mg/mL of Celecoxib in *Diluent*  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 215 nm  
**Column:** 4.6-mm × 25-cm; 5-µm packing L11  
**Column temperature:** 60°  
**Flow rate:** 1.5 mL/min  
**Injection size:** 25 µL  
**Run time:** About 1.5 times the celecoxib peak elution  
**System suitability**  
**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{14}F_3N_3O_2S$  in the portion of Celecoxib taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Inorganic Impurities

- HEAVY METALS:** NMT 20 ppm

**Diluent:** Acetone and water (17:3)

**Standard solution:** Dilute 1.0 mL of *Standard Lead Solution*, prepared as directed under *Heavy Metals* (231), *Special Reagents*, with *Diluent* to 20 mL.

**Sample solution:** Dissolve 0.50 g of Celecoxib in 20 mL of *Diluent*.

**Blank solution:** 20 mL of *Diluent*

#### Analysis

**Samples:** *Standard solution*, *Blank solution*, and *Sample solution*

To each solution, add 2 mL of pH 3.5 *Acetate Buffer*, prepared as directed under *Heavy Metals* (231), *Method I*. Mix, and add to each solution 1.2 mL of thioacetamide–glycerin base TS. Mix immediately, and allow to stand for 2 min. Pass the solutions through a filter of 0.45-µm pore size. Compare the spots on the filters obtained from each of the solutions.

**Acceptance criteria:** The brownish-black color of the spot resulting from the *Sample solution* is not more intense than that of the spot resulting from the *Standard solution*. The test is invalid if the *Standard solution* does not show a brownish-black color compared to the *Blank solution*.

- RESIDUE ON IGNITION** (281): NMT 0.2%, using a platinum crucible

#### Organic Impurities

##### PROCEDURE

**Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.5 µg/mL of USP Celecoxib RS in *Diluent*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

**Signal-to-noise ratio:** NLT 20, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Celecoxib taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response for each impurity in the *Sample solution*

- $r_s$  = peak response of celecoxib in the *Standard solution*  
 $C_s$  = concentration of celecoxib in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Celecoxib in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See *Table 1*.

[NOTE— Disregard any impurity peak less than 0.05%.]

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Celecoxib related compound A <sup>a</sup>	0.9	0.4
Celecoxib	1.0	—
Celecoxib related compound B <sup>b</sup>	1.1	0.10
Individual unspecified impurity	—	0.10
Total impurities	—	0.5

<sup>a</sup> 4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

<sup>b</sup> 4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

**SPECIFIC TESTS**

- WATER DETERMINATION, Method I (921):** NMT 0.5%, using a 400-mg sample

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store at room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Celecoxib RS
  - $p$ -[5- $p$ -Tolyl-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide.  
 $C_{17}H_{14}F_3N_3O_2S$  381.4
  - USP Celecoxib Related Compound A RS
  - 4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.  
 $C_{17}H_{14}F_3N_3O_2S$  381.4
  - USP Celecoxib Related Compound B RS
  - 4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.  
 $C_{17}H_{14}F_3N_3O_2S$  381.4

**Oxidized Cellulose****DEFINITION**

Oxidized Cellulose contains NLT 16.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

**IDENTIFICATION**

- A.**
  - Sample solution:** 200 mg in 10 mL of 0.25 N sodium hydroxide
  - Analysis 1:** Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.
  - Acceptance criteria 1:** The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.
  - Analysis 2:** Allow the resulting solution to stand for 10 min.
  - Acceptance criteria 2:** Any swollen fibers initially present are no longer visible.

**Analysis 3:** Acidify the resulting solution with 3 N hydrochloric acid.

**Acceptance criteria 3:** A flocculent white precipitate is formed.

**ASSAY****PROCEDURE**

**Solution A:** 20 mg/mL of calcium acetate

**Sample:** 500 mg, previously dried under vacuum over phosphorus pentoxide for 18 h

**Blank:** 50.0 mL of *Solution A*

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Place the *Sample* in a 125-mL conical flask.

Add 50.0 mL of *Solution A*, swirl until the sample is completely covered, allow the mixture to stand for 30 min, then add phenolphthalein TS. Titrate the solution with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 4.502 mg of carboxyl groups (COOH).

**Acceptance criteria:** 16.0%–24.0% on the dried basis

**IMPURITIES**

- RESIDUE ON IGNITION (281):** NMT 0.15%

**LIMIT OF NITROGEN**

**Solution A:** 40 mg/mL of boric acid

**Solution B:** Methyl red TS and bromocresol green TS (1:4)

**Sample:** 1 g, previously dried under vacuum over phosphorus pentoxide for 18 h

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.02 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask, add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

**Acceptance criteria:** NMT 0.5%

**LIMIT OF FORMALDEHYDE**

**Solution A:** Formaldehyde in water (1 in 40,000)

**Standard:** 0.50 mL of *Solution A*

**Sample:** 500 mg

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Vis

**Analytical wavelength:** 570 nm

**Blank:** Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

**Analysis:** Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

**Acceptance criteria:** 0.5%; the absorbance of the *Sample* is NMT the *Standard*.

**SPECIFIC TESTS**• **STERILITY TESTS** (71)

**Sample:** 250 mg

**Analysis:** Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

**Acceptance criteria:** Meets the requirements

• **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum over phosphorus pentoxide for 18 h.

**Acceptance criteria:** NMT 15.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve as described in *Injections* (1), *Containers for Sterile Solids*, protected from direct sunlight. Store in a cold place.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Cellulose meets the requirements for *Injections* (1), *Labeling*.

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**Oxidized Regenerated Cellulose**

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**DEFINITION**

Oxidized Regenerated Cellulose contains NLT 18.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

**IDENTIFICATION**

- **A.**

**Sample solution:** 200 mg in 10 mL of 0.25 N sodium hydroxide

**Analysis 1:** Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.

**Acceptance criteria 1:** The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.

**Analysis 2:** Allow the resulting solution to stand for 10 min.

**Acceptance criteria 2:** Any swollen fibers initially present are no longer visible.

**Analysis 3:** Acidify the resulting solution with 3 N hydrochloric acid.

**Acceptance criteria 3:** A flocculent white precipitate is formed.

**ASSAY**• **PROCEDURE**

**Sample:** 1 g of Oxidized Regenerated Cellulose, previously dried at 90° for 2 h

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Place the *Sample* in a 250-mL conical flask, add 10 mL of 0.5 N sodium hydroxide VS, swirl to dissolve, and add 100 mL of water. Immediately titrate with *Titrant* to a phenolphthalein endpoint. Perform a blank determination, and note the difference in volumes required. Each mL of the difference in volumes of 0.1 N hydrochloric acid consumed is equivalent to 4.50 mg of carboxyl groups (COOH).

**Acceptance criteria:** 18.0%–24.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.15%
- **LIMIT OF NITROGEN**

**Solution A:** 40 mg/mL of boric acid

**Solution B:** Methyl red TS and bromocresol green TS (1:4)

**Sample:** 1 g, previously dried

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.02 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint that persists for 30 s. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

**Acceptance criteria:** NMT 0.5%

• **LIMIT OF FORMALDEHYDE**

**Solution A:** Formaldehyde in water (1 in 40,000)

**Standard:** 0.50 mL of *Solution A*

**Sample:** 500 mg

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Vis

**Analytical wavelength:** 570 nm

**Blank:** Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

**Analysis:** Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

**Acceptance criteria:** 0.5% CH<sub>2</sub>O; the absorbance of the *Sample* is NMT the *Standard*.

**SPECIFIC TESTS**• **STERILITY TESTS** (71)

**Sample:** 250 mg

**Analysis:** Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

**Acceptance criteria:** Meets the requirements

• **LOSS ON DRYING** (731)

**Sample:** 150 mg

**Analysis:** Dry at 90° for 2 h.

**Acceptance criteria:** NMT 15.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve as described in *Injections* (1), *Containers for Sterile Solids*, protected from direct sunlight. Store at controlled room temperature.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Regenerated Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Regenerated Cellulose meets the requirements for *Injections* (1), *Labeling*.

## Cellulose Sodium Phosphate

» Cellulose Sodium Phosphate is prepared by phosphorylation of alpha cellulose. It has an inorganic bound phosphate content of not less than 31.0 percent and not more than 36.0 percent, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**pH** (791)—Place 3 g of it in a 100-mL beaker, add 60 mL of water, and stir occasionally for 5 minutes. Filter through a sintered-glass crucible. The pH of the filtrate is between 6.0 and 9.0.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

**Nitrogen**, Method I (461): not more than 1.0%.

### Calcium binding capacity—

**Standard calcium solution**—Transfer about 0.33 g of dried calcium carbonate, primary standard grade, accurately weighed, to a 250-mL beaker with the aid of a few mL of water, and dilute with water to about 50 mL. Carefully and dropwise add 2 N hydrochloric acid until all of the solid dissolves, and add 2 drops in excess. Heat the solution to boiling, and boil for 5 minutes. Cool the solution, and transfer to a 1000-mL volumetric flask. Dilute with water to volume, and mix. Calculate the molarity,  $M$ , of the solution taken by the formula:

$$g / 100.09$$

in which  $g$  is the weight, in g, of calcium carbonate taken.

**Standard edetate disodium titrant**—Dissolve 10 g of edetate disodium in 100 mL of water. Slowly add alcohol until the first permanent precipitate is formed. Filter, and discard the solid. Add an equal volume of alcohol to the filtrate. Filter the resulting precipitate, discard the filtrate, and wash the residue on the filter, first with acetone, then with ethyl ether. Dry at 80° for 4 days at about 50% relative humidity. Transfer about 3.72 g of this purified edetate disodium, accurately weighed, to a 1000-mL volumetric flask, and dissolve with water. Dilute with water to volume, and mix. Calculate the molarity,  $M_s$ , of the solution taken by the formula:

$$w / 372.24$$

in which  $w$  is the weight, in g, of the purified edetate disodium taken.

**Procedure**—Transfer  $0.15 \pm 0.02$  g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 150.0 mL of *Standard calcium solution*, and stir the mixture for 5 minutes on a magnetic stirrer. Filter, discarding the first few mL of the filtrate. To 50.0 mL of the filtrate add about 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with *Standard edetate disodium titrant* to a permanent deep blue color, and designate the number of mL consumed as  $V_s$ . Calculate the calcium binding capacity of the undried Cellulose Sodium Phosphate, in mmol per g, by the formula:

$$(150M - 3V_s M_s) / W$$

in which  $W$  is the weight, in g, of Cellulose Sodium Phosphate taken; and the other terms are as defined above. The calcium binding capacity, calculated on the dried basis, is not less than 1.8 mmol per g.

**Heavy metals**, Method III (231): 0.004%.

### Free phosphate—

**Standard preparation**—Prepare as directed under *Inorganic bound phosphate*.

**Test preparation**—Transfer about 2 g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 100 mL of water, accurately measured, stir, allow to stand for 5 minutes, stir again, and filter through moderately retentive filter paper, collecting the filtrate in a dry flask.

**Procedure**—Transfer 2.0 mL of the *Standard preparation* and 5.0 mL of the *Test preparation* to separate 100-mL volumetric flasks. Proceed as directed in the *Procedure under Inorganic bound phosphate*, beginning with "Treat each of these." Calculate the percentage of free phosphate taken by the formula:

$$(4000 / W)(A_u / A_s)$$

in which  $W$  is the weight, in mg, of undried Cellulose Sodium Phosphate taken: not more than 3.5%, calculated on the dried basis, is found.

### Sodium content—

**Standard stock solution**—Dissolve 508.5 mg of sodium chloride, previously dried at 105° for 2 hours, in 100 mL of water, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 200 µg of sodium.

**Standard preparations**—Transfer 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks, dilute each with water to volume, and mix.

**Test preparation**—Dissolve about 250 mg of Cellulose Sodium Phosphate, accurately weighed, in 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the emission of the *Test preparation* and of each *Standard preparation* at the sodium emission line of 589 nm, with a suitable flame photometer. Plot the emissions of the *Standard preparations* versus their concentration of sodium, and draw a straight line best fitting the four plotted points. From the graph so obtained determine the concentration of sodium in the *Test preparation*. Calculate the percentage of sodium in the undried Cellulose Sodium Phosphate. The content of sodium, calculated on the dried basis, is not less than 9.5% and not more than 13.0%.

### Inorganic bound phosphate—

**Standard preparation**—Transfer 358.2 mg of monobasic potassium phosphate, primary standard grade, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Each mL of this solution contains 1.0 mg of phosphate.

**Test preparation**—Transfer about 250 mg of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL conical flask. Rinse the bottom with a few mL of water. Add 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool the clear, almost colorless, solution, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, and mix.

**Procedure**—Transfer 2.0-mL portions of the *Standard preparation* and the *Test preparation* to separate 100-mL volumetric flasks. Treat each of these and a third flask, providing the blank, as follows: Add 10 mL of 5 N nitric acid, 10.0 mL of ammonium vanadate TS, and about 60 mL of water. Swirl, and add 10.0 mL of a freshly prepared solution of 2.5 g of ammonium molybdate in 50 mL of warm water. Dilute with water to volume, and mix. Concomitantly determine the absorbances,  $A_u$  and  $A_s$ , of the solutions from the *Standard preparation* and the *Test preparation*, respectively, at 400 nm with a suitable spectrophotometer, using the rea-

gent blank to set the instrument. Calculate the percentage of total phosphate taken by the formula:

$$(10,000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of Cellulose Sodium Phosphate taken. Calculate the percentage of inorganic bound phosphate in the undried Cellulose Sodium Phosphate by subtracting from this result the percentage of *Free phosphate*.

## Cellulose Sodium Phosphate for Oral Suspension

» Cellulose Sodium Phosphate for Oral Suspension contains Cellulose Sodium Phosphate. It has an inorganic bound phosphate content of not less than 28.0 percent and not more than 36.0 percent calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store in a refrigerator.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

### Calcium binding capacity—

*Standard calcium solution* and *Standard edetate disodium titrant*—Proceed as directed for *Calcium binding capacity* under *Cellulose Sodium Phosphate*.

*Procedure*—Transfer an accurately weighed amount of Oral Suspension, equivalent to about 0.15 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Procedure* in *Calcium binding capacity* under *Cellulose Sodium Phosphate*, beginning with “Add 150.0 mL of *Standard calcium solution*.” Calculate the calcium binding capacity, in mmol per g, of the portion of undried Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(150M_S - 3V_S M_S) / W$$

in which *W* is the weight, in g, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not less than 1.8 mmol per g, calculated on the dried basis, is found.

### Free phosphate—

*Standard preparation*—Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*.

*Test preparation*—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 2 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*, beginning with “Add 100 mL of water.”

*Procedure*—Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*. Calculate the percentage of free phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(4000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of undried Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not more than 6.0%, calculated on the dried basis, is found.

### Inorganic bound phosphate—

*Standard preparation*—Proceed as directed for *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*.

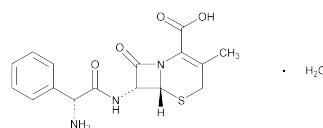
*Test preparation*—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 250 mg of cellulose sodium phosphate, to a 250-mL conical flask. Proceed as directed for *Test preparation* in *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*, beginning with “Rinse to the bottom.”

*Procedure*—Proceed as directed for *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*. Calculate the percentage of total phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(10,000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein. Calculate the percentage of inorganic bound phosphate in the portion of undried Cellulose Sodium Phosphate for Oral Suspension by subtracting from this result the percentage of free phosphate.

## Cephalexin



$C_{16}H_{17}N_3O_4S \cdot H_2O$  365.40

$C_{16}H_{17}N_3O_4S$  347.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrate, [6*R*-[6*α*,7*β* (*R*<sup>\*</sup>)]-; (6*R*,7*R*)-7-[(*R*)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [23325-78-2]. Anhydrous [15686-71-2].

### DEFINITION

Cephalexin has a potency of NLT 950 µg/mg and NMT 1030 µg/mg of  $C_{16}H_{17}N_3O_4S$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** 0.985 g/L of sodium-1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Cephalexin in water

**Sample solution:** 0.4 mg/mL of Cephalexin in *Mobile phase* from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in µg, of cephalexin  
(C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) per mg of the Cephalexin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cephalexin RS in the  
*Standard solution* (mg/mL)  
 $C_U$  = concentration of Cephalexin in the *Sample*  
*solution* (mg/mL)  
 $P$  = potency of cephalexin in USP Cephalexin RS  
 (µg/mg)

**Acceptance criteria:** 950–1030 µg/mg on the anhy-  
 drous basis

**IMPURITIES****Organic Impurities****• PROCEDURE 1**

**Solution A:** Dissolve 1 g of sodium 1-pentanesulfonate  
 in a mixture of 1000 mL of water and 15 mL of trieth-  
 ylamine. Adjust with phosphoric acid to a pH of 2.5 ±  
 0.1.

**Solution B:** Dissolve 1 g of sodium 1-pentanesulfonate  
 in a mixture of 300 mL of water and 15 mL of triethyl-  
 amine. Adjust with phosphoric acid to a pH of 2.5 ±  
 0.1, and add 350 mL of acetonitrile and 350 mL of  
 methanol.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

**Diluent:** 18 mg/mL of monobasic potassium phosphate  
 in water

**Standard solutions:** 0.08 mg/mL and 0.16 mg/mL of  
 cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) from USP Cephalexin RS in  
*Diluent*, taking into account the stated potency of the  
 USP Cephalexin RS

**Sample solution:** 5 mg/mL of Cephalexin in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1 of low acidity

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*  
 Plot the responses of the cephalexin peaks from the  
*Standard solutions* versus their concentrations, calcu-  
 lated on the anhydrous basis, in mg/mL, and draw a  
 straight line through the two points and zero. From  
 the line and the peak responses of the *Sample solu-*  
*tion*, determine the concentration,  $I$ , in mg/mL, of  
 each cephalexin-related substance of the *Sample solu-*  
*tion* other than the cephalexin peak.

Calculate the percentage of each cephalexin-related  
 substance:

$$\text{Result} = I/C \times 100$$

$I$  = concentration of each cephalexin-related  
 substance in the *Sample solution* as  
 determined from the calibration curve  
 (mg/mL)

$C$  = concentration of cephalexin from the *Sample*  
*solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 1.0% of any individual  
 cephalexin-related substance

**Total impurities:** NMT 5.0%

- **PROCEDURE 2: DIMETHYLANILINE** <223>: Meets the  
 requirement

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* <781S>: +149° to  
 +158°

**Sample solution:** 5 mg/mL, in pH 4.4 neutralized  
 phthalate buffer (see *Reagents, Indicators, and Solu-*  
*tions—Buffer Solutions*)

- **CRYSTALLINITY** <695>: Meets the requirements
- **pH** <791>: 3.0–5.5, in an aqueous suspension containing  
 50 mg/mL
- **WATER DETERMINATION**, *Method I* <921>: 4.0%–8.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
 USP Cephalexin RS

**Cephalexin Hydrochloride**

C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S · HCl · H<sub>2</sub>O 401.87  
 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-  
 [(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydro-  
 chloride, monohydrate, [6R-[6α,7β (R\*)]]-;  
 (6R,7R)-7-[(2R)-2-Amino-2-phenylacetamido]-3-methyl-  
 8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic  
 acid, monohydrochloride, monohydrate;  
 7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-car-  
 boxylic acid hydrochloride monohydrate [105879-42-3].

**DEFINITION**

Cephalexin Hydrochloride contains the equivalent of NLT  
 800 µg/mg and NMT 880 µg/mg of cephalexin  
 (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample*  
*solution* corresponds to that of the *Standard solution*, as  
 obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** <191>:  
 10 mg/mL meets the requirements

**ASSAY****• PROCEDURE**

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate  
 in a mixture of acetonitrile, methanol, triethylamine,  
 and water (20:10:3:170), adjusted with phosphoric acid  
 to a pH of 3.0 ± 0.1

**Standard stock solution:** 1 mg/mL of USP Cephalexin  
 RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile*  
*phase* from *Standard stock solution*

**Sample stock solution:** 1.15 mg/mL of Cephalexin Hy-  
 drochloride in water

**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile*  
*phase* from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in µg, of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) in each mg of Cephalexin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)  
 $C_U$  = concentration of Cephalexin Hydrochloride from the *Sample stock solution* (mg/mL)  
 $P$  = potency of cephalexin in USP Cephalexin RS (µg/mg)

**Acceptance criteria:** 800–880 µg/mg**IMPURITIES****Organic Impurities****• PROCEDURE 1**

**Solution A:** 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

**Solution B:** 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ , and add 350 mL of acetonitrile and 350 mL of methanol.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

**Diluent:** 18 mg/mL of monobasic potassium phosphate in water

**Standard solutions:** 0.08 mg/mL and 0.16 mg/mL of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS

**Sample solution:** 6 mg/mL of Cephalexin Hydrochloride in *Diluent***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; packing L1 of low acidity**Flow rate:** 1 mL/min**Injection size:** 20 µL**Analysis****Samples:** *Standard solutions* and *Sample solution*

Plot the responses of the cephalexin peaks of the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line and the peak responses of the *Sample solution*, determine the concentration,  $I$ , in mg/mL, of each cephalexin-related substance from the *Sample solution* other than the cephalexin peak.

Calculate the percentage of each cephalexin-related substance represented by each peak of the *Sample solution*, other than the cephalexin peak.

$$\text{Result} = (I/C) \times 100$$

$I$  = concentration of each cephalexin-related substance other than cephalexin in the *Sample solution* (mg/mL)

$C$  = concentration of cephalexin from the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 1.0% of any individual cephalexin-related substance is found.

**Total impurities:** NMT 5.0%

- **PROCEDURE 2: DIMETHYLANILINE** <223>: Meets the requirement

**SPECIFIC TESTS**

- **CRYSTALLINITY** <695>: Meets the requirements
- **pH** <791>: 1.5–3.0, in a solution containing 10 mg/mL
- **WATER DETERMINATION, Method I** <921>: 3.0%–6.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>: USP Cephalexin RS

**Cephalexin Capsules****DEFINITION**

Cephalexin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 Capsules in water. Sonicate, if necessary, to dissolve the cephalexin. Filter, if necessary, to obtain a clear solution.

**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; packing L1 of low acidity**Flow rate:** 1.5 mL/min**Injection size:** 20 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of cephalexin in the *Sample solution* (mg/mL)  
 $P$  = potency of cephalexin in USP Cephalexin RS (µg/mg)

F = conversion factor, 0.001 mg/μg  
**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

- **DISSOLUTION** <711>  
**Medium:** Water; 900 mL  
**Apparatus 1:** 100 rpm  
**Time:** 30 min  
**Standard solution:** 20 μg/mL of USP Cephalexin RS in *Medium*  
**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20 μg/mL.  
**Spectrometric conditions**  
 (See *Spectrophotometry and Light-Scattering* <851>.)  
**Mode:** UV  
**Analytical wavelength:** 262 nm  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
**Tolerances:** NLT 80% (Q) of the labeled amount of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
 USP Cephalexin RS

## Cephalexin for Oral Suspension

### DEFINITION

Cephalexin for Oral Suspension is a dry mixture of Cephalexin and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S per mL when constituted as directed in the labeling.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

- **PROCEDURE**  
**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1  
**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water  
**Standard solution:** Mix 10.0 mL of *Standard stock solution* with 15.0 mL of *Mobile phase*.  
**Sample stock solution:** Nominally equivalent to 1 mg/mL of cephalexin from Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.  
**Sample solution:** Mix 10.0 mL of *Sample stock solution* and 15.0 mL of *Mobile phase*.  
**Chromatographic system**  
 (See *Chromatography* <621>, *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 254 nm  
**Column:** 4.6-mm × 25-cm; packing L1 of low acidity  
**Flow rate:** 1.5 mL/min  
**Injection size:** 20 μL  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) in each mL of the constituted Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*
  - $r_S$  = peak response from the *Standard solution*
  - $C_S$  = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)
  - $C_U$  = nominal concentration of cephalexin from the *Sample stock solution* (mg/mL)
  - $P$  = potency of USP Cephalexin RS (μg/mg)
  - $F$  = conversion factor, 0.001 mg/μg
- Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** <905> For solid packaged in single-unit containers: meets the requirements
- **DELIVERABLE VOLUME** <698>: Meets the requirements

### SPECIFIC TESTS

- **PH** <791>: 3.0–6.0, constituted as directed in the labeling

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
 USP Cephalexin RS

## Cephalexin Tablets

### DEFINITION

Cephalexin Tablets are prepared from Cephalexin or Cephalexin Hydrochloride. They contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

- **PROCEDURE**  
**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170). Adjust with phosphoric acid to a pH of 3.0 ± 0.1.  
**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water  
**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*  
**Sample stock solution:** Equivalent to 1 mg/mL of cephalexin from combined contents of powdered Tablets (NLT 20) in water. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.  
**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*  
**Chromatographic system**  
 (See *Chromatography* <621>, *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 254 nm  
**Column:** 4.6-mm × 25-cm; packing L1 of low acidity  
**Flow rate:** 1.5 mL/min  
**Injection size:** 20 μL  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Relative standard deviation:** NMT 2.0%



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of cephalexin in the *Sample solution* (mg/mL)  
 $P$  = potency of cephalexin in USP Cephalexin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS**• **DISSOLUTION** <711>

**For Cephalexin**

**Medium:** Water; 900 mL

**Apparatus 1:** Use 40-mesh cloth and 100 rpm

**Time:** 30 min

**Standard solution:** 20  $\mu\text{g}/\text{mL}$  of USP Cephalexin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute, if necessary, with *Medium* to a concentration that is similar to the *Standard solution*.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 262 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 80% (Q) of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

**For Cephalexin hydrochloride**

**Medium, Standard solution, Sample solution, Spectrometric conditions, and Analysis:** Proceed as directed *For Cephalexin*.

**Apparatus 1:** Use 10-mesh cloth and 150 rpm.

**Time:** 45 min

**Tolerances:** NLT 75% (Q) of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states whether the Tablets contain Cephalexin or Cephalexin Hydrochloride.
- **USP REFERENCE STANDARDS** <11>  
USP Cephalexin RS

**Cephalexin Tablets for Oral Suspension****DEFINITION**

Cephalexin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine,

and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Nominally equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 powdered Tablets for Oral Suspension in water. Pass a portion of the solution through a filter having a 1- $\mu\text{m}$  or finer pore size.

**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) in each Tablet for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)  
 $C_U$  = nominal concentration of cephalexin in the *Sample stock solution* (mg/mL)  
 $P$  = potency of cephalexin in USP Cephalexin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISINTEGRATION** <701>: Tablets for Oral Suspension disintegrate in 3 min, using water at  $20 \pm 5^\circ$ .

• **DISSOLUTION** <711>

**Medium:** Water; 900 mL

**Apparatus 1:** Use 40-mesh cloth and 100 rpm.

**Time:** 30 min

**Standard solution:** 20  $\mu\text{g}/\text{mL}$  of USP Cephalexin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20  $\mu\text{g}/\text{mL}$ .

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 262 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

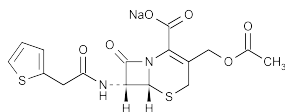
**Tolerances:** NLT 80% (Q) of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

- **DISPERSION FINENESS:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion is obtained that passes through a No. 25 sieve.
- **UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Cephalexin RS

## Cephalothin Sodium



$C_{16}H_{15}N_2NaO_6S_2$  418.42

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl)amino]-, monosodium salt, (6*R*-*trans*)-.

Monosodium (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(2-thienyl)-acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate acetate (ester) [58-71-9].

» Cephalothin Sodium contains the equivalent of not less than 850 µg of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cephalothin Sodium RS

USP Endotoxin RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 25 µg per mL.

*Medium:* water.

**B:** It responds to the tests for *Sodium* (191).

**Specific rotation** (781S): between +124° and +134°.

*Test solution:* a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.

**Crystallinity** (69S): meets the requirements.

**pH** (791): between 4.5 and 7.0, in a solution containing 250 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 1.5% of its weight.

**Chromatographic purity**—

*Mobile phase*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*, transfer 1.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Test solution*—Use the *Assay preparation* prepared as directed in the *Assay*.

*Procedure*—Proceed as directed for the *Procedure* in the *Assay*, except to inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* and to continue the chromatography of the *Test solution* for at least 4 times the retention time of the main cephalothin peak. The area of any peak in the chromatogram obtained from the *Test solution*, except the main peak, is not greater than the area of the main peak in the chromatogram obtained from the *Standard solution* (1.0%), and the sum of the areas of any such peaks is not greater than 3 times the area of the main peak in the chromatogram obtained from the *Standard solution* (3.0%). [NOTE—Any peak in the chromatogram obtained from the *Test solution* with an area less than one-tenth that of the main peak in the chromatogram obtained from the *Standard solution* is disregarded.]

**Other requirements**—Where the label states that Cephalothin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephalothin for Injection*. Where the label states that *Cephalothin Sodium* must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephalothin for Injection*.

**Assay**—

*Mobile phase*—Dissolve 17 g of sodium acetate in 790 mL of water, add 0.6 mL of glacial acetic acid, and if necessary adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of  $5.9 \pm 0.1$ . Add 150 mL of acetonitrile and 70 mL of alcohol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cephalothin Sodium RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL.

*Resolution solution*—Heat a 5-mL portion of the *Standard preparation* in a water bath at 90° for 10 minutes. Cool the solution, and immediately inject a portion of it into the chromatograph as directed under *Chromatographic system*.

*Assay preparation*—Transfer about 25 mg of Cephalothin Sodium, accurately weighed, to a 25-mL volumetric flask, add about 15 mL of *Mobile phase*, swirl to dissolve, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5 µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution between the two principal peaks is not less than 9.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) in each mg of Cephalothin Sodium taken by the formula:

$$25(CP / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cephalothin Sodium RS in the *Standard preparation*; *P* is the assigned potency, in µg of cephalothin per mg, of USP Cephalothin Sodium RS; *W* is the quantity, in mg, of Cephalothin Sodium taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephalothin Injection

» Cephalothin Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ).

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1). Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cephalothin Sodium RS

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.

**pH** (791): between 6.0 and 8.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification* test A under *Cephalothin Sodium* and meets the requirements for *Sterility* under *Cephalothin for Injection*.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cephalothin Sodium*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ), to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cephalothin Sodium*. Calculate the quantity, in  $\mu\text{g}$ , of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) in each mL of the Injection taken by the formula:

$$100(CP / V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cephalothin Sodium RS, in the *Standard preparation*; *P* is the assigned potency, in  $\mu\text{g}$  of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) per mg, of USP Cephalothin Sodium RS; *V* is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephalothin for Injection

» Cephalothin for Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ). It may contain Sodium Bicarbonate.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cephalothin Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Specific rotation** (781S): between  $+124^\circ$  and  $+134^\circ$ , calculated on the dried and sodium bicarbonate-free basis.

*Test solution*: a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.

**Bacterial endotoxins** (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements.

*Procedure for content uniformity*—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

**pH** (791): between 6.0 and 8.5, in the solution constituted as directed in the labeling.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Content of sodium bicarbonate (if present)**—Dissolve about 1 g of it, accurately weighed, in 50 mL of water. Add methyl orange TS, and titrate with 0.1 N sulfuric acid VS. Each mL of 0.1 N sulfuric acid is equivalent to 8.401 mg of  $\text{NaHCO}_3$ . Calculate the percentage of sodium bicarbonate, and use the value obtained to calculate the *Specific rotation* on the dried and sodium bicarbonate-free basis.

**Other requirements**—It meets the requirements for *Identification* test A and *Loss on drying* under *Cephalothin Sodium*. It meets also the requirements for *Labeling* under *Injections* (1).

**Assay**—

*Mobile phase, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cephalothin Sodium*.

*Standard preparation*—Dissolve a suitable quantity of USP Cephalothin Sodium RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 1 mg of cephalothin per mL.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.

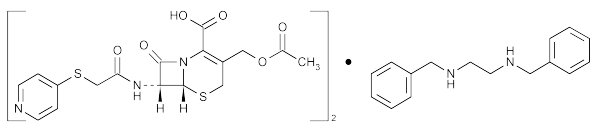
*Assay preparation 2* (where the label states the quantity of cephalothin in a given volume of constituted solution)—Constitute 1 container of Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured portion of the constituted solution quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.

*Procedure*—Proceed as directed in the *Assay* under *Cephalothin Sodium*. Calculate the quantity, in mg, of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) in the container, and in the portion of constituted solution taken by the formula:

$$(L / D)(CP / 1000)(r_U / r_S)$$

in which *L* is the labeled quantity of cephalothin in the container, or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of cephalothin in *Assay preparation 1* or in *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

## Cephapirin Benzathine



(C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>)<sub>2</sub> · C<sub>16</sub>H<sub>20</sub>N<sub>2</sub> 1087.27

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[4-(pyridinylthio)acetyl]amino]-, (6*R*-*trans*)-, compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1).

(6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio)-acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1) [97468-37-6].

» Cephapirin Benzathine contains the equivalent of not less than 715 µg and not more than 820 µg of cephapirin (C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>) per mg.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cephapirin Benzathine RS

USP Cephapirin Sodium RS

**Identification, Infrared Absorption** (197K).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.0 and 7.0, in a suspension (1 in 10).

**Water, Method I** (921): not more than 5.0%.

**Benzathine content**—Using about 1 g of Cephapirin Benzathine, accurately weighed, proceed as directed in the test for *Benzathine content* under *Penicillin G Benzathine*: between 20.0% and 24.0% of benzathine (C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>), calculated on the anhydrous basis, is found.

**Assay**—

**Solution A**—Transfer about 26.2 mL of acetic acid and about 99.12 g of potassium acetate to a 4-L volumetric flask. Add 2000 mL of water, and mix to dissolve. Dilute with water to volume, and pass through a 0.45-µm nylon filter.

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of **Solution A** and **Solution B** as directed for *Chromatographic system*. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Extraction solution**: a mixture of 400 mL of acetic acid and 600 mL of water.

**Dilution buffer**—Dissolve about 205 g of potassium acetate in about 800 mL of water. Adjust with acetic acid to a pH of 7.5 to 8.2. Dilute with water to 1000 mL, and pass through a 0.45-µm nylon filter.

**10% Acetic acid solution**—Add about 10.0 mL of acetic acid to a 100-mL volumetric flask. Mix, and dilute with water to volume.

**System suitability solution**—Dissolve an accurately weighed quantity of USP Cephapirin Sodium RS in 10% Acetic acid solution to prepare a solution containing a known concentration of about 2.0 mg per mL. Heat the solution at 50° for 12 to 18 hours.

**Standard preparation**—In duplicate, accurately weigh about 50 mg of USP Cephapirin Sodium RS, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and about 15.0 mL of *Dilution buffer*, and agitate to dissolve. Add 7.0 mL of acetonitrile, and mix well.

Allow the solution to return to room temperature, and dilute with water to volume.

**Assay preparation**—In duplicate, weigh about 60 mg of Cephapirin Benzathine, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer*, and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector, a 3.2-mm × 15-mm guard column that contains 7-µm packing L1 and a 3.9-mm × 15-cm analytical column that contains 4-µm packing L1. The flow rate is about 2.0 mL per minute, and the columns are heated to 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6	91.5	8.5	isocratic
6–10	91.5→80.0	8.5→20.0	linear
10–12	80.0	20.0	isocratic
12	80.0→91.5	20.0→8.5	return to initial
12–21	91.5	8.5	re-equilibration

Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak heights and valleys as directed for *Procedure*. Using the results from the *System suitability solution*, calculate the percentage of the height of the valley taken by the formula:

$$100(r_v / r_i)$$

in which  $r_v$  is the height of the valley between cephapirin and any impurity; and  $r_i$  is the impurity peak height. The percentage of the height of the valley is not more than 25% for the impurity peaks adjacent to the cephapirin peak. [NOTE—The *System suitability solution* is acceptable as long as the cephapirin peak is larger than the two peaks on either side of the cephapirin peak.] The relative standard deviation for replicate injections of the *Standard preparation* is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub> in each mg of Cephapirin Benzathine taken by the formula:

$$P(W_s / W_u)(V_u / V_s)(r_u / r_s)$$

in which  $P$  is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS;  $W_s$  and  $W_u$  are the quantities of USP Cephapirin Sodium RS and Cephapirin Benzathine, in mg, used to prepare the *Standard preparation* and the *Assay preparation*, respectively;  $V_s$  and  $V_u$  are the final volumes, in mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the average peak areas of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephapirin Benzathine Intramammary Infusion

» Cephapirin Benzathine Intramammary Infusion is a suspension of Cephapirin Benzathine in a suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity

of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ). It contains a suitable dispersing agent.

**Packaging and storage**—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.

**Labeling**—Label Intramammary Infusion to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cephapirin Benzathine RS

USP Cephapirin Sodium RS

**Identification**, *Infrared Absorption* (197K)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds to that of a similar dispersion of USP Cephapirin Benzathine RS in potassium bromide.

**Water**, *Method I* (921): not more than 1.0%, 10 mL of Intramammary Infusion being tested.

**Assay**—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Cephapirin Benzathine.

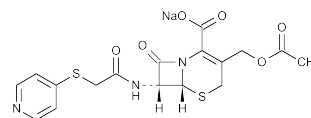
*Assay preparation*—Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 *n*-heptane and 1.5 mL of *Extraction solution*, cap, and vortex at high speed for 5 minutes. Centrifuge for 5 minutes at a sufficient speed to break the emulsion. Remove the aqueous layer, and pass through a 0.45- $\mu$ m nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

*Procedure*—Separately inject equal volumes (about 2  $\mu$ L) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each syringe of Intramammary Infusion taken by the formula:

$$15PW(V_U / V_S)(r_U / r_S)$$

in which *P* is the assigned potency, in  $\mu$ g cephapirin per mg, of USP Cephapirin Sodium RS; *W* is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation*; *V<sub>S</sub>* is the final volume, in mL, of the *Standard preparation*; *V<sub>U</sub>* is the entire volume of Intramammary Infusion, in mL, in one syringe; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak area and the average peak area of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephapirin Sodium



$C_{17}H_{16}N_3NaO_6S_2$  445.45

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[4-(pyridinylthio)acetyl]amino]-, monosodium salt, (6*R*-trans)-.

Monosodium (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio)acetamido]-5-thia-1-azabicyclo-[4.2.0]-oct-2-ene-2-carboxylate acetate (ester) [24356-60-3].

» Cephapirin Sodium has a potency equivalent to not less than 855  $\mu$ g and not more than 1000  $\mu$ g of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cephapirin Sodium RS

USP Endotoxin RS

**Identification**—

A: *Infrared Absorption* (197K).

B: It responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 6.5 and 8.5, in a solution containing 10 mg of cephapirin per mL.

**Water**, *Method I* (921): not more than 2.0%.

**Other requirements**—Where the label states that Cephapirin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephapirin for Injection*. Where the label states that Cephapirin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephapirin for Injection*.

**Assay**—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Cephapirin Benzathine.

*Assay preparation*—In duplicate, weigh about 50 mg of Cephapirin Sodium, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer*, and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

*Procedure*—Separately inject equal volumes (about 2  $\mu$ L) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu$ g, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each mg of Cephapirin Sodium taken by the formula:

$$P(W_S / W_U)(V_U / V_S)(r_U / r_S)$$

in which *P* is the assigned potency, in  $\mu$ g of cephapirin per mg, of USP Cephapirin Sodium RS; *W<sub>S</sub>* and *W<sub>U</sub>* are the quantities of USP Cephapirin Sodium RS and Cephapirin Sodium, in mg, used to prepare the *Standard preparation* and the *Assay preparation*, respectively; *V<sub>U</sub>* and *V<sub>S</sub>* are the final volumes, in mL, of the *Assay preparation* and the *Standard*

preparation, respectively; and  $r_u$  and  $r_s$  are the average peak areas of the cephapirin peaks obtained from the Assay preparation and the Standard preparation, respectively.

## Cephapirin for Injection

» Cephapirin for Injection contains an amount of Cephapirin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephapirin.

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under Injections (1).

**USP Reference standards** (11)—

USP Cephapirin Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for Constituted Solutions under Injections (1).

**Bacterial endotoxins** (85)—It contains not more than 0.17 USP Endotoxin Unit per mg of cephapirin.

**Sterility** (71)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the Identification tests and meets the requirements for Crystallinity, pH, and Water under Cephapirin Sodium. It meets also the requirements for Uniformity of Dosage Units (905) and Labeling under Injections (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, dimethylformamide, glacial acetic acid, and 11.7 N potassium hydroxide (1834:160:4:2). Make adjustments if necessary (see System Suitability under Chromatography (621)). Increase the proportion of dimethylformamide to decrease the retention time of cephapirin.

**Resolution solution**—Prepare a solution of Cephapirin Sodium in pH 2.0 hydrochloric acid buffer (see Buffer Solutions in the section Reagents, Indicators, and Solutions) containing about 1 mg per mL. Place 10 mL of this solution in a test tube, and heat at 95° for 10 minutes, accurately timed. Promptly cool the tube in an ice water bath. Dilute 5 mL of the cooled solution with Mobile phase to obtain 50 mL of Resolution solution.

**Standard preparation**—Transfer about 21 mg of USP Cephapirin Sodium RS, accurately weighed, to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix. This solution contains about 0.2 mg of cephapirin per mL.

**Assay preparation 1** (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cephapirin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution containing the equivalent of about 0.2 mg of cephapirin per mL.

**Assay preparation 2** (where the label states the quantity of cephapirin in a given volume of constituted solution)—Constitute Cephapirin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution containing the equivalent of about 0.2 mg of cephapirin per

mL. [NOTE—Use the Standard preparation and the Assay preparation within 1 hour.]

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution,  $R$ , between the cephapirin peak and the peak having a retention time of about 0.9 relative to that of cephapirin is not less than 0.9. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative retention times are about 0.9 for cephapirin lactone and 1.0 for cephapirin; the column efficiency determined from the cephapirin peak is not less than 1200 theoretical plates; the tailing factor for the cephapirin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the Standard preparation and the appropriate Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_u / r_s)$$

in which  $L$  is the labeled quantity, in mg, of cephapirin in the single-dose container, or in the volume of constituted solution taken;  $D$  is the concentration, in mg per mL, of cephapirin in Assay preparation 1 or in Assay preparation 2, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution;  $C$  is the concentration, in mg per mL, of USP Cephapirin Sodium RS in the Standard preparation;  $P$  is the potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; and  $r_u$  and  $r_s$  are the cephapirin peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Cephapirin Sodium Intramammary Infusion

» Cephapirin Sodium Intramammary Infusion is a suspension of Cephapirin Sodium in a suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ). It contains a suitable dispersing agent.

**Packaging and storage**—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cephapirin Sodium RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds

to that of a similar dispersion of USP Cephapirin Sodium RS in potassium bromide.

**Water, Method I** (921): not more than 1.0%, 10 mL of Intramammary Infusion being tested.

#### Assay—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system—*Proceed as directed in the Assay under *Cephapirin Benzathine*.

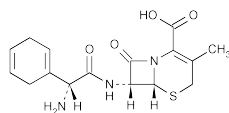
*Assay preparation—*Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 mL *n*-heptane and 1.0 mL of *Extraction solution*, cap, and mix on a vortex mixer at high speed for 5 minutes. Centrifuge for 5 minutes at a speed sufficient to break the emulsion. Remove the aqueous layer, and pass through a 0.45- $\mu$ m nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

*Procedure—*Separately inject equal volumes (about 2  $\mu$ L) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephalapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each syringe of Intramammary Infusion taken by the formula:

$$10PW(V_U / V_S)(r_U / r_S)$$

in which *P* is the assigned potency, in  $\mu$ g of cephalapirin per mg, of USP Cephapirin Sodium RS; *W* is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation*; *V<sub>S</sub>* is the final volume, in mL, of the *Standard preparation*; *V<sub>U</sub>* is the entire volume of Intramammary Infusion, in mL, in one syringe; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak area and the average peak area of the cephalapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine



$C_{16}H_{19}N_3O_4S$  349.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(amino-1,4-cyclohexadien-1-ylacetyl)amino]-3-methyl-8-oxo-, [6*R*-[6 $\alpha$ ,7 $\beta$ (*R*\*)]]-

(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [38821-53-3].

Monohydrate 367.43 [31828-50-9(non-stoichiometric hydrate)].

Dihydrate 385.44 [58456-86-3].

» Cephadrine has a potency of not less than 900  $\mu$ g and not more than 1050  $\mu$ g of total cephalosporins per mg, calculated as the sum of cephaladrine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ), calculated on the anhydrous basis.

**Packaging and storage—**Preserve in tight containers.

**Labeling—**Where it is the dihydrate form, the label so indicates. Where the quantity of cephaladrine is indicated in the labeling of any preparation containing Cephadrine, this shall be understood to be in terms of anhydrous cephaladrine ( $C_{16}H_{19}N_3O_4S$ ). Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### USP Reference standards (11)—

USP Cephadrine RS

USP Cephalixin RS

USP Endotoxin RS

**Identification, Infrared Absorption** (197K).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 3.5 and 6.0, in a solution containing 10 mg per mL.

**Water, Method I** (921): not more than 6.0%, except that if it is the dihydrate form, the limit is between 8.5% and 10.5%.

**Limit of cephalixin—**Using the chromatogram of the *Assay preparation* obtained in the Assay, calculate the percentage of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) in the portion of Cephadrine taken by the formula:

$$100(r_{UX} / r_U)$$

in which *r<sub>UX</sub>* is the cephalixin peak response in the chromatogram obtained from the *Assay preparation*, and *r<sub>U</sub>* is the sum of the cephalixin and cephaladrine peak responses in the chromatogram obtained from the *Assay preparation*: not more than 5.0%, calculated on the anhydrous basis, is found.

**Other requirements—**Where the label states that Cephadrine is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephadrine for Injection*. Where the label states that Cephadrine must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephadrine for Injection*.

#### Assay—

*Mobile phase—*Prepare a mixture of water, methanol, 0.5 M sodium acetate, and 0.7 N acetic acid (782:200:15:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Filter the solution through a filter of 1  $\mu$ m or finer porosity, and degas before use.

*Standard preparation—*Dissolve an accurately weighed quantity of USP Cephadrine RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

*Resolution solution—*Prepare a solution in *Mobile phase* containing in each mL about 0.5 mg of USP Cephadrine RS and 0.5 mg of USP Cephalixin RS.

*Assay preparation—*Transfer about 50 mg of Cephadrine, accurately weighed, to a 100-mL volumetric flask, add about 30 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.8 for cephalixin and 1.0 for cephaladrine; and the resolution, *R*, between the cephalixin peak and the cephaladrine peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of total cephalosporins (sum of cephradine and cephalixin) in each mg of the Cephadrine taken by the formula:

$$100(CP / M)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; M is the quantity, in mg, of Cephadrine taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the sums of the cephradine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine Capsules

» Cephadrine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The quantity of cephradine stated in the labeling is in terms of anhydrous cephradine ( $C_{16}H_{19}N_3O_4S$ ).

**USP Reference standards** (11)—

USP Cephadrine RS

USP Cephalixin RS

**Identification**—Mix the contents of 1 Capsule with water to obtain a solution having a concentration of about 3 mg of cephradine per mL, and filter (*test solution*). Place a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of binder-free silica gel in a chamber containing a mixture of *n*-hexane and tetradecane (95:5) to a depth of about 1 cm, allow the solvent front to move the length of the plate, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10  $\mu$ L each of the *test solution* and a *Standard solution* containing 3 mg of USP Cephadrine RS per mL. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and a 1 in 15 solution of ninhydrin in acetone (60:40:1.5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, dry the plate for 10 minutes at 110°, and examine the chromatogram: the  $R_f$  value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

**Dissolution** (711)—

*Medium*: 0.12 N hydrochloric acid; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}N_3O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cephadrine RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, of the mixed contents of 4 Capsules in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: the Capsule contents lose not more than 7.0% of their weight.

**Assay**—

*Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the Assay under Cephadrine.

*Assay preparation*—Transfer, as completely as possible, the contents of not fewer than 20 Capsules to a suitable tared container, determine the average weight per Capsule, and mix the combined contents. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of cephradine, to a 250-mL volumetric flask, add 50 mL of *Mobile phase*, sonicate for about 15 minutes, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this mixture through a filter having a porosity of 0.5  $\mu$ m or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard cephradine preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalixin) in the portion of Capsules taken by the formula:

$$0.25CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; and  $r_U$  and  $r_S$  are the sums of the cephradine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine for Injection

» Cephadrine for Injection contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under *Injections* (1).

**USP Reference standards** (11)—

USP Cephadrine RS

USP Cephalixin RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—Dilute the contents of 1 container of Cephadrine for Injection with water to obtain a test solution containing about 3 mg of cephradine per mL. Proceed as directed in the *Identification* test under *Cephadrine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of cephradine.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 8.0 and 9.6, in a solution containing 10 mg per mL.



**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 5.0% of its weight.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling under Injections* (1).

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cephadrine*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cephadrine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephadrine per mL.

*Assay preparation 2* (where the label states the quantity of cephadrine in a given volume of constituted solution)—Constitute Cephadrine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephadrine per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and *Assay preparation 1* or *Assay preparation 2* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephadrine (sum of cephadrine and cephalixin) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(CP)(L / 1000D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; L is the labeled quantity, in mg of cephadrine, in the container taken to prepare *Assay preparation 1*, or in the volume of constituted solution taken to prepare *Assay preparation 2*; D is the concentration, in mg of cephadrine per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephadrine and cephalixin peak responses obtained from *Assay preparation 1* or *Assay preparation 2* and the *Standard preparation*, respectively.

## Cephadrine for Oral Suspension

» Cephadrine for Oral Suspension is a dry mixture of Cephadrine and one or more suitable buffers, colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of cephadrine, calculated as the sum of cephadrine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cephadrine RS  
USP Cephalixin RS

**Identification**—Constitute 1 container of Cephadrine for Oral Suspension as directed in the labeling. Mix a portion of the resulting suspension with water to obtain a concentration of about 3 mg of cephadrine per mL, and filter (*test solution*). Proceed as directed in the *Identification* test under *Cephadrine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the  $R_f$  value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 3.5 and 6.0, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 1.5%.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cephadrine*.

*Assay preparation*—Constitute Cephadrine for Oral Suspension as directed in the labeling. Dilute an accurately measured volume of the suspension so obtained, freshly mixed and free from air bubbles, quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephadrine per mL. Filter a portion of this mixture through a filter having a porosity of 0.5  $\mu$ m or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephadrine (sum of cephadrine and cephalixin) in each mL of constituted Oral Suspension taken by the formula:

$$(CP)(L / 1000D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; L is the labeled quantity, in mg of cephadrine, in each mL of the constituted Oral Suspension; D is the concentration, in mg of cephadrine per mL, of the *Assay preparation*, based on the labeled quantity of cephadrine per mL of constituted Oral Suspension and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephadrine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine Tablets

» Cephadrine Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephadrine, calculated as the sum of cephadrine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cephadrine RS  
USP Cephalixin RS

**Identification**—Mix a quantity of finely powdered Tablets with water to obtain a concentration of about 3 mg of cephadrine per mL, and filter (*Test solution*). Proceed as directed in the *Identification* test under *Cephadrine Capsules*, beginning with "Place a suitable thin-layer chromatographic

plate": the  $R_F$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

#### Dissolution <711>—

*Medium:* 0.12 N hydrochloric acid; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 60 minutes.

*Procedure*—Determine the amount of  $C_{16}H_{19}N_3O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a *Standard solution* having a known concentration of USP Cephadrine RS in the same medium.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 6.0%.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under Cephadrine.

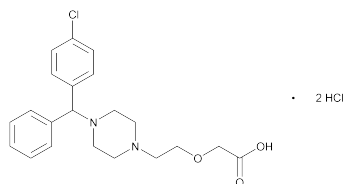
*Assay preparation*—Place not less than 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, sufficient to yield a concentration of not less than 5 mg of cephadrine per mL, and blend for  $4 \pm 1$  minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with *Mobile phase* to obtain an *Assay preparation* containing about 0.5 mg of cephadrine per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephadrine (sum of cephadrine and cephalixin) in each Tablet taken by the formula:

$$(CP)(L / 1000D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; L is the labeled quantity, in mg of cephadrine, in each Tablet; D is the concentration, in mg of cephadrine per mL, of the *Assay preparation*, based on the labeled quantity per Tablet, the number of Tablets taken, and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephadrine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cetirizine Hydrochloride



$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$  461.81  
 ( $\pm$ )-[2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride;  
 ( $\pm$ )-[2-[4-(*p*-Chloro- $\alpha$ -phenylbenzyl)-1-piperazinyl]ethoxy]acetic acid, dihydrochloride [83881-52-1].

#### DEFINITION

Cetirizine Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ , calculated on the dried basis.

#### IDENTIFICATION

##### A. INFRARED ABSORPTION <197K>

- **B. IDENTIFICATION TEST—GENERAL, Chloride** (191): Meets the requirements

#### ASSAY

##### PROCEDURE

**Mobile phase:** Acetonitrile, water, and 1 M sulfuric acid (93:6.6:0.4)

**Standard solution:** 0.5 mg/mL USP Cetirizine Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.5 mg/mL Cetirizine Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L3

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$  in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **HEAVY METALS, Method I** (231): 10 ppm

##### Organic Impurities

[NOTE—It is recommended that *Test 2* be performed if either cetirizine ethanol (2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol) or cetirizine acetic acid (2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid) may be present in the test substance.]

##### PROCEDURE 1

**Mobile phase and Sample solution:** Proceed as directed in the Assay.

**System suitability solution:** 4  $\mu$ g/mL each of USP Cetirizine Hydrochloride RS and USP Cetirizine Related Compound A RS in *Mobile phase*

**Standard solution:** 0.5  $\mu$ g/mL of USP Cetirizine Hydrochloride RS in *Mobile phase*

**Chromatographic system:** Prepare as directed in the Assay.

(See *Chromatography* (621), *System Suitability*.)

**Run time:** Three times the retention time of cetirizine

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for cetirizine, *System suitability solution*

**Resolution:** NLT 2.0 between cetirizine and cetirizine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0% cetirizine, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Cetirizine hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response for each impurity from the *Sample solution*  
 $r_S$  = peak response for cetirizine from the *Standard solution*  
 $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Impurity Table 1* for values)

**Acceptance criteria:** See *Impurity Table 1*.

**Total impurities:** NMT 0.3%. [NOTE— Disregard peaks below 0.02%.]

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-CBH <sup>a</sup>	0.3	1.4	0.1
Dimer <sup>b</sup>	0.5	1.8	0.1
2-Chlorocetirizine <sup>c</sup>	0.85	0.49	0.1
Cetirizine related compound A <sup>d</sup>	0.9	0.95	0.1
Cetirizine	1.0	—	—
Deschlorocetirizine <sup>e</sup>	1.4	0.45	0.1
CBHP <sup>f</sup>	1.45	1.6	0.1
Any individual unspecified impurity	—	1.0	0.1

<sup>a</sup> 4-Chlorobenzhydrol.

<sup>b</sup> 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.

<sup>c</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>d</sup> 2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid, ethyl ester (cetirizine ethyl ester).

<sup>e</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.

<sup>f</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

#### • PROCEDURE 2

**Solution A:** 2 g/L tetrabutyl ammonium hydrogen sulfate and 3 g/L of monobasic sodium phosphate monohydrate in water. Adjust with 1 N sodium hydroxide to a pH of  $2.8 \pm 0.05$ .

**Solution B:** Methanol

**Buffer:** 1.4 g/L monobasic sodium phosphate monohydrate and 2.7 g/L of dibasic sodium phosphate heptahydrate. Adjust with either 1 N sodium hydroxide or 10% phosphoric acid to a pH of  $6.9 \pm 0.1$ .

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	58	42	1.2
40	58	42	1.2
68	20	80	1.5
108	20	80	1.5
110	58	42	1.2
120	58	42	1.2

**Standard solution:** 2 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** 2 mg/mL cetirizine hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 232 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2

**Column efficiency:** NLT 6000 theoretical plates

**Relative standard deviation:** NMT 5.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response for each impurity from the *Sample solution*  
 $r_S$  = peak response for cetirizine from the *Standard solution*  
 $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Impurity Table 2* for values)

**Acceptance criteria:** See *Impurity Table 2*.

**Total impurities:** NMT 0.3%. [NOTE— Disregard peaks below 0.05%.]

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Deschlorocetirizine <sup>a</sup>	0.35	0.56	0.1
Cetirizine ethanol <sup>b</sup>	0.53	1.2	0.1
CBHP <sup>c</sup>	0.66	1.3	0.1
2-Chlorocetirizine <sup>d</sup>	0.70	0.52	0.1
Cetirizine methyl ester <sup>e</sup>	0.81	0.96	0.1
3-Chlorocetirizine <sup>f</sup>	0.87	0.52	0.1
Cetirizine	1.0	—	—
Cetirizine acetic acid <sup>g</sup>	1.15	0.97	0.1
Cetirizine N-oxide <sup>h</sup>	1.25	0.81	0.1
4-CBH <sup>i</sup>	1.55	1.2	0.1
4-Chlorobenzophenone <sup>j</sup>	1.66	0.50	0.1

<sup>a</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.

<sup>b</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

<sup>c</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

<sup>d</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>e</sup> Methyl 2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetate.

<sup>f</sup> 2-[2-[4-[(3-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>g</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.

<sup>h</sup> 2-[2-[4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl]ethoxy]acetic acid N<sup>1</sup>-oxide.

<sup>i</sup> 4-Chlorobenzhydrol.

<sup>j</sup> (4-Chlorophenyl)phenylmethanone.

<sup>k</sup> 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.

Impurity Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizine dimer <sup>k</sup>	2.48	1.4	0.1
Any individual unspecified impurity	—	1.0	0.10

- a 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.  
b 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.  
c 1-[(4-Chlorophenyl)phenylmethyl]piperazine.  
d 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.  
e Methyl 2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetate.  
f 2-[2-[4-[(3-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.  
g 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.  
h 2-[2-[4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl]ethoxy]acetic acid N<sup>1</sup>-oxide.  
i 4-Chlorobenzhydrol.  
j (4-Chlorophenyl)phenylmethanone.  
k 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.

**SPECIFIC TESTS**

- **pH (791):** 1.2–1.8, in an aqueous solution 1 in 20
- **Loss on Drying (731):** Dry a sample at 105° to a constant weight: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store at room temperature.
- **LABELING:** Label it to indicate with which impurity procedures the article complies.
- **USP REFERENCE STANDARDS (11)**  
USP Cetirizine Hydrochloride RS  
USP Cetirizine Related Compound A RS  
(RS)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid ethyl ester dihydrochloride.  
C<sub>23</sub>H<sub>29</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl 489.86

**Cetirizine Hydrochloride Oral Solution****DEFINITION**

Cetirizine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

**ASSAY**• **PROCEDURE**

**Solution A:** Acetonitrile

**Solution B:** 1.36 g/L of monobasic potassium phosphate in water. Adjust with a 2% solution of phosphoric acid in water to a pH of 3.5 ± 0.05.

**Diluent:** Acetonitrile and water (3:7)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	5	95
15	5	95

Time (min)	Solution A (%)	Solution B (%)
22	25	75
35	25	75
40	5	95
50	5	95

**Standard stock solution:** 5 mg/mL of USP Cetirizine Hydrochloride RS in water

**Standard solution:** 0.1 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*, from the *Standard stock solution*

**Sample solution:** Transfer an amount of Oral Solution to a suitable volumetric flask to obtain a nominal concentration of 0.1 mg/mL of cetirizine hydrochloride. Dissolve in 60% of the flask volume of *Diluent* by swirling. Sonicate 3 min, and dilute with *Diluent* to volume. Pass through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 233 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L10

**Column temperature:** 50°

**Flow rate:** 2 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DELIVERABLE VOLUME (698):** Meets the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE**

**Solution A:** Transfer 50 mL of water to a 100-mL volumetric flask, add 5.5 mL of sulfuric acid, and dilute with water to volume.

**Mobile phase:** Acetonitrile, water, and *Solution A* (965:33:1)

**Diluent:** Acetonitrile and water (7:13)

**Standard solution:** 6 μg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** 0.6 mg/mL of cetirizine hydrochloride in *Diluent*. Transfer an amount of Oral Solution to a suitable volumetric flask, dissolve in *Diluent*, sonicate for 10 min, and dilute with *Diluent* to volume. Pass through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L3

**Column temperature:** 30°

Flow rate: 2 mL/min

Injection size: 10 µL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 5.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for cetirizine from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Impurity Table 1*.

Total impurities: NMT 0.8%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetirizine acetic acid <sup>a</sup>	0.69	P <sup>b</sup>
2-Chlorocetirizine <sup>c</sup>	0.83	P
Cetirizine	1.00	—
Cetirizineethanol <sup>d</sup>	1.30	P
Ethoxycetirizine <sup>e</sup>	1.38	P
CBHP <sup>f</sup>	1.52	P
Propylene glycol ester of cetirizine (diastereomer 1) <sup>g</sup>	1.53	0.2
Propylene glycol ester of cetirizine (diastereomer 2) <sup>g</sup>	1.61	0.2
Deschlorocetirizine <sup>h</sup>	1.65	P
Glyceryl ester of cetirizine <sup>i</sup>	2.20	0.5
Any individual unspecified impurity	—	0.2

<sup>a</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.

<sup>b</sup> P = Process impurity. Provided for information only; the content is not calculated and not reported. The content is controlled in the drug substance monograph.

<sup>c</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>d</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

<sup>e</sup> 2-[2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]ethoxy]acetic acid (ethoxycetirizine).

<sup>f</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

<sup>g</sup> 2-Hydroxypropyl 2-(2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetate.

<sup>h</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.

<sup>i</sup> 2,3-Dihydroxypropyl 2-(2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetate.

#### SPECIFIC TESTS

• **PH** (791): 4.0–5.1

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 10 cfu/mL. It meets the requirements of the tests for absence of *Escherichia coli*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature or in a cold place.
- **USP REFERENCE STANDARDS** (11)  
USP Cetirizine Hydrochloride RS

## Cetirizine Hydrochloride Tablets

#### DEFINITION

Cetirizine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ .

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** 2 N sulfuric acid and water (2:33)

**Buffer:** 2.9 mL/L of phosphoric acid in water

**Mobile phase:** Acetonitrile and *Buffer* (3:7)

**Diluent:** Acetonitrile, *Solution A*, and water (100:1:100)

**Standard solution:** 0.2 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** 0.2 mg/mL of cetirizine hydrochloride in *Diluent*, from NLT 20 powdered Tablets. [NOTE—Sonicate, if necessary.]

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

**Run time:** 1.3 times the retention time of cetirizine

##### System suitability

Sample: *Standard solution*

##### Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

##### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

Medium: Water; 900 mL, degassed

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 2.9 mL/L of phosphoric acid in water

Mobile phase: Acetonitrile and *Buffer* (2:3)

**Standard solution:** 11 µg/mL of USP Cetirizine Hydrochloride RS in water. This solution can be stored for 48 h at room temperature.

**Sample solution:** Pass a portion of the solution under test through a suitable 0.45-µm filter.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 50 μL**Run time:** 1.3 times the retention time of cetirizine**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%

Calculate the percentage of cetirizine dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 $r_U$  = peak response from the *Standard solution* $r_S$  = peak response from the *Sample solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $L$  = Tablet label claim, mg $V$  = volume of *Medium*, 900 mL**Tolerances:** NLT 80% (Q) of the labeled amount of cetirizine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE****Solution A:** 2 N sulfuric acid and water (2:33)**Buffer:** 3.4 g/L of tetrabutyl ammonium hydrogen sulfate in water**Diluent:** Acetonitrile, *Solution A*, and water (910:27:63)**Mobile phase:** Acetonitrile, *Solution A*, and *Buffer* (93:5:2)**Standard solution:** 1.5 μg/mL of USP Cetirizine Hydrochloride RS in *Diluent***Sample solution:** 0.5 mg/mL of cetirizine hydrochloride in *Diluent*, from NLT 20 powdered Tablets. [NOTE—Sonicate, if necessary.]**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.0-mm × 25-cm; 5-μm packing L3**Flow rate:** 0.8 mL/min**Injection size:** 20 μL**Run time:** 2.5 times the retention time of cetirizine**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 10.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response for cetirizine from the *Standard solution* $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor (see *Impurity Table 1*)**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 0.8%**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizine lactose ester <sup>a</sup>	0.56	1.0	0.40
Cetirizine	1.0	—	—
Cetirizine ethanol <sup>b</sup>	1.67	1.2	0.15
Any unspecified degradation product	—	—	0.2

<sup>a</sup> 6-O-[2-(2-{4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)-acetyl]-β-D-galactopyranosyl-(1→4)β-D-glucopyranose.<sup>b</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store below 30°.
- **USP REFERENCE STANDARDS** <11>  
USP Cetirizine Hydrochloride RS

## Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets

**DEFINITION**Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride (C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl) and pseudoephedrine hydrochloride (C<sub>10</sub>H<sub>15</sub>NO · HCl).**IDENTIFICATION**

- The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **CETIRIZINE HYDROCHLORIDE****Buffer:** 3.5 g/L of monobasic ammonium phosphate and 1.0 g/L of tetrabutylammonium bisulfate in water. Adjust with phosphoric acid to a pH of 2.5.**Diluent:** Methanol and *Buffer* (2:3)**Solution A:** Acetonitrile, methanol, and *Buffer* (9:2:29)**Solution B:** Acetonitrile**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27.0	100	0
30.0	0	100
30.1	100	0
35.0	100	0

**Standard stock solution:** 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*. [NOTE—Sonicate to dissolve.]**Standard solution:** 0.025 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution***Sample solution:** 0.025 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 22.5% of the final flask volume. Sonicate for NLT 20

min with vigorous swirling every 5 min. To the solution add a volume of *Buffer* equal to 26% of the final flask volume. Allow the solution to equilibrate to room temperature. Dilute with *Diluent* to volume. Pass a portion through a membrane filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  15-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 30°

**Autosampler temperature:** 5°

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cetirizine from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### • PSEUDOEPHEDRINE HYDROCHLORIDE

**Buffer:** 0.8 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of 4.5.

**Mobile phase:** Acetonitrile and *Buffer* (3:7)

**Standard solution:** 0.5 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*. [NOTE—Sonicate to dissolve.]

**Sample stock solution:** 2.4 mg/mL of pseudoephedrine hydrochloride (from 5 finely powdered Tablets) prepared as follows. Dissolve the crushed Tablets first in acetonitrile, using 24% of the final flask volume. Sonicate for NLT 15 min. To the solution add a volume of *Buffer* equal to 56% of the final flask volume. Sonicate for NLT 15 min. Shake the flask for NLT 10 min. Allow the solution to equilibrate to room temperature. Dilute with *Mobile phase* to volume. Centrifuge a portion for 15 min to obtain a clear supernatant.

**Sample solution:** 0.5 mg/mL of pseudoephedrine hydrochloride in *Mobile phase*, from the *Sample stock solution*. Pass the solution through a membrane filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L9

**Flow rate:** 1.5 mL/min

**Injection size:** 25  $\mu$ L

**Run time:** 2 times the retention time of pseudoephedrine

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of pseudoephedrine from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 500 mL, deaerated

**Apparatus 1:** 100 rpm

**Time:** 30 min for cetirizine hydrochloride and 30 min (used only for adjustments in the calculations), 1, 2, and 6 h for pseudoephedrine hydrochloride

**Buffer:** 0.77 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of  $4.5 \pm 0.05$ .

**Mobile phase:** Acetonitrile and *Buffer* (3:7)

**Standard stock solution:** 0.5 mg/mL of USP Cetirizine Hydrochloride RS in water

**Standard solution:** 0.24 mg/mL of USP Pseudoephedrine Hydrochloride RS and 0.01 mg/mL of USP Cetirizine Hydrochloride RS in *Medium* from the *Standard stock solution*

**Sample solution:** At the times specified, withdraw 5 mL of the solution under test, and pass through a suitable filter of 0.45- $\mu$ m pore size, discarding the first few mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV, 230 nm for cetirizine hydrochloride, 254 nm for pseudoephedrine hydrochloride

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L9

**Flow rate:** 1.5 mL/min

**Injection size:** 25  $\mu$ L

**Run time:** 2 times the retention time of pseudoephedrine

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both cetirizine and pseudoephedrine

**Relative standard deviation:** NMT 2.0% for both cetirizine and pseudoephedrine

Calculate the percentage of cetirizine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of cetirizine from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of cetirizine hydrochloride in the *Standard solution* (mg/mL)

L = cetirizine hydrochloride Tablet label claim (mg)

V = volume of *Medium*, 500 mL

Calculate the percentage of pseudoephedrine hydrochloride dissolved at each time point:

$$Q_{30} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_1 = (Q_{30} \times 5/500) + [(r_U/r_S) \times (C_S/L) \times 495 \times 100]$$

$$Q_2 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + [(r_U/r_S) \times (C_S/L) \times 490 \times 100]$$

$$Q_6 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + (Q_2 \times 5/490) + [(r_U/r_S) \times (C_S/L) \times 485 \times 100]$$

$r_U$  = peak response of pseudoephedrine from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of pseudoephedrine hydrochloride in the *Standard solution* (mg/mL)

L = pseudoephedrine hydrochloride Tablet label claim (mg)

V = initial volume of *Medium*, 500 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of cetirizine hydrochloride is dissolved in 30 min. The percentage of the labeled amount of pseudoephedrine hydrochloride dissolved at times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	30%–50%
2	50%–70%
6	NLT 80%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE 1: CETIRIZINE HYDROCHLORIDE RELATED COMPOUNDS

Buffer, Diluent, Solution A and Solution B: Proceed as directed in the *Assay for Cetirizine hydrochloride*.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27	100	0
45	60	40
65	60	40
65.1	100	0
75	100	0

**Standard stock solution:** 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*. [NOTE—Sonicate to dissolve.]

**Standard solution:** 1 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution*

**Sample stock solution:** 0.5 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 70% of the final flask volume. Sonicate for 15

min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

**Sample solution:** 0.2 mg/mL of cetirizine hydrochloride in *Buffer*, from the *Sample stock solution*. Pass a portion through a suitable membrane filter of 0.45-µm pore size.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

**Column temperature:** 30°

**Autosampler temperature:** 5°

**Flow rate:** 1 mL/min

**Injection size:** 25 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 1300 theoretical

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 5.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the individual impurity from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

### Acceptance criteria:

[NOTE—Disregard any peak less than 0.05% of the main peak.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.8%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizineethanol <sup>a</sup>	0.54	1.4	0.3
Chlorobenzhydryl piperazine (CBHP) <sup>b</sup>	0.57	1.5	0.3
Cetirizine	1.0	—	—
Cetirizine acetic acid <sup>c</sup>	1.30	1.1	0.3
Cetirizine N-Oxide <sup>d</sup>	1.47	1.2	0.3
Any unspecified degradation product	—	1.0	0.2

<sup>a</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

<sup>b</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

<sup>c</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.

<sup>d</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]-1-oxide-1-piperazinyl]ethoxy]acetic acid.

#### • PROCEDURE 2: PSEUDOEPHEDRINE HYDROCHLORIDE RELATED COMPOUNDS

**Buffer:** 11.2 g/L of monohydrate sodium perchlorate in water. Adjust with hydrochloric acid to a pH of 2.7.



**Solution A:** Methanol and Buffer (3:17)  
**Solution B:** Methanol and Buffer (1:1)  
**Diluent:** *Solution A*  
**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
35	28	72

**Standard stock solution:** 0.48 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*

**Standard solution:** 4.8 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent* from the *Standard stock solution*

**System suitability stock solution:** 49 µg/mL of ephedrine in *Diluent* from USP Ephedrine Sulfate RS

**System suitability solution:** 1.96 µg/mL of ephedrine and 0.46 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Standard stock solution* from the *System suitability stock solution* and the *Standard stock solution*, respectively

**Sample stock solution:** 2.4 mg/mL of pseudoephedrine hydrochloride (from NMT 25 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 75% of the final flask volume. Sonicate for NLT 15 min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

**Sample solution:** 0.48 mg/mL of pseudoephedrine hydrochloride in *Diluent*, from the *Sample stock solution*. Pass a portion through a suitable membrane filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 212 nm

**Column:** 4.6-mm × 25-cm; 4-µm packing L11

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 30 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.3 between ephedrine and pseudoephedrine, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the individual impurity from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 2*)

#### Acceptance criteria

[NOTE—Disregard any peak less than 0.05% of the main peak.]

**Individual impurities:** See *Impurity Table 2*.

**Total pseudoephedrine related impurities:** NMT 0.5%

**Total cetirizine and pseudoephedrine related impurities:** NMT 1.0%

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ephedrine <sup>a</sup>	0.95	—	—
Pseudoephedrine	1.0	—	—
Methcathinone <sup>b</sup>	1.1	1.1	0.2
Any unspecified degradation product	—	1.0	0.2

\* For system suitability and identification purposes.

<sup>a</sup> [R-(R\*,S\*)]-α-[1-(Methylamino)ethyl]-benzenemethanol.

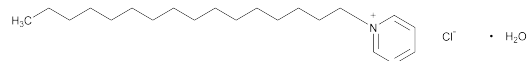
<sup>b</sup> 2-Methylamino-1-phenylpropan-1-one.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>  
 USP Cetirizine Hydrochloride RS  
 USP Ephedrine Sulfate RS  
 USP Pseudoephedrine Hydrochloride RS

## Cetylpyridinium Chloride



$C_{21}H_{38}ClN \cdot H_2O$  358.00

$C_{21}H_{38}ClN$  339.99

Pyridinium, 1-hexadecyl-, chloride, monohydrate;  
 1-Hexadecylpyridinium chloride monohydrate [6004-24-6].  
 Anhydrous [123-03-5].

#### DEFINITION

Cetylpyridinium Chloride contains NLT 99.0% and NMT 102.0% of cetylpyridinium chloride ( $C_{21}H_{38}ClN$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B. ULTRAVIOLET ABSORPTION** <197U>  
**Sample solution:** 40 µg/mL in water  
**Acceptance criteria:** Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** <191>

**Sample solution:** 2 mg/mL in water

**Acceptance criteria:** A 10-mL portion of the *Sample solution* meets the requirements, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

#### ASSAY

##### • PROCEDURE

**Sample solution:** Dissolve 200 mg of Cetylpyridinium Chloride in 75 mL of water. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (1 in 2000), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 in 1000).

**Analysis:** Titrate the *Sample solution* with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating

vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 6.800 mg of cetylpyridinium chloride ( $C_{21}H_{38}ClN$ ).

**Acceptance criteria:** 99.0%–102.0% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2% on the anhydrous basis
- **HEAVY METALS**, *Method II* (231): NMT 20 ppm
- **ORGANIC IMPURITIES, PYRIDINE**  
**Sample solution:** Dissolve 1 g in 10 mL of sodium hydroxide solution (1 in 10) without heating.  
**Acceptance criteria:** The odor of pyridine is not immediately perceptible.

#### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* (741): 80°–84°, the preliminary drying treatment being omitted
- **ACIDITY**  
**Analysis:** Dissolve 500 mg in 50 mL of water, add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide.  
**Acceptance criteria:** NMT 2.5 mL is required for neutralization.
- **WATER DETERMINATION**, *Method I* (921): 4.5%–5.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Cetylpyridinium Chloride RS

## Cetylpyridinium Chloride Lozenges

#### DEFINITION

Cetylpyridinium Chloride Lozenges contain NLT 90.0% and NMT 125.0% of the labeled amount of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ) in a suitable molded base.

#### IDENTIFICATION

- **A.**  
**Eluting solvent:** Alcohol and 1.2 N hydrochloric acid (7:3)  
**Chromatographic column:** Pack a pledget of fine glass wool in the base of a 10-mm × 200-mm chromatographic tube. Add styrene–divinylbenzene cation-exchange resin (strong acid form) to form a uniform column 12 cm in height, and top the column with a pledget of fine glass wool.  
**Standard solution:** 5 µg/mL of USP Cetylpyridinium Chloride RS in *Eluting solvent*  
**Sample solution:** Dissolve nominally 500 µg of cetylpyridinium chloride from NLT 20 finely powdered Lozenges in 50 mL of water. Immediately transfer this solution to the *Chromatographic column*, and discard the eluate. Wash the column, successively, with 200 mL of water, 100 mL of alcohol, 100 mL of water, and 100 mL of 3 N hydrochloric acid. Discard the washings. Elute the column with 80 mL of *Eluting solvent*. Collect the eluate in a 100-mL volumetric flask, and dilute with the *Eluting solvent* to volume.  
**Instrumental conditions**  
*(See Spectrophotometry and Light-Scattering (851).)*  
**Mode:** UV  
**Wavelength range:** 225–300 nm  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**0.004 M sodium lauryl sulfate:** Dissolve 1.15 g of sodium lauryl sulfate in 500 mL of water. Add 2 mL of sulfuric acid, and dilute with water to 1000 mL.

**Standardization of 0.004 M sodium lauryl sulfate:** Determine the molarity of the solution as follows. To a glass-stoppered 100-mL cylinder transfer 10.0 mL of 0.004 M cetylpyridinium chloride (1.432 mg/mL of USP Cetylpyridinium Chloride RS). Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Titrate with the sodium lauryl sulfate solution with frequent vigorous shaking until the chloroform layer acquires the first permanent orange-pink color. Calculate the molarity, and restandardize before each use. [NOTE—Sulfuric acid is included in this solution to inhibit precipitate formation. If a precipitate forms under storage, discard the solution, and prepare and standardize a fresh solution of 0.004 M sodium lauryl sulfate.]

**Sample solution:** Nominally 0.1 mg/mL of cetylpyridinium chloride prepared as follows. Dissolve an accurately determined number of Lozenges (about 100) in about 400 mL of water in a 500-mL volumetric flask, and dilute with water to volume. Transfer a measured aliquot of this solution, equivalent to about 10 mg of cetylpyridinium chloride, to a glass-stoppered, 100-mL cylinder. Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Insert the stopper, and shake until the chloroform layer develops a bright yellow color.

**Analysis:** Titrate with 0.004 M sodium lauryl sulfate, shaking thoroughly after each addition, until the chloroform layer develops the first permanent orange-pink color. Each mL of 0.004 M sodium lauryl sulfate is equivalent to 1.432 mg of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

**Acceptance criteria:** 90.0%–125.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Cetylpyridinium Chloride RS

## Cetylpyridinium Chloride Topical Solution

#### DEFINITION

Cetylpyridinium Chloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

#### IDENTIFICATION

- **A.**  
**Standard solution:** 40 µg/mL of USP Cetylpyridinium Chloride RS in water  
**Sample solution:** 40 µg/mL of cetylpyridinium chloride from Topical Solution diluted with water  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**  
**Sample solution:** Evaporate on a steam bath a volume of Topical Solution equivalent to 500 mg of cetylpyridinium chloride from Topical Solution to one-half of its original volume.  
**Acceptance criteria:** The *Sample solution* meets the requirements, except that when silver nitrate TS is added,

turbidity is produced rather than a curdy white precipitate.

## ASSAY

### • PROCEDURE

**Sample solution:** Add a volume of Topical Solution nominally equivalent to 150 mg of cetylpyridinium chloride to a glass-stoppered, 500-mL graduated cylinder. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (1 in 2000), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 in 1000).

**Analysis:** Titrate the *Sample solution* with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 7.160 mg of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

**Acceptance criteria:** 95.0%–105.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)  
USP Cetylpyridinium Chloride RS

## Activated Charcoal

### DEFINITION

Activated Charcoal is the residue from the destructive distillation of various organic materials, treated to increase its adsorptive power.

### IMPURITIES

#### • RESIDUE ON IGNITION (281)

**Sample:** 0.50 g

**Acceptance criteria:** NMT 4.0%

#### • ACID-SOLUBLE SUBSTANCES

**Sample:** 1.0 g

**Analysis:** Boil the *Sample* with a mixture of 20 mL of water and 5 mL of hydrochloric acid for 5 min. Filter into a tared porcelain crucible, and wash the residue with 10 mL of hot water, adding the washing to the filtrate. To the combined filtrate and washing add 1 mL of sulfuric acid. Evaporate to dryness, and ignite to constant weight.

**Acceptance criteria:** The residue weighs NMT 35 mg (NMT 3.5%).

#### • CHLORIDE AND SULFATE, Chloride (221)

**Sample solution:** A 10-mL portion of the filtrate obtained in the test for *Reaction*

**Acceptance criteria:** The *Sample solution* shows no more chloride than is contained in 1.5 mL of 0.020 N hydrochloric acid (NMT 0.2%).

#### • CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** A 10-mL portion of the filtrate obtained in the test for *Reaction*

**Acceptance criteria:** The *Sample solution* shows no more sulfate than is contained in 1.0 mL of 0.020 N sulfuric acid (NMT 0.2%).

#### • SULFIDE

**Sample:** 0.50 g

**Analysis:** Place the *Sample* in a small conical flask. Add 20 mL of water and 5 mL of hydrochloric acid, and boil gently.

**Acceptance criteria:** The escaping vapors do not darken paper moistened with lead acetate TS.

#### • CYANOGEN COMPOUNDS

**Sample:** 5 g

**Analysis:** Place the *Sample*, 50 mL of water, and 2 g of tartaric acid in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of

1 N sodium hydroxide and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distill about 25 mL. Dilute the distillate with water to 50 mL, and mix. To 25 mL of the diluted distillate add 12 drops of ferrous sulfate TS, heat the mixture almost to boiling, cool, and add 1 mL of hydrochloric acid.

**Acceptance criteria:** No blue color is produced.

#### • HEAVY METALS (231)

**Sample:** 1.0 g

**Test preparation:** Boil the *Sample* with a mixture of 20 mL of 3 N hydrochloric acid and 5 mL of bromine TS for 5 min. Filter, and wash the charcoal and the filter with 50 mL of boiling water. Evaporate the filtrate and washing to dryness, and to the residue add 1 mL of 1 N hydrochloric acid, 20 mL of water, and 5 mL of sulfuric acid. Boil the solution until all of the sulfur dioxide is expelled. Filter if necessary, and dilute with water to 50 mL. To 20 mL of the solution add water to make 25 mL.

**Acceptance criteria:** NMT 50 ppm

#### • UNCARBONIZED CONSTITUENTS

**Sample:** 0.25 g

**Analysis:** Boil the *Sample* with 10 mL of 1 N sodium hydroxide for 5 s, and filter.

**Acceptance criteria:** The filtrate is colorless.

## SPECIFIC TESTS

### • ADSORPTIVE POWER

#### Alkaloids

**Sample:** 1 g, previously dried at 120° for 4 h

**Analysis:** Shake the *Sample* with a solution of 100 mg of strychnine sulfate in 50 mL of water for 5 min, and filter through a dry filter, rejecting the first 10 mL of the filtrate. To a 10-mL portion of the subsequent filtrate add 1 drop of hydrochloric acid and 5 drops of mercuric-potassium iodide TS.

**Acceptance criteria:** No turbidity is produced.

#### Dyes

**Sample:** 250 mg

**Analysis:** Pipet 50 mL of methylene blue solution (1 in 1000) into each of two glass-stoppered, 100-mL flasks. Add the *Sample* to one of the flasks, insert the stopper in the flask, and shake for 5 min. Filter the contents of each flask through a dry filter, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrates into two 250-mL volumetric flasks. Add to each flask 50 mL of sodium acetate solution (1 in 10), mix, and add from a buret 35.0 mL of 0.1 N iodine VS, swirling the mixture during the addition. Insert the stoppers in the flasks, and allow them to stand for 50 min, shaking them vigorously at 10-min intervals. Dilute each mixture with water to volume, mix, allow to stand for 10 min, and filter through dry filters, rejecting the first 30 mL of each filtrate. Titrate the excess iodine in a 100-mL aliquot of each subsequent filtrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Calculate the mL of 0.1 N iodine consumed in each titration.

**Acceptance criteria:** The difference between the two volumes is NLT 0.7 mL.

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

### • REACTION

**Sample:** 3.0 g

**Analysis:** Boil the *Sample* with 60 mL of water for 5 min. Allow to cool, restore the original volume by the addition of water, and filter.

**Acceptance criteria:** The filtrate is colorless and is neutral to litmus.

• **LOSS ON DRYING (731):** Dry a sample at 120° for 4 h: it loses NMT 15.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Chloral Hydrate**

$C_2H_3Cl_3O_2$  165.40  
1,1-Ethenediol, 2,2,2-trichloro-.  
Chloral hydrate [302-17-0].

» Chloral Hydrate contains not less than 99.5 percent and not more than 102.5 percent of  $C_2H_3Cl_3O_2$ .

**Packaging and storage**—Preserve in tight containers.

**Identification**—Transfer to a 125-mL conical flask a portion of a solution in water equivalent to about 1 mg of chloral hydrate, and add water to bring the volume to about 10 mL. Add 10 mL of 1-ethylquinazolinium iodide solution (15 in 1000), which has been filtered through a 0.45- $\mu$ m filter. Add 60 mL of isopropyl alcohol, 5 mL of an aqueous 0.1 M monoethanolamine solution, and 15 mL of water. Mix, and heat in a water bath at 60° for 15 minutes: a blue color develops.

**Acidity**—A 1 in 20 solution in alcohol does not at once redden moistened blue litmus paper.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—To a 1 in 10 solution in alcohol add a few drops of silver nitrate TS: any opalescence produced does not exceed that of a control containing 0.10 mL of 0.020 N hydrochloric acid (0.007%).

**Readily carbonizable substances** (271)—Shake 500 mg, at intervals of 5 minutes during 1 hour, with 5 mL of sulfuric acid in a glass-stoppered cylinder that previously has been rinsed with sulfuric acid, and transfer the mixture to a comparison vessel: the mixture has no more color than *Matching Fluid P*.

**Assay**—Dissolve about 4 g of Chloral Hydrate, accurately weighed, in 10 mL of water, add 30.0 mL of 1 N sodium hydroxide VS, and allow the mixture to stand for 2 minutes. Add a few drops of phenolphthalein TS, and titrate the residual alkali at once with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide corresponds to 165.4 mg of  $C_2H_3Cl_3O_2$ .

**Chloral Hydrate Capsules**

» Chloral Hydrate Capsules contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of  $C_2H_3Cl_3O_2$ .

**Packaging and storage**—Preserve in tight containers, preferably at controlled room temperature.

**Identification**—The contents of the Capsules respond to the *Identification* test under *Chloral Hydrate*.

**Dissolution** (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting

rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Place a counted number of Capsules, equivalent to about 2.5 g of chloral hydrate, in a glass-stoppered, 250-mL flask, add 25 mL of water, insert the stopper in the flask, and heat on a steam bath with frequent swirling until the Capsules are dissolved. Cool to room temperature, add 25 mL of neutralized alcohol and 20.0 mL of 1 N sodium hydroxide VS, mix, and allow the mixture to stand for 4 minutes. Add phenolphthalein TS, and titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 165.4 mg of  $C_2H_3Cl_3O_2$ .

**Chloral Hydrate Oral Solution**

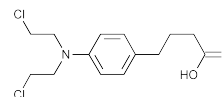
» Chloral Hydrate Oral Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of chloral hydrate ( $C_2H_3Cl_3O_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—It meets the requirements for the *Identification* test under *Chloral Hydrate*.

**Assay**—Transfer 25.0 mL of Oral Solution to a 250-mL conical flask with the aid of several portions of water. Add 30.0 mL of 1 N sodium hydroxide VS, and mix. After the mixture has stood for 2 minutes, add 5 drops of phenolphthalein TS, and immediately titrate the excess sodium hydroxide with 1 N sulfuric acid VS. Designate the volume of 1 N sodium hydroxide VS consumed as *A*. Transfer 5.0 mL of Oral Solution to a second 250-mL conical flask with the aid of several portions of water. Add 10 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Designate the volume of 0.1 N sodium hydroxide VS consumed as *B*. Calculate the weight, in mg, of chloral hydrate ( $C_2H_3Cl_3O_2$ ) in the amount of Oral Solution taken by the first titration by the formula:

$$165.4(A - 0.5B).$$

**Chlorambucil**

$C_{14}H_{19}Cl_2NO_2$  304.21  
Benzenebutanoic acid, 4-[[bis(2-chloroethyl)amino]-  
4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyric acid  
[305-03-3].

» Chlorambucil contains not less than 98.0 percent and not more than 101.0 percent of  $C_{14}H_{19}Cl_2NO_2$ , calculated on the anhydrous basis.

**Caution**—Great care should be taken to prevent inhaling particles of Chlorambucil and exposing the skin to it.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorambucil RS

**Identification**—

**A:** *Infrared Absorption* (197S): 1 in 125 solution, carbon disulfide, 1-mm cell.

**B:** Dissolve 50 mg in 5 mL of acetone, and dilute with water to 10 mL. Add 1 drop of 2 N sulfuric acid, then add 4 drops of silver nitrate TS: no opalescence is observed immediately (*absence of chloride ion*). Warm the solution on a steam bath: opalescence develops (*presence of ionizable chlorine*).

**Melting range** (741): between 65° and 69°.

**Water, Method I** (921): not more than 0.5%.

**Assay**—Dissolve about 200 mg of Chlorambucil, accurately weighed, in 10 mL of acetone, add 10 mL of water, and titrate with 0.1 N sodium hydroxide VS, using phenolphthalein TS as the indicator. Each mL of 0.1 N sodium hydroxide is equivalent to 30.42 mg of C<sub>14</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>2</sub>.

## Chlorambucil Tablets

» Chlorambucil Tablets contain not less than 85.0 percent and not more than 110.0 percent of the labeled amount of C<sub>14</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>2</sub>.

**Packaging and storage**—Preserve coated Tablets in well-closed containers; preserve uncoated Tablets in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorambucil RS

USP Propylparaben RS

**Identification**—Shake a quantity of finely powdered Tablets, equivalent to about 16 mg of chlorambucil, with 20 mL of carbon disulfide. Filter, evaporate to dryness, and dissolve the residue in 2 mL of carbon disulfide: the resulting solution responds to *Identification test A* under *Chlorambucil*.

**Disintegration** (701)—Place 1 Tablet in each of the six tubes of the basket, and if the Tablet has a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Operate the apparatus, using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 30 minutes of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets. If the Tablets have not disintegrated completely, substitute simulated intestinal fluid TS maintained at 37 ± 2° as the immersion fluid, and continue the test for a total period of time, including previous exposure to water and simulated gastric fluid TS, equal to 45 minutes. Lift the basket from the fluid, and observe the Tablets: all of the Tablets have disintegrated completely. If 1 or 2 Tablets fail to disintegrate completely, repeat the test on 12 additional Tablets: not less than 16 of the total of 18 Tablets tested disintegrate completely.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Internal standard solution**—Transfer about 20 mg of USP Propylparaben RS to a 50-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix.

**Standard preparation**—Dissolve in alcohol a suitable quantity of USP Chlorambucil RS, accurately weighed, and prepare, by quantitative dilution, a solution in alcohol having a

known concentration of about 1 mg per mL. Transfer 2.0 mL of the solution to a 100-mL volumetric flask containing about 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Dilute with alcohol to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of chlorambucil, to a 100-mL volumetric flask containing about 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Sonicate for 5 minutes, dilute with alcohol to volume, and mix. Filter through a medium-porosity, sintered-glass filtering funnel, maintaining reduced pressure for the minimum necessary time in order to avoid solvent loss of evaporation.

**Mobile phase**—Mix 500 mL of alcohol with 1.0 mL of glacial acetic acid in a 1-liter volumetric flask, dilute with water to volume, and mix. The alcohol concentration may be varied to meet system suitability requirements and to provide a suitable elution time for chlorambucil. Degas the solution at a pressure of approximately 250 mm of mercury for 2 minutes.

**Chromatographic system**—Typically, a high-pressure liquid chromatograph, operated at room temperature, is fitted with a 25- or 30-cm × 2-mm stainless steel column packed with spherical silica microbeads, 5 μm to 10 μm in diameter, to which is bonded a nominal 10% or 20% (w/w) octadecyl silane. The mobile phase is maintained at a flow rate capable of giving the required resolution (see *System suitability test*) and a suitable elution time. An UV detector that monitors absorption at the 254-nm wavelength is used.

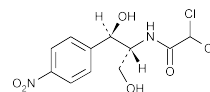
**System suitability test**—Chromatograph 6 to 10 injections of the *Standard preparation*, and measure the peak responses as directed under *Procedure*. Calculate the response factor, *P<sub>S</sub>*, and the resolution factor, *R* (see *Chromatography* (621)). The response factor for 6 to 8 injections does not vary by more than 2.0% relative standard deviation and the resolution factor is not less than 2.0.

**Procedure**—Introduce equal volumes (10 to 12 μL) of the *Standard preparation* and the *Assay preparation* into the high-pressure liquid chromatograph by means of a suitable sampling valve or high-pressure microsyringe, and measure the peak responses at identical retention times obtained with each preparation. Calculate the quantity, in mg, of C<sub>14</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>2</sub> in the portion of Tablets taken by the formula:

$$0.1C(P_U / P_S)$$

in which *C* is the concentration, in μg per mL, of USP Chlorambucil RS in the *Standard preparation*, and *P<sub>U</sub>* and *P<sub>S</sub>* are the response factors of the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol



C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub> 323.13

Acetamide, 2,2-dichloro-*N*-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-, [*R*-(*R*<sup>\*</sup>, *R*<sup>\*</sup>)]-

*D*-threo-(-)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide [56-75-7].

» Chloramphenicol contains not less than 97.0 percent and not more than 103.0 percent of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 149° and 153°.

**Specific rotation** (781S): between +17.0° and +20.0°.

*Test solution:* 50 mg, undried, per mL, in dehydrated alcohol.

**Crystallinity** (695): meets the requirements.

**Bacterial endotoxins** (85)—Where Chloramphenicol is intended for use in preparing injectable dosage forms, it contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility** (71)—Where the label states that Chloramphenicol is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use 1 g of solid specimen.

**pH** (791): between 4.5 and 7.5, in an aqueous suspension containing 25 mg per mL.

**Chromatographic purity**—Dissolve an accurately weighed quantity of Chloramphenicol in methanol to obtain a test solution containing 10 mg per mL. Prepare a solution of USP Chloramphenicol RS in methanol containing 10 mg per mL (*Standard solution A*). Dilute portions of *Standard solution A* quantitatively with methanol to obtain *Standard solution B* containing 100 µg per mL and *Standard solution C* containing 50 µg per mL. Apply separate 20-µL portions of the test solution and *Standard solutions B* and *C* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (79:14:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light: any spot other than the principal spot obtained from the test solution does not exceed in size or intensity the principal spot obtained from *Standard solution B* (1%), and the sum of the impurities represented by all of the spots other than the principal spot, based on a comparison of the intensities of such spots with the intensities of the principal spots obtained from *Standard solutions B* and *C*, does not exceed 2%.

**Assay**—

*Mobile phase*—Prepare a suitable filtered mixture of water, methanol, and glacial acetic acid (55:45:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 80 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer about 200 mg of Chloramphenicol, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of

this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub> in the portion of Chloramphenicol taken by the formula:

$$2.5C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol Capsules

» Chloramphenicol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Chloramphenicol RS in the same *Medium*.

*Tolerances*—Not less than 85% (Q) of the labeled amount of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub> is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately counted number of Chloramphenicol Capsules, equivalent to about

2500 mg of chloramphenicol, to a 1000-mL volumetric flask, add 100 mL of water, and heat on a steam bath until the Capsules have disintegrated. Add 300 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each Capsule taken by the formula:

$$20(C / N)(r_u / r_s)$$

in which *N* is the number of Capsules taken, and the other terms are as defined therein.

## Chloramphenicol Cream

» Chloramphenicol Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in collapsible tubes or in tight containers.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Transfer about 40 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately weighed quantity of Chloramphenicol Cream, equivalent to about 40 mg of chloramphenicol, to a 100-mL volumetric flask, add about 80 mL of methanol, and sonicate for about 10 minutes. Cool to room temperature, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Cream taken by the formula:

$$0.5C(r_u / r_s)$$

in which the terms are as defined therein.

## Chloramphenicol Injection

» Chloramphenicol Injection is a sterile solution of Chloramphenicol in one or more suitable solvents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ . It may contain suitable buffers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 1 mL from each container being transferred directly to the membrane filter.

**pH** (791): between 5.0 and 8.0, in a solution diluted with water (1:1).

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Chloramphenicol*.

*Assay preparation*—Transfer an accurately measured volume of Chloramphenicol Injection, equivalent to about 200 mg of chloramphenicol, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a 0.5- $\mu$ m or finer porosity filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each mL of the Injection taken by the formula:

$$2.5(C / V)(r_u / r_s)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Chloramphenicol Ophthalmic Ointment

» Chloramphenicol Ophthalmic Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to

that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Metal particles**—It meets the requirements under *Metal Particles in Ophthalmic Ointments* (751).

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 25 mg of chloramphenicol, to a suitable conical flask, add 20 mL of cyclohexane, mix, and sonicate for about 2 minutes. Add 60 mL of methanol, and mix. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume, and mix. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol. Transfer 10.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Ophthalmic Ointment taken by the formula:

$$0.25C(r_u / r_s)$$

in which the terms are as defined therein.

## Chloramphenicol Ophthalmic Solution

» Chloramphenicol Ophthalmic Solution is a sterile solution of Chloramphenicol. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers, and store in a refrigerator until dispensed. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

**Labeling**—The labeling states that there is a 21-day beyond-use period after dispensing.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.0 and 7.5, except that in the case of Ophthalmic Solution that is unbuffered or is labeled for veterinary use it is between 3.0 and 6.0.

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100  $\mu$ g per mL. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C / V)(r_u / r_s)$$

in which *V* is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

## Chloramphenicol for Ophthalmic Solution

» Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ , when constituted as directed.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.1 and 7.5, in an aqueous solution containing 5 mg of chloramphenicol per mL.

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100  $\mu$ g per mL. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.



**Assay preparation**—Transfer the contents of 1 container of Chloramphenicol for Ophthalmic Solution to a suitable container with the aid of *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 100 µg of chloramphenicol per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the container of Chloramphenicol for Ophthalmic Solution taken by the formula:

$$(L / D)C(r_u / r_s)$$

in which *L* is the labeled quantity, in mg, of chloramphenicol in the container, *D* is the concentration, in µg per mL, of chloramphenicol in the *Assay preparation*, based on the labeled quantity and the extent of dilution, and the other terms are as defined therein.

## Chloramphenicol Oral Solution

» Chloramphenicol Oral Solution is a solution of Chloramphenicol in a suitable solvent. It contains one or more suitable buffers and preservatives. It has a potency of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only and that it is not to be used in animals raised for food production.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—Prepare a *Test solution* containing 20 µg per mL chloramphenicol from Oral Solution diluted with water. The ultraviolet absorption spectrum of the *Test solution* exhibits maxima and minima only at the same wavelength as that of a similar solution of USP Chloramphenicol RS, concomitantly measured.

**pH** (791): between 5.0 and 8.5, when diluted with an equal volume of water.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Oral Solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Chloramphenicol Otic Solution

» Chloramphenicol Otic Solution is a sterile solution of Chloramphenicol in a suitable solvent. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to

that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 8.0, when diluted with an equal volume of water.

**Water, Method I** (921): not more than 2.0%.

**Assay**—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately measured volume of Otic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each mL of the Otic Solution taken by the formula:

$$0.5(C / V)(r_u / r_s)$$

in which *V* is the volume, in mL, of Otic Solution taken, and the other terms are as defined therein.

## Chloramphenicol Tablets

» Chloramphenicol Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Tablets to indicate that they are for veterinary use only and are not to be used in animals raised for food production.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Disintegration** (701): 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol*.

*Standard preparation*—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of

the powder, equivalent to about 500 mg of chloramphenicol, to a 200-mL volumetric flask, add 80 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Tablets taken by the formula:

$$4C(r_U / r_S)$$

in which the terms are as defined therein.

### Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension

» Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension is a sterile, dry mixture of Chloramphenicol and Hydrocortisone Acetate with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ), when constituted as directed.

**Labeling**—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.

#### USP Reference standards (11)—

USP Chloramphenicol RS

USP Hydrocortisone Acetate RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.1 and 7.5, in an aqueous suspension containing 5 mg of chloramphenicol per mL.

#### Assay—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Chloramphenicol*.

**Standard preparation**—Transfer about 37.5 mg of USP Chloramphenicol RS and 37.5 mg of USP Hydrocortisone Acetate RS, both accurately weighed, *I* being the ratio of the labeled amount, in mg, of hydrocortisone acetate to the labeled amount, in mg, of chloramphenicol in the Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, to a 100-mL volumetric flask, add 15 mL of water and 75 mL of methanol, sonicate for a few seconds, dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

**Assay preparation**—Transfer the contents of an accurately counted number of containers of Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, equivalent to about 37.5 mg of chloramphenicol, to a 100-mL volumetric

flask with the aid of 5 mL of water for each 12.5 mg of chloramphenicol contained therein. Wash each container with methanol, and add the washings to the volumetric flask. Dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in each container taken by the formula:

$$0.5(C / N)(r_U / r_S)$$

in which *N* is the number of containers taken, and the other terms are as defined therein. Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in each container taken by the formula:

$$500(C / N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*, *N* is the number of containers taken, and *r<sub>U</sub>* and *r<sub>S</sub>* are the hydrocortisone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment

» Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) and not less than 90.0 percent and not more than 125.0 percent of the labeled amount of polymyxin B.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

#### USP Reference standards (11)—

USP Chloramphenicol RS

USP Polymyxin B Sulfate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for chloramphenicol*.

**Sterility** (71): meets the requirements.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

#### Assay for chloramphenicol—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Chloramphenicol*.

**Standard preparation**—Proceed as directed for *Standard preparation* in the *Assay* under *Chloramphenicol Ophthalmic Ointment*.

**Assay preparation**—Using Ophthalmic Ointment, proceed as directed for *Assay preparation* in the *Assay* under *Chloramphenicol Ophthalmic Ointment*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Ophthalmic Ointment taken by the formula:

$$0.25C(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for polymyxin**—Proceed as directed for polymyxin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ophthalmic Ointment, equivalent to about 5000 Polymyxin B Units, shaken in a separator containing about 50 mL of ether and extracted with four 20-mL portions of *Buffer No. 6*. Combine the aqueous extracts in a 100-mL volumetric flask, dilute with *Buffer No. 6* to volume, and mix. Dilute an accurately measured portion of this solution quantitatively with *Buffer No. 6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Chloramphenicol, Polymyxin B Sulfate, and Hydrocortisone Acetate Ophthalmic Ointment

» Chloramphenicol, Polymyxin B Sulfate, and Hydrocortisone Acetate Ophthalmic Ointment contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), not less than 90.0 percent and not more than 125.0 percent of the labeled amount of polymyxin B, and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ).

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Polymyxin B Sulfate RS

USP Hydrocortisone Acetate RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation* as obtained in the *Assay for chloramphenicol and hydrocortisone acetate*.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Metal particles**—It meets the requirements under *Metal Particles in Ophthalmic Ointments* (751).

**Assay for polymyxin**—Proceed with Ophthalmic Ointment as directed for polymyxin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ophthalmic Ointment, equivalent to about 5000 Polymyxin B Units, shaken in a separator containing about 50 mL of ether and extracted with four 20-mL portions of *Buffer No. 6*. Combine the aqueous extracts in a 100-mL volumetric flask, dilute, if necessary, with *Buffer No. 6* to volume, and mix. Dilute an accurately measured portion of the resulting solution quantitatively with *Buffer No. 6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for chloramphenicol and hydrocortisone acetate**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol*.

*Standard preparation*—Transfer about 25 mg of USP Chloramphenicol RS and 25J mg of USP Hydrocortisone Acetate RS, both accurately weighed, *J* being the ratio of the labeled amount, in mg, of hydrocortisone acetate to the labeled amount, in mg, of chloramphenicol per g of Ophthalmic Ointment, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and

mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Using Ophthalmic Ointment, proceed as directed for *Assay preparation* in the *Assay under Chloramphenicol Ophthalmic Ointment*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Chloramphenicol*. Calculate the quantity, in mg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the portion of Ophthalmic Ointment taken by the formula:

$$0.25C(r_U / r_S)$$

in which the terms are as defined therein. Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in the portion of Ophthalmic Ointment taken by the formula:

$$250C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*, and the other terms are as defined therein.

### Chloramphenicol and Prednisolone Ophthalmic Ointment

» Chloramphenicol and Prednisolone Ophthalmic Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of prednisolone ( $C_{21}H_{28}O_5$ ).

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Prednisolone RS

**Identification**—

**A:** Transfer a quantity of Ophthalmic Ointment, equivalent to about 20 mg of chloramphenicol, to a screw-capped test tube, add 5 mL of 5 N sodium hydroxide and 2 mL of pyridine, and shake. Place the tube in a water bath at 50° for 20 minutes: a reddish brown color develops in the pyridine layer.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for chloramphenicol*.

**C:** Transfer a quantity of Ophthalmic Ointment, equivalent to about 1.5 mg of prednisolone, to a screw-capped test tube, add 10 mL of methylene chloride, and shake to disperse. Heat at 60° for 15 minutes, and allow to cool while shaking for about 30 minutes. Allow to separate, draw off the upper ointment layer, and retain the lower methylene chloride layer. Apply, in portions, 0.4 mL each of the methylene chloride test solution and a Standard solution of USP Prednisolone RS in chloroform containing 0.5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow each portion to dry before adding the next portion to the same spot. Develop the chromatogram in a chromatographic chamber lined with paper and equilibrated with a solvent system consisting of a mixture of chloroform and acetone (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber,

mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Metal particles**—It meets the requirements under *Metal Particles in Ophthalmic Ointments* (751).

#### Assay for chloramphenicol—

*Methanol-water solution and Mobile phase*—Proceed as directed in the *Assay for prednisolone*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Methanol-water solution* to obtain a solution having a known concentration of about 0.3 mg per mL.

*Assay preparation*—Transfer an accurately weighed portion of Ophthalmic Ointment, equivalent to about 3.0 mg of chloramphenicol, to a screw-capped test tube. Add 10 mL of *n*-heptane, and shake by mechanical means until the substance is dissolved. Add 10.0 mL of *Methanol-water solution*, and shake by mechanical means for 30 seconds. Allow the layers to separate, and carefully remove the upper phase. Centrifuge the lower phase for 15 minutes, and use the clear portion as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the peak for chloramphenicol obtained from the Ophthalmic Ointment, at a retention time corresponding to that of the peak obtained from the Reference Standard, exhibits baseline separation from the adjacent prednisolone peak, and the relative standard deviation of replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Ophthalmic Ointment taken by the formula:

$$10C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chloramphenicol RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for prednisolone—

*Methanol-water solution*—Mix 4 volumes of methanol with 1 volume of water, and mix.

*Mobile phase*—Dissolve 0.68 g of sodium acetate trihydrate in 400 mL of water in a 1000-mL graduated cylinder, adjust with glacial acetic acid to a pH of 4.0, and dilute with water to 500 mL. Dilute with methanol to 1000 mL, and mix. Filter this solution through a membrane filter (1  $\mu$ m or finer porosity), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Prednisolone RS in *Methanol-water solution* to obtain a solution having a known concentration of about 0.2 mg per mL.

*Assay preparation*—Transfer an accurately weighed portion of Ophthalmic Ointment, equivalent to about 2.0 mg of Prednisolone, to a screw-capped test tube. Add 10 mL of *n*-heptane, and shake by mechanical means until the substance is dissolved. Add 10.0 mL of *Methanol-water solution*, and shake by mechanical means for 30 seconds. Allow the layers to separate, and carefully remove the upper phase. Centrifuge the lower phase for 15 minutes, and use the clear portion as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the peak for prednisolone obtained from the Ophthalmic Ointment, at a retention time corresponding to that of the peak obtained from the *Reference Standard*, exhibits baseline separation from the adjacent chloramphenicol peak, and the relative standard deviation of replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 15  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{28}O_5$  in the portion of Ophthalmic Ointment taken by the formula:

$$10C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Prednisolone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol Palmitate

$C_{27}H_{42}Cl_2N_2O_6$  561.54

Hexadecanoic acid, 2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl ester, [ $R$ -( $R^*$ ,  $R^*$ )]-, *D*-threo-(-)-2,2-Dichloro-*N*-[ $\beta$ -hydroxy- $\alpha$ -(hydroxymethyl)-*p*-nitrophenethyl]acetamide  $\alpha$ -palmitate [530-43-8].

» Chloramphenicol Palmitate has a potency equivalent to not less than 555  $\mu$ g and not more than 595  $\mu$ g of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chloramphenicol Palmitate RS

**Identification**—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Melting range** (741): between 87° and 95°.

**Specific rotation** (781S): between +21° and +25°.

*Test solution*: 50 mg, undried, per mL, in dehydrated alcohol.

**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry it to constant weight over phosphorus pentoxide in vacuum at a pressure not exceeding 5 mm of mercury: it loses not more than 0.5% of its weight.

**Acidity**—Dissolve 1.0 g by heating at 35° with 5 mL of a 1:1 mixture of 80 percent alcohol and ether, previously neutralized using phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS, using phenolphthalein TS, until on gentle shaking a pink color persists for not less than 30 seconds: not more than 0.4 mL is consumed.

**Free chloramphenicol**—Dissolve 1.0 g in 80 mL of xylene with the aid of gentle warming. Cool, and extract with three 15-mL portions of water, combining the aqueous extracts and discarding the xylene. Dilute the combined aqueous extracts with water to 50 mL, extract with 10 mL of toluene, allow to separate, and discard the toluene. Centrifuge a portion of the aqueous solution, and determine the absorbance of the clear solution at the wavelength of maxi-

mum absorbance at about 278 nm, using a suitable spectrophotometer, and using as a reagent blank to set the instrument to zero the solution obtained by the same procedure without the specimen: the absorbance is not more than 0.268 (0.045%).

#### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of methanol, water, and glacial acetic acid (172:27:1).

**Standard preparation**—Transfer about 65 mg of USP Chloramphenicol Palmitate RS to a 50-mL volumetric flask, add about 40 mL of methanol and 1 mL of glacial acetic acid, and sonicate for a few minutes. Dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Using about 65 mg of Chloramphenicol Palmitate, accurately weighed, prepare as directed under *Standard preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains 10-μm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2400 theoretical plates, and the relative standard deviation for replicate injections is not more than 0.5%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μg, of chloramphenicol (C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>) equivalent in each mg of specimen taken by the formula:

$$(W_S / W_U)(P_S)(r_U / r_S)$$

in which  $W_S$  and  $W_U$  are the quantities, in mg, of USP Chloramphenicol Palmitate RS and Chloramphenicol Palmitate taken, respectively;  $P_S$  is the designated chloramphenicol equivalent, in μg per mg, of USP Chloramphenicol Palmitate RS; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol Palmitate Oral Suspension

» Chloramphenicol Palmitate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloramphenicol (C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>). It contains one or more suitable buffers, colors, flavors, preservatives, and suspending agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Chloramphenicol Palmitate RS

USP Chloramphenicol Palmitate Polymorph A RS

USP Chloramphenicol Palmitate Nonpolymorph A RS

**Identification**—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Uniformity of dosage units <905>—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>: meets the requirements.

**pH** <791>: between 4.5 and 7.0.

#### Limit of polymorph A—

**Standard preparation**—Prepare a dry mixture of 1 part by weight of USP Chloramphenicol Palmitate Polymorph A RS and 9 parts by weight of USP Chloramphenicol Palmitate Nonpolymorph A RS. Prepare a 1 in 3 mineral oil dispersion of this mixture, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.

**Test preparation**—Place 20 mL of previously mixed Oral Suspension in a 50-mL centrifuge tube, add 20 mL of water, and mix. Centrifuge, and discard the supernatant. Add 20 mL of water to the residue in the centrifuge tube, mix, centrifuge, and discard the supernatant. Repeat this washing two times. Dry the residue in vacuum over silica gel for not less than 14 hours. Prepare a 1 in 3 mineral oil dispersion of the dried residue, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.

**Procedure**—Concomitantly record the absorption spectra of the *Standard preparation* and the *Test preparation* from about 11 μm to about 13 μm, with a suitable IR absorption spectrophotometer, using an empty cell to set the instrument to 100 percent transmittance. Adjust the cell thickness of the *Standard preparation* and of the *Test preparation* so that transmittances of 20% to 30% are obtained at 12.3 μm. On each spectrum, draw a straight baseline between the absorption minima at wavelengths of about 11.3 μm and 12.65 μm. Draw straight lines, perpendicular to the wavelength scale, at the wavelengths of maximum absorption at about 11.65 μm and 11.86 μm, intersecting both the baseline and the spectrum. Determine the absorbance ratio:

$$(A_{11.65a} - A_{11.65b}) / (A_{11.86a} - A_{11.86b})$$

in which the parenthetical expressions are the differences in absorbance values obtained at the wavelengths indicated by the subscripts for the spectrum (a) and at the point of intersection of the perpendicular line with the baseline (b). The absorbance ratio of the *Test preparation* is greater than that of the *Standard preparation*, corresponding to not more than 10% of polymorph A.

#### Assay—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Chloramphenicol Palmitate*.

**Assay preparation**—Transfer an accurately measured volume of Oral Suspension, well-shaken and free from air bubbles, equivalent to about 160 mg of chloramphenicol, to a 200-mL volumetric flask containing about 20 mL of methanol, add 4 mL of glacial acetic acid, dilute with methanol to volume, and mix. Filter about 20 mL of this solution through glass-fiber filter paper. Transfer 10.0 mL of the filtrate to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol Palmitate*. Calculate the quantity, in mg, of chloramphenicol (C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>) equivalent in each mL of Oral Suspension taken by the formula:

$$0.004(W_S / V)(P_S)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Oral Suspension taken, and the other terms are as defined therein.

## Chloramphenicol Sodium Succinate

$C_{15}H_{15}Cl_2N_2NaO_8$  445.18

Butanedioic acid, mono[2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl] ester, monosodium salt, [*R*-(*R*\*,*R*\*)]-.

D-threo-(-)-2,2-Dichloro-N-[[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]acetamide α-(sodium succinate) [982-57-0].

» Chloramphenicol Sodium Succinate has a potency equivalent to not less than 650 µg and not more than 765 µg of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Identification**—The *Assay preparation* exhibits an absorption maximum at a wavelength of about 276 nm, as obtained in the *Assay*.

**Specific rotation** (781S): between +5.0° and +8.0°.

*Test solution*: 50 mg per mL.

**pH** (791): between 6.4 and 7.0, in a solution containing the equivalent of 250 mg of chloramphenicol per mL.

**Water, Method I** (921): not more than 5.0%.

**Limit of free chloramphenicol**—

*Mobile phase*—Prepare a filtered and degassed mixture of 0.05 M monobasic ammonium phosphate, previously adjusted with 10% (v/v) phosphoric acid to a pH of  $2.5 \pm 0.1$ , and methanol (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase* to obtain a solution having a known concentration of about 6 µg per mL. Pass this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate.

*Test solution*—Transfer about 33 mg of Chloramphenicol Sodium Succinate, accurately weighed, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the two major peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not less than 1750 theoretical plates; the resolution, *R*, between the two peaks is not less than 2.0; and the tailing factor is not more than 1.2. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not less than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the

portion of Chloramphenicol Sodium Succinate taken by the formula:

$$5000(C / WQ)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard solution*; *W* is the quantity, in mg, of Chloramphenicol Sodium Succinate taken to prepare the *Test solution*; *Q* is the quantity, in µg, of chloramphenicol in each mg of Chloramphenicol Sodium Succinate taken, as obtained in the *Assay*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

**Other requirements**—Where the label states that Chloramphenicol Sodium Succinate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Chloramphenicol Sodium Succinate for Injection*. Where the label states that Chloramphenicol Sodium Succinate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Chloramphenicol Sodium Succinate for Injection*.

**Assay**—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 20 µg per mL.

*Assay preparation*—Dissolve an accurately weighed quantity of Chloramphenicol Sodium Succinate in water, and dilute quantitatively with water to obtain a solution having a concentration equivalent to about 20 µg of chloramphenicol per mL.

*Procedure*—Concomitantly determine the absorbance of the *Standard preparation*, at the wavelength of maximum absorbance at about 278 nm, and the absorbance of the *Assay preparation*, at the wavelength of maximum absorbance at about 276 nm, in 1-cm cells, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in µg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in each mg of Chloramphenicol Sodium Succinate taken by the formula:

$$(CP / W)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation*; *P* is the potency, in µg per mg, of USP Chloramphenicol RS; *W* is the weight, in µg, of Chloramphenicol Sodium Succinate taken in each mL of the *Assay preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol Sodium Succinate for Injection

» Chloramphenicol Sodium Succinate for Injection contains an amount of Chloramphenicol Sodium Succinate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Limit of free chloramphenicol—**

*Mobile phase, Standard solution, and Chromatographic system*—Proceed as directed in the test for *Limit of free chloramphenicol* under *Chloramphenicol Sodium Succinate*.

*Test solution*—Dissolve the contents of 1 container in a volume of *Mobile phase* to obtain a solution containing the equivalent of about 100 mg of chloramphenicol per mL. Dilute this solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.5 mg of chloramphenicol per mL. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the specimen taken by the formula:

$$0.1(C/D)(r_U/r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard solution*; *D* is the concentration, in mg per mL, of chloramphenicol equivalent in the *Test solution*, based on the labeled quantity in the container and the extent of dilution; and  $r_U$  and  $r_S$  are the chloramphenicol peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

**Other requirements**—It meets the requirements of the tests for *Identification*, *Specific rotation*, *pH*, and *Water* under *Chloramphenicol Sodium Succinate*.

**Assay—**

*Standard preparation*—Proceed as directed in the *Assay* under *Chloramphenicol Sodium Succinate*.

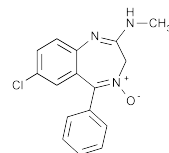
*Assay preparation*—Constitute 1 container of Chloramphenicol Sodium Succinate for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution having a concentration of about 20  $\mu$ g of chloramphenicol per mL.

*Procedure*—Proceed as directed in the *Assay* under *Chloramphenicol Sodium Succinate*. Calculate the quantity, in mg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), in each mL of the constituted Chloramphenicol Sodium Succinate for Injection taken by the formula:

$$(L/D)(CP/1000)(A_U/A_S)$$

in which *L* is the labeled quantity, in mg, of chloramphenicol in each mL of constituted solution; *D* is the concentration, in  $\mu$ g per mL, of chloramphenicol in the *Assay preparation*, on the basis of the labeled quantity of chloramphenicol in each mL of constituted solution and the extent of dilution; *C* is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard preparation*; *P* is the potency, in  $\mu$ g per mg, of USP Chloramphenicol RS; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Chlordiazepoxide



$C_{16}H_{14}ClN_3O$  299.75

3*H*-1,4-Benzodiazepin-2-amine, 7-chloro-*N*-methyl-5-phenyl-, 4-oxide.

7-Chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide [58-25-3].

» Chlordiazepoxide contains not less than 98.0 percent and not more than 102.0 percent of  $C_{16}H_{14}ClN_3O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_{10}ClNO$  231.68

USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS

7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepine-2-one 4-oxide.

$C_{15}H_{11}ClN_2O^2$  286.72

**Identification—**

**A:** *Infrared Absorption* <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**C:** To about 20 mg add 5 mL of hydrochloric acid and 10 mL of water, and heat to boiling to effect hydrolysis. To the cooled solution add 2 mL of sodium nitrite solution (1 in 1000), shake, add 1 mL of ammonium sulfamate solution (1 in 200), then shake for 2 minutes, and add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000): a reddish violet color is produced.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 0.3% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals**, *Method II* <231>: 0.002%.

**Related compounds**—Transfer 50.0 mg to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and apply 50  $\mu$ L of the supernatant to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Apply to the same plate 10  $\mu$ L of an acetone solution containing 100  $\mu$ g per mL of USP Chlordiazepoxide Related Compound A RS and 10  $\mu$ L of an acetone solution containing 10  $\mu$ g per mL of USP 2-Amino-5-chlorobenzophenone RS. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in ethyl acetate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with 2 N sulfuric acid, drying at 105° for 15 minutes, and then spraying in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000). Any spots from the test solution are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the Standard solutions, corresponding to not more than

0.1% of chlordiazepoxide related compound A, and to not more than 0.01% of 2-amino-5-chlorobenzophenone.

**Assay**—[NOTE—Use low-actinic glassware in this procedure.]

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlordiazepoxide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 200 µg per mL.

**Assay preparation**—Transfer about 100 mg of Chlordiazepoxide, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, sonicate for 5 minutes, dilute with *Mobile phase* to volume, and mix. Pass this solution through a membrane filter having a 0.5-µm or finer porosity. Transfer 10 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the column efficiency is not less than 3600 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O in the portion of Chlordiazepoxide taken by the formula:

$$0.5C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of the USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlordiazepoxide Tablets

» Chlordiazepoxide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS

C<sub>13</sub>H<sub>10</sub>ClNO 231.68

USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS  
7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

C<sub>15</sub>H<sub>11</sub>ClN<sub>2</sub>O<sup>2</sup> 286.72

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**B:** A portion of finely powdered Tablets, equivalent to about 20 mg of chlordiazepoxide, responds to *Identification* test C under *Chlordiazepoxide*.

**Dissolution** (711)—

**Medium:** simulated gastric fluid TS, prepared without pepsin; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O dissolved from UV absorbances at the wavelength of maximum absorbance at about 309 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chlordiazepoxide RS in the same medium.

**Tolerances**—Not less than 85% (Q) of the labeled amount of C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to about 25 mg of chlordiazepoxide, to a 10-mL conical flask, and proceed as directed in the test for *Related compounds* under *Chlordiazepoxide*, beginning with “add 2.5 mL of acetone,” except to use 20 µL of an acetone solution containing 1 mg per mL of USP Chlordiazepoxide Related Compound A RS instead of 10 µL of an acetone solution containing 100 µg per mL of the Reference Standard, and except to use 5 µL of an acetone solution containing 100 µg per mL of USP 2-Amino-5-chlorobenzophenone RS instead of 10 µL of an acetone solution containing 10 µg per mL of the Reference Standard. Any spots from the test solution are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the Standard solutions, corresponding to not more than 4.0% of chlordiazepoxide related compound A, and to not more than 0.1% of 2-amino-5-chlorobenzophenone.

**Assay**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Chlordiazepoxide*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of chlordiazepoxide, to a 25-mL volumetric flask, add 20 mL of *Mobile phase*, sonicate for 5 minutes to dissolve, dilute with *Mobile phase* to volume, mix, and filter through a 5-µm membrane filter, discarding the first 5 mL of the filtrate.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chlordiazepoxide*. Calculate the quantity, in mg, of C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O in the portion of Tablets taken by the formula:

$$25C(r_U / r_S)$$

## Chlordiazepoxide and Amitriptyline Hydrochloride Tablets

» Chlordiazepoxide and Amitriptyline Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlordiazepoxide (C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O) and an amount of amitriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amitriptyline (C<sub>20</sub>H<sub>23</sub>N).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS

C<sub>13</sub>H<sub>10</sub>ClNO 231.68

USP Amitriptyline Hydrochloride RS



USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS  
7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-  
2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O^2$  286.72

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* are the same as those of the *Standard preparation* as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: simulated gastric fluid TS, prepared without pepsin; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the absorbances of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, and Standard solutions having known concentrations of USP Chlordiazepoxide RS and USP Amitriptyline Hydrochloride RS in the same *Medium*, at wavelengths of 239 nm and 309 nm, using *Dissolution Medium* as the blank. Calculate the percentage of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) dissolved by the formula:

$$100(CD / L)(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlordiazepoxide RS in the *Standard solution* determined at the wavelength of 309 nm;  $D$  is the dilution factor of the diluted solution under test;  $L$  is the labeled amount, in mg, of chlordiazepoxide in each Tablet; and  $A_U$  and  $A_S$  are the absorbances of the solution under test and the chlordiazepoxide *Standard solution*, respectively, at the wavelength of 309 nm.

Calculate the percentage of amitriptyline ( $C_{20}H_{23}N$ ) dissolved by the formula:

$$100(277.40/313.86)(CD / L)(A_X / A_S)$$

in which 277.40 and 313.86 are the molecular weights of amitriptyline and amitriptyline hydrochloride, respectively;  $C$  is the concentration, in mg per mL, of USP Amitriptyline Hydrochloride RS in the amitriptyline *Standard solution*;  $D$  is the dilution factor of the diluted solution under test;  $L$  is the labeled amount, in mg, of amitriptyline in each Tablet;  $A_S$  is the absorbance of the amitriptyline *Standard solution* at the wavelength of 239 nm; and  $A_X$  is defined by the formula:

$$A_{U239} - A_{U309} (C_{309} A_{S239} / C_{239} A_{S309})$$

in which  $A_{U239}$  and  $A_{U309}$  are the absorbances of the solution under test measured at 239 nm and 309 nm, respectively;  $C_{309}$  and  $C_{239}$  are the concentrations, in mg per mL, of USP Chlordiazepoxide RS in the *Standard solution* obtained at wavelengths of 309 and 239 nm, respectively; and  $A_{S239}$  and  $A_{S309}$  are the absorbances of the chlordiazepoxide *Standard solution* determined at the wavelengths indicated in the subscripts. [NOTE—All of the chlordiazepoxide measurements may be made with either a single *Standard solution* or two separate *Standard solutions*.]

**Tolerances**—Not less than 85% ( $Q$ ) of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) and an amount of amitriptyline hydrochloride equivalent to not less than 85% ( $Q$ ) of the labeled amount of amitriptyline ( $C_{20}H_{23}N$ ) are dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to chlordiazepoxide and to amitriptyline.

**Related compounds**—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to about 25 mg of chlordiazepoxide, to a 10-mL conical flask, add 2.5 mL of

acetone, and shake. Allow any undissolved particles to settle, and apply 50  $\mu$ L of the supernatant to a thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Apply to the same plate 20  $\mu$ L of an acetone solution containing 1 mg per mL of USP Chlordiazepoxide Related Compound A RS and 10  $\mu$ L of an acetone solution containing 50  $\mu$ g per mL of USP 2-Amino-5-chlorobenzophenone RS. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in ethyl acetate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with 2 N sulfuric acid, drying at 105° for 15 minutes, and then spraying in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000): any spots from the test solution are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*, corresponding to not more than 4.0% of chlordiazepoxide related compound A, and not more than 0.1% of 2-amino-5-chlorobenzophenone.

**Assay**—[NOTE—Use low-actinic glassware in this procedure.]

*pH 2.5 Buffer*—Mix 10.5 mL of 0.20 N sodium hydroxide with 100 mL of a solution consisting of 0.04 M acetic acid, 0.04 M phosphoric acid, and 0.04 M boric acid (prepared by dissolving 2.402 g of glacial acetic acid, 4.612 g of phosphoric acid, and 2.473 g of boric acid in sufficient water to obtain 1000 mL of solution).

*Solvent mixture*—Prepare a mixture consisting of *pH 2.5 Buffer*, tetrahydrofuran, and methanol (5:4:1).

*Mobile phase*—Prepare a solution of 0.01 M sodium lauryl sulfate in *Solvent mixture*.

*Standard preparation*—Dissolve accurately weighed quantities of USP Chlordiazepoxide RS and USP Amitriptyline Hydrochloride RS in *Solvent mixture*, and quantitatively dilute with *Solvent mixture* to obtain a solution having known concentrations of about 1 mg of USP Chlordiazepoxide RS per mL and about 2.8 mg of USP Amitriptyline Hydrochloride RS per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of chlordiazepoxide and about 125 mg of amitriptyline, to a 50-mL volumetric flask, add *Solvent mixture* to volume, sonicate to disperse the mixture, and allow undissolved particles to settle. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. Pass a portion of this solution through a filter having a 5- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and record the chromatogram. Measure the peak responses at approximate retention times of 5 minutes for chlordiazepoxide and 7 minutes for amitriptyline hydrochloride. Calculate the quantity, in mg, of

chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) in the portion of Tablets taken by the formula:

$$0.5[C(r_U / r_S)]_Z$$

and calculate the quantity, in mg, of amitriptyline ( $C_{20}H_{23}N$ ) in the portion of Tablets taken by the formula:

$$(277.40/313.86)[(0.5C)(r_U / r_S)]_Y$$

in which Z denotes chlordiazepoxide; Y denotes amitriptyline hydrochloride; 277.40 is the molecular weight of amitriptyline; 313.86 is the molecular weight of amitriptyline hydrochloride; C is the concentration, in  $\mu\text{g}$  per mL, of USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlordiazepoxide Hydrochloride

$C_{16}H_{14}ClN_3O \cdot HCl$  336.22  
3H-1,4-Benzodiazepin-2-amine, 7-chloro-N-methyl-5-phenyl-4-oxide, monohydrochloride.  
7-Chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepine 4-oxide monohydrochloride [438-41-5].

» Chlordiazepoxide Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{16}H_{14}ClN_3O \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards (11)—

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68  
USP Chlordiazepoxide Hydrochloride RS  
USP Chlordiazepoxide Related Compound A RS  
7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O^2$  286.72  
USP Endotoxin RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**C:** To about 20 mg add 5 mL of hydrochloric acid and 10 mL of water, and heat to boiling to effect hydrolysis. To the cooled solution add 2 mL of sodium nitrite solution (1 in 1000), 1 mL of ammonium sulfamate solution (1 in 200), and 1 mL of N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000): a reddish violet color is produced.

**Melting range**, *Class I* (741): between  $212^\circ$  and  $218^\circ$ , with decomposition.

**Loss on drying** (731)—Dry it in vacuum over phosphorus pentoxide at  $60^\circ$  for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Related compounds**—It meets the requirements of the test for *Related compounds* under *Chlordiazepoxide*.

**Other requirements**—Where the label states that Chlordiazepoxide Hydrochloride is sterile, it meets the re-

quirements for *Sterility Tests* (71) and *Labeling* under *Injections* (1), and *Bacterial endotoxins* under *Chlordiazepoxide Hydrochloride for Injection*. Where the label states that Chlordiazepoxide Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Chlordiazepoxide Hydrochloride for Injection*.

**Assay**—[NOTE—Use low-actinic glassware in this procedure.] Proceed as directed in the *Assay* under *Chlordiazepoxide*, except to use USP Chlordiazepoxide Hydrochloride RS when preparing the *Standard preparation*.

## Chlordiazepoxide Hydrochloride Capsules

» Chlordiazepoxide Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{14}ClN_3O \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68  
USP Chlordiazepoxide Hydrochloride RS  
USP Chlordiazepoxide Related Compound A RS  
7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O^2$  286.72

### Identification—

**A:** Transfer a portion of the Capsule powder, equivalent to about 60 mg of chlordiazepoxide hydrochloride, to a 100-mL flask. Add methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Pipet 5 mL of the clear filtrate into a 100-mL volumetric flask, and add a 1 in 360 solution of sulfuric acid in dehydrated alcohol to volume. Pipet 10 mL of this solution into a 50-mL volumetric flask, and dilute with the same acidified alcohol to volume. The resulting solution exhibits maxima at  $245 \pm 2$  nm and  $311 \pm 2$  nm, and the ratio  $A_{245}/A_{311}$  is between 2.90 and 3.45, using the acidified alcohol as the blank and a 1-cm cell.

**B:** A portion of the contents of Capsules responds to *Identification test C* under *Chlordiazepoxide Hydrochloride*.

### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Measure the amount in solution in filtered portions of the *Dissolution Medium*, suitably diluted, in 1-cm cells at the wavelength of maximum absorbance at about 245 nm, with a suitable spectrophotometer, in comparison with a Standard solution of known concentration of USP Chlordiazepoxide Hydrochloride RS. Remove the contents of 12 Capsules as completely as possible with the aid of a current of air. Dissolve the empty capsule shells in 900 mL of *Dissolution Medium*. Filter a portion of the solution, and determine the absorbance at the same dilution and in the same manner as for the Capsules, making any necessary modifications.

**Tolerances**—Not less than 85% (Q) of the labeled amount of  $C_{16}H_{14}ClN_3O \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—[NOTE—Use low-actinic glassware in this procedure.] Transfer the contents of 1 Cap-

sule to a 200-mL volumetric flask, dissolve in and dilute with water to volume, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the filtrate quantitatively and stepwise with 0.1 N hydrochloric acid to obtain a solution having a concentration of about 6 µg of chlordiazepoxide hydrochloride per mL. Dissolve a suitable quantity of USP Chlordiazepoxide Hydrochloride RS, accurately weighed, in 0.1 N hydrochloric acid to obtain a Standard solution having a known concentration of about 6 µg per mL. Concomitantly determine the absorbances of the two solutions in 1-cm cells at the wavelength of maximum absorbance at about 245 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{16}H_{14}ClN_3O \cdot HCl$  in the Capsule by the formula:

$$(T / D)C(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of chlordiazepoxide hydrochloride in the Capsule; *D* is the concentration, in µg per mL, of chlordiazepoxide hydrochloride in the test solution, based on the labeled quantity per Capsule and the extent of dilution; *C* is the concentration, in µg per mL, of USP Chlordiazepoxide Hydrochloride RS in the Standard solution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the contents of the Capsule and the Standard solution, respectively.

**Related compounds**—Proceed as directed for *Related compounds* under *Chlordiazepoxide Hydrochloride*, but use an accurately weighed portion of Capsule contents, equivalent to about 25 mg of chlordiazepoxide hydrochloride, and use 15 µL of a 1 in 1000 solution of USP Chlordiazepoxide Related Compound A RS in acetone and 10 µL of a 1 in 20,000 solution of USP 2-Amino-5-chlorobenzophenone RS in acetone. Not more than 3.0% of chlordiazepoxide related compound A is found, and not more than 0.1% of 2-amino-5-chlorobenzophenone is found.

**Assay**—[NOTE—Use low-actinic glassware in this procedure.] Proceed as directed in the Assay under *Chlordiazepoxide Tablets* except to use an accurately weighed portion of capsule contents equivalent to about 5 mg of chlordiazepoxide hydrochloride and USP Chlordiazepoxide Hydrochloride RS in preparing the *Standard preparation*.

## Chlordiazepoxide Hydrochloride for Injection

» Chlordiazepoxide Hydrochloride for Injection is Chlordiazepoxide Hydrochloride suitable for parenteral use.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), protected from light.

**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68

USP Chlordiazepoxide Hydrochloride RS

USP Chlordiazepoxide Related Compound A RS  
7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O_2$  286.72

USP Endotoxin RS

**Completeness of solution** (641)—It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Labeling* under *Injections* (1).

**Bacterial endotoxins** (85)—It contains not more than 3.57 USP Endotoxin Units per mg of chlordiazepoxide hydrochloride.

**pH** (791): between 2.5 and 3.5, in a solution (1 in 100).

**Other requirements**—It responds to the *Identification* tests and meets the requirements of the tests for *Loss on drying* and *Heavy metals* under *Chlordiazepoxide Hydrochloride*, and the test for *Related compounds* under *Chlordiazepoxide*. It meets also the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and *Labeling* under *Injections* (1).

**Assay**—Proceed with Chlordiazepoxide Hydrochloride for Injection as directed in the Assay under *Chlordiazepoxide*, except to use USP Chlordiazepoxide Hydrochloride RS to prepare the *Standard preparation*.

## Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules

### DEFINITION

Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) and clidinium bromide ( $C_{22}H_{26}BrNO_3$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

[NOTE—Use low-actinic glassware.]

**Buffer:** Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with 1 N sulfuric acid to a pH of  $3.8 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer* (6:24:70)

**Diluent:** Methanol and water (1:1)

**Standard solution:** 0.1 mg/mL of USP Chlordiazepoxide Hydrochloride RS and 0.05 mg/mL of USP Clidinium Bromide RS in *Diluent*

**Sample solution:** Weigh the contents of NLT 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an amount equivalent to about 5 mg of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) to a 50-mL volumetric flask. Add about 25 mL of *Diluent*, sonicate for 5 min, and shake by mechanical means for 10 min. Dilute with *Diluent* to volume, and filter, discarding the first 20 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 212 nm

**Column:** 8-mm × 10-cm; packing L1

**Flow rate:** 3 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

Relative standard deviation: NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlordiazepoxide hydrochloride from the *Sample solution*  
 $r_S$  = peak response of chlordiazepoxide hydrochloride from the *Standard solution*  
 $C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clidinium bromide from the *Sample solution*  
 $r_S$  = peak response of clidinium bromide from the *Standard solution*  
 $C_S$  = concentration of USP Clidinium Bromide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clidinium bromide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION, *Procedure for a Pooled Sample* (711)

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Buffer:** Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with dilute sulfuric acid to a pH of  $3.8 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer* (6:18:75)

**Standard solution:** Prepare a solution having known concentrations of USP Chlordiazepoxide Hydrochloride RS and USP Clidinium Bromide RS in *Medium*.

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Combine equal volumes of the filtered solutions and use the pooled sample for the analysis. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 212 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.6 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

Relative standard deviation: NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the average percentage of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) or clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Sample solution*  
 $r_S$  = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Standard solution*  
 $C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS or USP Clidinium Bromide RS in the *Standard solution* (mg/mL)  
 $L$  = chlordiazepoxide hydrochloride or clidinium bromide label claim (mg)  
 $V$  = volume of *Medium* (mL), 900

**Tolerances:** NLT 75% (Q) each of the labeled amounts of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) and clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • LIMIT OF CHLORDIAZEPOXIDE RELATED COMPOUND A AND 2-AMINO-5-CHLOROBENZOPHENONE

**Standard solution A:** 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

**Standard solution B:** 50  $\mu$ g/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

**Sample solution:** Transfer an amount equivalent to 25 mg of chlordiazepoxide hydrochloride from Capsule contents to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 50  $\mu$ L for the *Sample solution*, 15  $\mu$ L for *Standard solution A*, and 10  $\mu$ L for *Standard solution B*

**Developing solvent system:** Ethyl acetate

**Spray reagent:** 2 N sulfuric acid

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*. Dry at 105° for 15 min, and then spray in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000).

**Acceptance criteria:** Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*, corresponding to NMT 3.0% of chlordiazepoxide related compound A and to NMT 0.1% of 2-amino-5-chlorobenzophenone.

##### • LIMIT OF CLIDINIUM BROMIDE RELATED COMPOUND A

**Extracting solvent mixture:** Dehydrated alcohol and cyclohexane (1:1)

**Identification solution:** Dissolve 50 mg of USP Clidinium Bromide RS in 1 mL of 0.1 N methanolic hydrochloric acid. To this solution add 20  $\mu$ L of a solution



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$  in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlorhexidine from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = concentration of Chlorhexidine Acetate in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.15%

**Organic Impurities****• PROCEDURE**

**Solution A and Solution B:** Proceed as directed in the *Assay*.

**Diluent:** 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
16	45	55
21	45	55
22	100	0
27	100	0

**Sample solution:** 2 mg/mL of Chlorhexidine Acetate in *Solution A*

**Standard solution A:** 0.06 mg/mL of Chlorhexidine Acetate in *Solution A*, from *Sample solution*

**Standard solution B:** 1.2  $\mu\text{g/mL}$  of Chlorhexidine Acetate in *Solution A*, from *Standard solution A*

**System suitability solution:** 10 mg of USP Chlorhexidine Related Compounds RS to a 10-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Diluent* to volume.

**Chromatographic system:** Proceed as directed in the *Assay*. [NOTE—Injection size is 20  $\mu\text{L}$ .]

**System suitability**

**Sample:** *System suitability solution*

[NOTE— The relative retention times for the main related compound peak and chlorhexidine are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** The two peaks between the main related compound peak and the chlorhexidine peak should be at least partially resolved from each other and completely resolved from the chlorhexidine peak.

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of chlorhexidine from *Standard solution A*

$C_S$  = concentration of chlorhexidine acetate in *Standard solution A* ( $\mu\text{g/mL}$ )

$C_U$  = concentration of Chlorhexidine Acetate in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria**

**Total impurities:** NMT 3.0%. [NOTE— Disregard any peak less than the area of the chlorhexidine peak as obtained from *Standard solution B*.]

**SPECIFIC TESTS****• LIMIT OF *p*-CHLOROANILINE**

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0  $\mu\text{g/mL}$  of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** 2.0 mg/mL of Chlorhexidine Acetate in *Solution A*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria**

**Total impurities:** The *p*-chloroaniline peak area of the *Sample solution* is NMT the *p*-chloroaniline peak area in the *Standard solution* (NMT 500 ppm).

- **LOSS ON DRYING** (731): Dry a sample at 105° to constant weight: it loses NMT 3.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Chlorhexidine Acetate RS

USP Chlorhexidine Related Compounds RS

USP *p*-Chloroaniline RS

**Chlorhexidine Acetate Topical Solution****DEFINITION**

Chlorhexidine Acetate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine acetate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Acetate** (191): Meets the requirements of the lanthanum nitrate test  
**Sample:** Evaporate or dilute a volume of Topical Solution containing the equivalent of about 5 mg of chlorhexidine acetate to about 5 mL.

**ASSAY****• PROCEDURE**

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 40 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample solution:** Nominally 40 µg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 20 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$  in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of chlorhexidine from the *Sample solution*

$r_S$  = peak area of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of chlorhexidine acetate in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

## IMPURITIES

### Organic Impurities

#### • PROCEDURE: LIMIT OF *p*-CHLOROANILINE

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** Nominally 2.0 mg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 200 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (NMT 500 ppm, calculated with reference to the nominal content of chlorhexidine acetate).

## SPECIFIC TESTS

### • pH <791>: 5.0–7.0

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers, protected from light.

### • LABELING: Label it to indicate that it is for veterinary use only.

### • USP REFERENCE STANDARDS <11>

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

## Chlorhexidine Gluconate Oral Rinse

» Chlorhexidine Gluconate Oral Rinse is prepared from Chlorhexidine Gluconate Solution. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ).

**Packaging and storage—**Preserve in tight containers, protected from light, at controlled room temperature.

**Labeling—**Oral Rinse intended solely for veterinary use is so labeled. Oral Rinse intended for human use is labeled to indicate it is to be expectorated and not swallowed after rinsing.

### USP Reference standards <11>—

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

USP Potassium Gluconate RS

### Identification—

**A:** The retention time of the major peak for chlorhexidine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** To a volume of Oral Rinse, equivalent to about 10 mg of chlorhexidine gluconate, add 5 mL of a solution of cetyltrimethylammonium bromide (1 in 100), 1 mL of 10 N sodium hydroxide, and 1 mL of bromine TS: a deep red color is produced.

**C:** Undiluted Oral Rinse used as the test solution meets the requirements for *Identification test B* under *Calcium Gluconate*, except that a *Standard solution* containing about 0.6 mg of USP Potassium Gluconate RS per mL is used and 15 µL of the test solution and the *Standard solution* are applied to the thin-layer chromatographic plate.

**pH <791>:** between 5.0 and 7.0.

### Limit of *p*-chloroaniline—

*Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system—*Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*.

*Standard solutions—*Prepare as directed for *Standard solutions* in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution*.

*Test solution—*Transfer 10.0 mL of Oral Rinse to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Procedure—*Proceed as directed in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution*. Calculate the quantity, in µg per mL, of *p*-chloroaniline in the Oral Rinse taken by the formula:

$$2.5C.$$

The limit is 3.0 µg per mL.

### Content of alcohol—

*Internal standard solution—*Dilute 25 mL of *n*-propyl alcohol with water to 500 mL.

*Standard solution—*Transfer about 0.25 g of dehydrated alcohol, accurately weighed, to a 28-mL screw capped vial containing about 3 mL of water. Add 5.0 mL of *Internal standard solution*, and dilute with water to almost fill the

vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

**Test solution**—Transfer about 2.5 g of Oral Rinse, accurately weighed, to a 28-mL screw-capped vial. Add 5.0 mL of *Internal standard solution*, and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm  $\times$  30-m column, the internal wall of which is coated with a 1.5- $\mu$ m film of liquid phase G27. The column is maintained at about 150° between periods of use. The injection port is equipped with a split injection port with a split ratio of 10:1. The injection port and the detector block temperatures are maintained at about 250° and 275°, respectively. At the time of use the initial column temperature is maintained at about 35° until the alcohol peaks elute, then is increased at a rate of 30° per minute to a final temperature of about 225°. The carrier gas is helium. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for alcohol and about 1.5 for *n*-propyl alcohol; the resolution, *R*, between alcohol and *n*-propyl alcohol is not less than 2; the tailing factor for the alcohol peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 0.5  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the Oral Rinse taken by the formula:

$$(W_S / W_U)(R_U / R_S)$$

in which *W<sub>S</sub>* is the weight, in g, of dehydrated alcohol taken to prepare the *Standard solution*; *W<sub>U</sub>* is the weight, in g, of Oral Rinse taken to prepare the *Test solution*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of alcohol to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution*, respectively: between 90.0% and 115.0% of the labeled amount of alcohol (C<sub>2</sub>H<sub>5</sub>OH) is found.

#### Assay—

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay under *Chlorhexidine Gluconate Solution*.

**Assay preparation**—Transfer 5.0 mL of Oral Rinse to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Procedure**—Proceed as directed in the Assay under *Chlorhexidine Gluconate Solution*. Calculate the percentage (w/v) of chlorhexidine gluconate (C<sub>22</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>10</sub> · 2C<sub>6</sub>H<sub>12</sub>O<sub>7</sub>) in the portion of Oral Rinse taken by the formula:

$$(897.76/625.55)(C/500)(r_U / r_S)$$

in which the terms are as defined therein.

### Chlorhexidine Gluconate Solution

C<sub>22</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>10</sub> · 2C<sub>6</sub>H<sub>12</sub>O<sub>7</sub> 897.76  
2,4,11,13-Tetraazatetradecanediimidamide, *N,N'*-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate; 1,1'-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] di-D-gluconate [18472-51-0].

#### DEFINITION

Chlorhexidine Gluconate Solution is an aqueous solution of chlorhexidine gluconate. It contains NLT 19.0% and NMT 21.0% of C<sub>22</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>10</sub> · 2C<sub>6</sub>H<sub>12</sub>O<sub>7</sub> (w/v).

#### IDENTIFICATION

##### A. INFRARED ABSORPTION <197K>

**Standard solution:** 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

**Sample solution:** To 1 mL of Solution add 40 mL of water, and cool in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution produces a red color on thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

##### B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST <201>

**Standard solution:** 20 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Dilute 10 mL of Solution with water to 50 mL. This solution contains 40 mg/mL of chlorhexidine gluconate.

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in color, size, and *R<sub>f</sub>* value to that from the *Standard solution*.

#### ASSAY

##### PROCEDURE

**Diluent:** 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Mix the resulting solution and acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS and 1  $\mu$ g/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample stock solution:** Transfer 5.0 mL of Solution to a 250-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Solution A*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)



**Mode:** LC  
**Detector:** UV 239 nm  
**Column:** 4.6-mm × 25-cm; base-deactivated 5-μm packing L1  
**Column temperature:** 40°  
**Flow rate:** 1.5 mL/min  
**Injection size:** 50 μL

**System suitability**

[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 2.0% from the chlorhexidine peak, and NMT 5.0% from the *p*-chloroaniline peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (0.25 \times C_S) \times (M_{r1}/M_{r2})$$

$r_U$  = peak area response of chlorhexidine from the *Sample solution*

$r_S$  = peak area response of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (μg/mL)

$M_{r1}$  = molecular weight of chlorhexidine gluconate, 897.76

$M_{r2}$  = molecular weight of chlorhexidine acetate, 625.55

**Acceptance criteria:** 19.0%–21.0% (w/v)

**IMPURITIES****Organic Impurities****• PROCEDURE 1**

**Diluent, Solution A, Solution B, and Mobile phase:** Proceed as directed in the *Assay*.

**Sample stock solution:** Transfer 5.0 mL of Solution to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 2 mg/mL of chlorhexidine gluconate.

**Reference solution A:** Transfer 3.0 mL of the *Sample solution* to a 100-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 0.06 mg/mL of chlorhexidine gluconate.

**Reference solution B:** Transfer 2.0 mL of *Reference solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 0.0012 mg/mL of chlorhexidine gluconate.

**System suitability solution:** Transfer 10 mg of USP Chlorhexidine Related Compounds RS to a 10-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Diluent* to volume.

**Chromatographic system:** Proceed as directed in the *Assay*, except the *Injection size* and chromatograph are programmed as shown in the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
16	45	55
21	45	55
22	100	0
27	100	0

**Injection size:** 20 μL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for the main related compound peak and chlorhexidine are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** The two peaks between the main related compound peak and the chlorhexidine peak should be at least partially resolved from each other and completely resolved from the chlorhexidine peak.

**Analysis**

**Samples:** *Sample solution*, *Reference solution A*, and *Reference solution B*

Examine the chromatogram from the *Sample solution*.

**Acceptance criteria:** The sum of the peak areas, other than chlorhexidine and any peak areas less than that obtained for chlorhexidine in the chromatogram from *Reference solution B*, is NMT the peak area for chlorhexidine in the chromatogram from *Reference solution A* (3.0%).

**• PROCEDURE 2: LIMIT OF *p*-CHLOROANILINE**

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0 μg/mL of USP *p*-Chloroaniline RS in *Diluent*

**Sample stock solution:** Transfer 5.0 mL of Solution to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 10.0 mL of *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 0.4 mg/mL of chlorhexidine gluconate.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** NMT 500 μg/mL

The *p*-chloroaniline peak area response of the *Sample solution* is NMT the *p*-chloroaniline peak area response of the *Standard solution*.

**SPECIFIC TESTS**

**• SPECIFIC GRAVITY (841):** 1.06–1.07

**• PH (791):** 5.5–7.0, when diluted 1 in 20 with water

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Chlorhexidine RS  
 USP Chlorhexidine Acetate RS  
 USP Chlorhexidine Related Compounds RS  
 USP *p*-Chloroaniline RS  
 USP Potassium Gluconate RS

## Chlorhexidine Gluconate Topical Solution

**DEFINITION**

Chlorhexidine Gluconate Topical Solution is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ).

**IDENTIFICATION**

**• A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**• B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Nominally 20 mg/mL of chlorhexidine gluconate from the Topical Solution

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in color, size, and  $R_f$  value to that from the *Standard solution*.

## ASSAY

### • PROCEDURE

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS and 1  $\mu$ g/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS in *Solution A*.

**Sample solution:** Nominally about 80  $\mu$ g/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak

## Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of chlorhexidine from the *Sample solution*

$r_S$  = peak area of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of chlorhexidine gluconate in the *Sample solution* ( $\mu$ g/mL)

$M_{r1}$  = molecular weight of chlorhexidine gluconate, 897.76

$M_{r2}$  = molecular weight of chlorhexidine acetate, 625.55

**Acceptance criteria:** 90.0%–110.0%

## IMPURITIES

### Organic Impurities

#### • PROCEDURE: LIMIT OF *p*-CHLOROANILINE

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0  $\mu$ g/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** Nominally 0.4 mg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

#### Acceptance criteria

The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (equivalent to NMT 500 ppm in the portion of Chlorhexidine Gluconate Solution used to prepare the Topical Solution).

## SPECIFIC TESTS

• **pH** <791>: 5.0–7.0

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

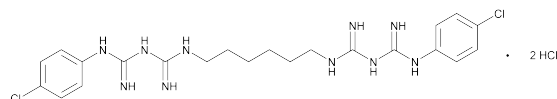
• **USP REFERENCE STANDARDS** <11>

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

USP Potassium Gluconate RS

## Chlorhexidine Hydrochloride



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$

578.37

2,4,11,13-Tetraazatetradecanediimidamide, *N,N'*-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride; 1,1'-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] dihydrochloride [3697-42-5].

## DEFINITION

Chlorhexidine Hydrochloride contains NLT 98.0% and NMT 101.0% of  $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ , calculated on the dried basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)

**Sample:** Dissolve 0.3 g in 10 mL of 6 N hydrochloric acid. Add 40 mL of water, filter if necessary, and cool the solution in ice. Add 10 N sodium hydroxide dropwise with stirring until the solution is alkaline to thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105°.

**Standard:** 5 mg/mL USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

• **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements**ASSAY**• **PROCEDURE**

**Solution A:** 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of acetonitrile and this solution (3:7).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**Standard solution:** 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

**System suitability solution:** 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** 50 µg/mL of Chlorhexidine Hydrochloride in *Solution A*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 2.0% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>22</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>10</sub> · 2HCl in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of chlorhexidine from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (µg/mL)

$M_{r1}$  = molecular weight of chlorhexidine hydrochloride, 578.37

$M_{r2}$  = molecular weight of chlorhexidine acetate, 625.55

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES****Inorganic Impurities**• **RESIDUE ON IGNITION** (281): NMT 0.1%**Organic Impurities**• **PROCEDURE**

**Solution A and Solution B:** Proceed as directed in the *Assay*.

**Diluent:** 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
16	45	55
21	45	55
22	100	0
27	100	0

**Sample solution:** 2 mg/mL of Chlorhexidine Hydrochloride in *Solution A*

**Standard solution A:** 0.06 mg/mL of Chlorhexidine Hydrochloride in *Solution A*, from the *Sample solution*

**Standard solution B:** 1.2 µg/mL of Chlorhexidine Hydrochloride in *Solution A*, from *Standard solution A*

**System suitability solution:** Transfer 10 mg of USP Chlorhexidine Related Compounds RS to a 10-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Diluent* to volume.

**Chromatographic system:** Proceed as directed in the *Assay*. [NOTE—Injection size is 20 µL.]

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for the main related compound peak and chlorhexidine are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** The two peaks between the main related compound peak and the chlorhexidine peak should be at least partially resolved from each other and completely resolved from the chlorhexidine peak.

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of any impurity in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of chlorhexidine from *Standard solution A*

$C_S$  = concentration of Chlorhexidine Hydrochloride in *Standard solution A* (mg/mL)

$C_U$  = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Total impurities:** NMT 3.0%. [NOTE—Disregard any peak less than the area of the chlorhexidine peak as obtained from *Standard solution B*.]

**SPECIFIC TESTS**• **LIMIT OF *p*-CHLOROANILINE**

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** 2.0 mg/mL of Chlorhexidine Hydrochloride in *Solution A*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria**

**Total impurities:** The *p*-chloroaniline peak area in the chromatogram of the *Sample solution* is NMT the *p*-chloroaniline peak area in the *Standard solution* (NMT 500 ppm).

- **LOSS ON DRYING (731):** Dry a sample at 105° to constant weight: it loses NMT 1.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Chlorhexidine RS

USP Chlorhexidine Acetate RS

USP Chlorhexidine Related Compounds RS

USP *p*-Chloroaniline RS

**Chlorophyllin Copper Complex Sodium****DEFINITION**

Chlorophyllin Copper Complex Sodium contains sodium salts of copper-chelated chlorophyll derivatives. It contains no artificial coloring.

**IDENTIFICATION**

- **A. SPECTROPHOTOMETRY AND LIGHT-SCATTERING (851)** (in the visible region)

**Sample solution:** 10 µg/mL

**Medium:** pH 7.5 phosphate buffer, prepared by mixing 0.15 M dibasic sodium phosphate and 0.15 M monobasic potassium phosphate (21:4)

**Acceptance criteria:** The ratio of  $A_{405}/A_{630}$  is 3.0–3.9.

**OTHER COMPONENTS**• **CONTENT OF TOTAL COPPER**

**Stock solution 1:** 1000 µg/mL of copper. Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. [NOTE—Store in a polyethylene bottle.]

**Stock solution 2:** 10 µg/mL of copper. Transfer 5.0 mL of *Stock solution 1* into a 500-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** Transfer 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Stock solution 2* to separate 100-mL volumetric flasks, and dilute the contents of each flask with water to volume. These *Standard solutions* contain 0.5, 1.0, 1.5, and 2.0 µg/mL of copper, respectively.

**Sample solution:** Transfer 100 mg of previously dried Chlorophyllin Copper Complex Sodium to a Kjeldahl flask. Add 2.0 mL of sulfuric acid, 1.0 mL of nitric acid, and 1.0 mL of hydrogen peroxide, and carefully heat under a fume hood until a light green color is obtained. [NOTE—If the solution has any hint of a brown tint, continue to add 0.5-mL portions of nitric acid until a green color is obtained.] Cool, transfer the contents quantitatively to a 1000-mL volumetric flask with several portions of water, dilute the contents of the flask with water to volume, and mix. Transfer 10.0 mL of this

solution to a 50-mL volumetric flask, and dilute with water to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Copper hollow-cathode

**Flame:** Air–acetylene

**Analytical wavelength:** Copper emission line of 324.8 nm

**Blank:** Water

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of copper, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

*C* = concentration of the *Sample solution* determined from the graph (µg/mL)

*W* = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)

*V* = final volume of *Sample solution*, 5000 mL

*F* = conversion factor, 1000 µg/mg

**Acceptance criteria:** NLT 4.25% on the dried basis

• **CONTENT OF CHELATED COPPER**

**Analysis:** Calculate the percentage of chelated copper in the portion of Chlorophyllin Copper Complex Sodium taken by subtracting the percentage of ionic copper found in the test for *Limit of Ionic Copper* from the percentage of total copper found in the test for *Content of Total Copper*.

**Acceptance criteria:** NLT 4.0% on the dried basis

• **CONTENT OF SODIUM**

**Standard stock solution:** 100 µg/mL of sodium. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer to a 1000-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent solution (1 in 500). To each flask add, respectively, 2.5, 5.0, 10.0, and 15.0 mL of the *Standard stock solution*, and dilute with water to volume. These *Standard solutions* contain 2.5, 5.0, 10.0, and 15.0 µg/mL of sodium, respectively.

**Blank:** Transfer 10 mL of a nonionic wetting agent solution (1 in 500) into a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 1000-mL volumetric flask. Add 100 mL of a solution of nonionic wetting agent (1 in 500) and 400 mL of water, and shake by mechanical means for 5 min. Dilute with water to volume.

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*

Set the flame photometer for maximum transmission at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage of transmittance of the other *Standard solutions*, and plot the percentage of transmittance versus the concentration, in µg/mL, of sodium. Read the percentage of transmittance of the *Sample solution*, and from the graph read the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

- $C$  = concentration of the *Sample solution* determined from the graph ( $\mu\text{g/mL}$ )  
 $W$  = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)  
 $V$  = volume of *Sample solution*, 1000 mL  
 $F$  = conversion factor, 1000  $\mu\text{g/mg}$

Acceptance criteria: 5%–7% on the dried basis

- **NITROGEN DETERMINATION**, *Method I* (461): NLT 4.0%

## IMPURITIES

### • LIMIT OF IONIC COPPER

**Standard solutions:** Prepare as directed in the test for *Content of Total Copper*.

**Sample solution:** Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 150-mL conical flask. Add 75 mL of water, and shake by mechanical means for 3 min. Adjust with 1 N hydrochloric acid to a pH of 3.0, transfer the suspension thus obtained to a 100-mL volumetric flask, and dilute with water to volume. Filter this suspension, discarding the first 10 mL of the filtrate. Use the clear filtrate for analysis.

### Analysis

**Samples:** *Standard solutions* and *Sample solution*  
 Proceed as directed in the test for *Content of Total Copper*.

Calculate the percentage of ionic copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

- $C$  = concentration of the *Sample solution* determined from the graph ( $\mu\text{g/mL}$ )  
 $W$  = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (in mg on the dried basis)  
 $V$  = volume of *Sample solution*, 100 mL  
 $F$  = conversion factor, 1000  $\mu\text{g/mg}$

Acceptance criteria: NMT 0.25% on the dried basis

- **RESIDUE ON IGNITION** (281): NMT 30% on the dried basis
- **ARSENIC**, *Method II* (211): NMT 3 ppm
- **LEAD** (251): NMT 10 ppm
- **IRON** (241): NMT 0.50%

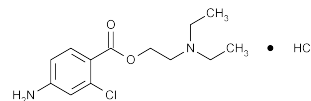
## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Escherichia coli* and *Salmonella* species.
- **pH** (791): 9.5–10.7, in a solution (1 in 100)
- **LOSS ON DRYING** (731): Dry a sample at 150° for 2 h: it loses NMT 5% of its weight.
- **TEST FOR FLUORESCENCE**  
**Sample solution:** 10 mg/mL  
**Analysis:** Apply 10  $\mu\text{L}$  of *Sample solution* on filter paper, allow to dry, and examine the area of application under long-wavelength UV light through a red optical filter.  
**Acceptance criteria:** No fluorescence is visible.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

## Chloroprocaine Hydrochloride



$\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_2 \cdot \text{HCl}$  307.22

Benzoic acid, 4-amino-2-chloro-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl 4-amino-2-chlorobenzoate monohydrochloride [3858-89-7].

» Chloroprocaine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_2 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
 USP Chloroprocaine Hydrochloride RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10  $\mu\text{g}$  per mL.

*Medium:* pH 4.5 buffer solution [prepared by dissolving 13.61 g of monobasic potassium phosphate in 750 mL of water, adjusting to a pH of  $4.5 \pm 0.1$  with potassium hydroxide solution (1 in 180) and diluting with water to 1000 mL]. Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** It meets the requirements of the tests for *Chloride* (191).

**Melting range**, *Class I* (741): between 173° and 176°.

**Acidity**—Dissolve 1.0 g in 25 mL of water, add 2 drops of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 1.8 mL is required to produce a yellow color.

**Loss on drying** (731)—Dry about 500 mg, accurately weighed, at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

### Related compounds—

**4-Amino-2-chlorobenzoic acid**—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ( $\text{C}_7\text{H}_6\text{ClNO}_2$ ) in the Chloroprocaine Hydrochloride taken by the formula:

$$10,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation*;  $W$  is the quantity, in mg, of Chloroprocaine Hydrochloride taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.625% is found.

### Assay—

**Mobile phase**—Dissolve 800 mg of sodium 1-heptanesulfonate in 740 mL of water, add 200 mL of acetonitrile, 50 mL of methanol, and 10 mL of glacial acetic acid, and mix. Filter and degas this solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard 4-amino-2-chlorobenzoic acid solution**—Dissolve an accurately weighed quantity of recrystallized 4-amino-2-chlorobenzoic acid in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

**Standard preparation**—Transfer about 50 mg of USP Chloroprocaine Hydrochloride RS, accurately weighed, to a

50-mL volumetric flask containing 5.0 mL of *Standard 4-amino-2-chlorobenzoic acid solution*, add 15 mL of methanol, swirl to dissolve, dilute with water to volume, and mix. This solution contains about 1 mg of USP Chlorprocaine Hydrochloride RS and 0.02 mg of 4-amino-2-chlorobenzoic acid per mL.

**System suitability solution**—Mix equal volumes of *Standard 4-amino-2-chlorobenzoic acid solution* and *Standard preparation*.

**Assay preparation**—Transfer about 100 mg of Chlorprocaine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in 40 mL of methanol, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.35 for 4-amino-2-chlorobenzoic acid and 1.0 for chlorprocaine; and the resolution,  $R_s$ , between 4-amino-2-chlorobenzoic acid and chlorprocaine is not less than 5.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation determined from chlorprocaine obtained from replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for 4-amino-2-chlorobenzoic acid and chlorprocaine. Calculate the quantity, in mg, of  $C_{13}H_{19}ClN_2O_2 \cdot HCl$  in the portion of Chlorprocaine Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorprocaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the chlorprocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorprocaine Hydrochloride Injection

» Chlorprocaine Hydrochloride Injection is a sterile solution of Chlorprocaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of chlorprocaine hydrochloride ( $C_{13}H_{19}ClN_2O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** <11>—  
USP Chlorprocaine Hydrochloride RS

**Identification**—Dissolve 60 mg of USP Chlorprocaine Hydrochloride RS in 10 mL of water in a 60-mL separator, and in a second 60-mL separator mix a volume of Injection, equivalent to 60 mg of chlorprocaine hydrochloride, with sufficient water to obtain 10 mL of solution. Add 5 mL of dilute ammonium hydroxide (4 in 10) to each, mix, and immediately extract each with four 10-mL portions of chloroform, passing the extracts from the Reference Standard and the test specimen through cotton filters into separate 50-mL volumetric flasks. Dilute each with chloroform to volume, and mix. Add a mixture of chloroform and methanol (4:1) to a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* <621>),

cover the chamber, and allow the system to equilibrate for 15 minutes. Apply separately 10-µL portions of the chloroform solutions obtained from the Reference Standard and the test specimen to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution ( $R_f$  about 0.40). After viewing, spray the chromatogram with iodoplatinate TS: violet-blue colored spots, characteristic of tertiary nitrogen compounds, are visible.

**pH** <791>: between 2.7 and 4.0.

### Related compounds—

**4-Amino-2-chlorobenzoic acid**—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ( $C_7H_6ClNO_2$ ) in the chlorprocaine hydrochloride contained in the Injection taken by the formula:

$$10,000(C / W_o)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation*;  $W_o$  is the quantity, in mg, of chlorprocaine hydrochloride in the portion of Injection taken to prepare the *Assay preparation*, determined as directed in the *Assay preparation*; and  $r_U$  and  $r_S$  are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay—

**Mobile phase**, *Standard 4-amino-2-chlorobenzoic acid solution*, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay under Chlorprocaine Hydrochloride*.

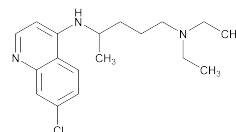
**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chlorprocaine hydrochloride, to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix.

**Procedure**—Proceed as directed in the *Assay under Chlorprocaine Hydrochloride*. Calculate the quantity, in mg, of chlorprocaine hydrochloride ( $C_{13}H_{19}ClN_2O_2 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Chloroquine



$C_{18}H_{26}ClN_3$  319.87  
1,4-Pentanediamine,  $N^4$ -(7-chloro-4-quinolinyl)- $N^1,N^1$ -diethyl-;  
7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline [54-05-7].

**DEFINITION**

Chloroquine contains NLT 98.0% and NMT 102.0% of chloroquine ( $C_{18}H_{26}ClN_3$ ), calculated on the dried basis.

**IDENTIFICATION**

- A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)**

**Standard solution:** Proceed as directed in the chapter except use chloroform instead of carbon disulfide in the test.

**Sample solution:** 8.75 mg/mL of chloroquine prepared as follows. Dissolve 35 mg of Chloroquine in 4 mL of chloroform. Pass through a dry filter.

**Analysis:** Determine the absorption of the *Standard solution* and *Sample solution* without delay in 1-mm cells between 7 and 15  $\mu$ m, using chloroform in a matched cell as a blank.

**Acceptance criteria:** The IR absorption spectrum of the *Sample solution* so obtained exhibits maxima only at the same wavelengths as that of the *Standard solution*.

- B. ULTRAVIOLET ABSORPTION (197U)**

**Medium:** Dilute hydrochloric acid (1 in 1000)

**Sample solution:** 10  $\mu$ g/mL

**Acceptance criteria:** The ratio  $A_{343}/A_{329}$  is 1.00–1.15.

**ASSAY**

- PROCEDURE**

**Sample solution:** Dissolve 250 mg of Chloroquine in 50 mL of glacial acetic acid. Add crystal violet TS.

**Analysis:** Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.99 mg of chloroquine ( $C_{18}H_{26}ClN_3$ ).

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- RESIDUE ON IGNITION (281):** NMT 0.2%

**SPECIFIC TESTS**

- MELTING RANGE OR TEMPERATURE (741):** 87°–92°

- LOSS ON DRYING (731)**

**Analysis:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** NMT 2.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

- USP REFERENCE STANDARDS (11)**

USP Chloroquine Phosphate RS

## Chloroquine Hydrochloride Injection

$C_{18}H_{26}ClN_3 \cdot 2HCl$  392.79

1,4-Pentanediamine, *N*-(7-chloro-4-quinolinyl)-*N*′, *N*′-diethyl-, dihydrochloride;

7-(Chloro-4-[[4-diethylamino]-1-methylbutyl]amino]quinoline dihydrochloride [3545-67-3].

**DEFINITION**

Chloroquine Hydrochloride Injection is a sterile solution of Chloroquine in Water for Injection prepared with the aid of Hydrochloric Acid. It contains NLT 47.5 mg and NMT 52.5 mg of chloroquine hydrochloride ( $C_{18}H_{26}ClN_3 \cdot 2HCl$ ) in each mL.

**IDENTIFICATION**

- A. ULTRAVIOLET ABSORPTION**

**Sample solution:** Prepare as directed in the Assay.

**Standard solution:** 7.5  $\mu$ g/mL of USP Chloroquine Phosphate RS prepared similarly to the *Sample solution*

**Instrumental conditions**

**Mode:** UV

**Wavelength range:** 329–343 nm

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

$A_{343}$  = absorbance of the *Sample solution* at 343 nm

$A_{329}$  = absorbance of the *Sample solution* at 329 nm

**Acceptance criteria:** The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.

- B.**

**Sample solution:** 20 mL of a 1-mg/mL solution of chloroquine hydrochloride from Injection, diluted with water

**Analysis 1:** To the *Sample solution* add 5 mL of trinitrophenol TS.

**Acceptance criteria 1:** A yellow precipitate is formed.

**Analysis 2:** Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel.

[CAUTION—Picrates may explode.]

**Acceptance criteria 2:** The precipitate melts at 205°–210°.

- C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

**ASSAY**

- PROCEDURE**

**Diluent 1:** Dilute hydrochloric acid (1 in 100)

**Diluent 2:** Dilute hydrochloric acid (1 in 1000)

**Standard solution:** 10  $\mu$ g/mL of USP Chloroquine Phosphate RS in *Diluent 2*

**Sample stock solution:** 150  $\mu$ g/mL of chloroquine hydrochloride prepared as follows. Transfer a volume of Injection, nominally 150 mg of chloroquine hydrochloride, to a 1-L volumetric flask, and dilute with water to volume.

**Sample solution:** 7.5  $\mu$ g/mL of chloroquine hydrochloride prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100.0-mL volumetric flask, add 10.0 mL of *Diluent 1*, and dilute with water to volume.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maxima at about 343 nm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration, in mg/mL, of chloroquine hydrochloride ( $C_{18}H_{26}ClN_3 \cdot 2HCl$ ) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (V/V_i) \times (M_{r1}/M_{r2})$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)

$D$  = dilution factor for the preparation of the *Sample solution*, 20

$V$  = volume of the *Sample stock solution*, 1000 mL

$V_i$  = volume of Injection taken for the *Sample stock solution* (mL)

$M_{r1}$  = molecular weight of chloroquine hydrochloride, 392.79

$M_{r2}$  = molecular weight of chloroquine phosphate, 515.86

**Acceptance criteria:** 47.5–52.5 mg/mL

**SPECIFIC TESTS**

- pH (791):** 5.5–6.5

- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.7 USP Endotoxin Unit/mg of chloroquine hydrochloride.

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections* <1>.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** <11>  
USP Chloroquine Phosphate RS  
USP Endotoxin RS

### Chloroquine Phosphate

$C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$  515.86

1,4-Pentanediamine, *N*-(7-chloro-4-quinolinyl)-*N*′, *N*′-diethyl-, phosphate (1:2).

7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline phosphate (1:2) [50-63-5].

» Chloroquine Phosphate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Chloroquine Phosphate RS

USP Hydroxychloroquine Sulfate RS

#### Identification—

**A:** It meets the requirements under *Identification—Organic Nitrogenous Bases* <181>, chloroform being substituted for carbon disulfide in the test.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

*Medium:* dilute hydrochloric acid (1 in 1000).

*Ratio:*  $A_{343}/A_{329}$ , between 1.00 and 1.15.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** <731>—Dry it at 105° for 16 hours: it loses not more than 2.0% of its weight.

#### Assay—

**Buffer solution**—Accurately weigh about 13.6 g of monobasic potassium phosphate, and dissolve in 2 L of water. Add 2.0 mL perchloric acid, mix, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.5$ . Pass the solution through a membrane filter having a 0.45-µm porosity.

**Mobile phase**—Prepare a mixture of *Buffer solution* and methanol (78:22). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer an accurately weighed quantity of USP Chloroquine Phosphate RS to a suitable volumetric flask, and dissolve in and dilute with water to volume to obtain a solution having a concentration of about 0.15 mg per mL of chloroquine phosphate.

**Assay preparation**—Transfer an accurately weighed quantity of Chloroquine Phosphate to a suitable volumetric flask, and dissolve in and dilute with water to volume to obtain a solution having a concentration of about 0.15 mg per mL of chloroquine phosphate.

**System suitability solution**—Transfer accurately weighed quantities of USP Hydroxychloroquine Sulfate RS and USP Chloroquine Phosphate RS to a suitable volumetric flask, and dissolve in and dilute with water to volume to obtain a solution having concentrations of about 0.015 mg per mL of hydroxychloroquine sulfate and 0.15 mg per mL of chloroquine phosphate.

**Chromatography system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 224-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at  $25 \pm 5^\circ$ . Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for chloroquine phosphate and 0.8 for hydroxychloroquine sulfate; the resolution, *R*, between chloroquine phosphate and hydroxychloroquine sulfate is not less than 1.5; the column efficiency is not less than 2000 theoretical plates; for both compounds, the tailing factor is not more than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$  in the portion of Chloroquine Phosphate taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which 100 is the percent conversion factor;  $C_S$  is the concentration, in mg per mL, of USP Chloroquine Phosphate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Chloroquine Phosphate in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Add the following:

### ▲Chloroquine Phosphate Oral Suspension

#### DEFINITION

Chloroquine Phosphate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ).

Prepare Chloroquine Phosphate Oral Suspension 15 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Chloroquine Phosphate tablets <sup>a</sup>	1.5 g
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Aralen 500-mg tablets, Sanofi-Winthrop, NY.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a chloroquine phosphate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

#### ASSAY

##### • PROCEDURE

**Buffer solution:** 20 mM 1-heptanesulfonic acid adjusted to a pH of 3.4

**Mobile phase:** Acetonitrile and *Buffer solution* (34:66). Filter and degas.

**Standard solution:** 150 µg/mL of USP Chloroquine Phosphate RS in *Mobile phase*

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of the Oral Suspension



into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 340 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for chloroquine phosphate is about 9.4 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 2% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of chloroquine phosphate in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of chloroquine phosphate in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **PH** <791>: 4.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled cold temperature or controlled room temperature
- **USP REFERENCE STANDARDS** <11>  
USP Chloroquine Phosphate RS ▲<sub>USP36</sub>

## Chloroquine Phosphate Tablets

#### DEFINITION

Chloroquine Phosphate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ).

#### IDENTIFICATION

##### • A. ULTRAVIOLET ABSORPTION

**Standard solution:** 7.5 μg/mL of USP Chloroquine Phosphate RS in water

**Sample solution:** Nominally 7.5 μg/mL of chloroquine phosphate from a filtered solution of finely powdered Tablets in water

#### Instrumental conditions

**Mode:** UV

**Wavelength range:** 329–343 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

$A_{343}$  = absorbance of the *Sample solution* at 343 nm

$A_{329}$  = absorbance of the *Sample solution* at 329 nm

**Acceptance criteria:** The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.

##### • B.

**Sample solution:** 20 mL of a filtered 1-mg/mL chloroquine phosphate solution from finely powdered Tablets in water

**Analysis 1:** To the *Sample solution* add 5 mL of trinitrophenol TS.

**Acceptance criteria 1:** A yellow precipitate is formed.

**Analysis 2:** Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel.

[CAUTION—Picrates may explode.]

**Acceptance criteria 2:** The precipitate melts at 205°–210°.

##### • C.

The retention time of the chloroquine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45-μm pore size.

**Mobile phase:** Methanol and *Buffer* (22:78)

**System suitability solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Standard solution:** 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Sample solution:** Nominally 0.15 mg/mL of chloroquine phosphate in water prepared as follows. Transfer nominally 7.5 mg of chloroquine phosphate from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with water to volume. Sonicate for 20 min. Pass 10 mL through a nylon filter of 0.2-μm pore size, discarding the first 4 mL, and use 2 mL for the *Analysis*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for the amodiaquine and chloroquine peaks

**Relative standard deviation:** NMT 2.0% for the amodiaquine and chloroquine peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chloroquine phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

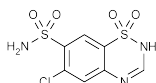
## PERFORMANCE TESTS

- **DISSOLUTION** (711)  
 Medium: Water; 900 mL  
 Apparatus 2: 100 rpm  
 Time: 45 min  
 Detector: UV  
 Analytical wavelength: 343 nm  
 Standard solution: USP Chloroquine Phosphate RS in Medium  
 Sample solution: Dilute with Medium to a concentration that is similar to the Standard solution.  
 Tolerances: NLT 75% (Q) of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Amodiaquine Hydrochloride RS  
 USP Chloroquine Phosphate RS

## Chlorothiazide



$C_7H_6ClN_3O_4S_2$  295.72  
 2 *H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.  
 6-Chloro-2 *H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [58-94-6].

» Chlorothiazide contains not less than 98.0 percent and not more than 102.0 percent of  $C_7H_6ClN_3O_4S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Benzothiadiazine Related Compound A RS  
 4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $C_6H_8ClN_3O_4S_2$  285.73  
 USP Chlorothiazide RS

### Identification—

**A:** *Infrared Absorption* (197M): previously dried at 105° for 1 hour.

**B:** *Ultraviolet Absorption* (197U)—

Solution: 10 µg per mL.

Medium: sodium hydroxide solution (1 in 250).

Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Loss on drying** (731)—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride**—Dissolve 1.00 g in a mixture of 10 mL of water and 10 mL of sodium hydroxide solution (1 in 10). Cool in an ice bath, and add 20 mL of water and 5 mL of nitric acid. A flocculent, white precipitate is formed. Titrate potentiometrically with 0.050 N silver nitrate, using a silver–silver chloride electrode system: not more than 0.28 mL is required (0.05%).

**Selenium** (291): 0.003%.

**Heavy metals**, Method II (231): 0.001%.

### Related compounds—

*Mobile phase*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 1.5 µg per mL.

*Test solution*—Proceed as directed for Assay preparation in the Assay.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0 for chlorothiazide. Calculate the quantity, in mg, of benzothiadiazine related compound A in the portion of Chlorothiazide taken by the formula:

$$0.2C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of benzothiadiazine related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is present.

### Assay—

*Mobile phase*—Prepare a suitable degassed mixture of 0.1 M monobasic sodium phosphate and acetonitrile (9:1), adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Resolution solution*—Dissolve quantities of USP Reference Standards in *Mobile phase* to obtain solutions having known concentrations of about 0.15 mg per mL of USP Chlorothiazide RS and about 1.5 µg per mL of USP Benzothiadiazine Related Compound A RS.

*Standard preparation*—[NOTE—A volume of acetonitrile not exceeding 10% of the total volume of solution may be used to dissolve the reference standard.] Dissolve an accurately weighed quantity of USP Chlorothiazide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL.

*Assay preparation*—Transfer about 30 mg of Chlorothiazide, accurately weighed, to a 200-mL volumetric flask, dissolve in a small volume of acetonitrile, not exceeding 10% of the total volume of the solution, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.5%. Chromatograph the *Resolution solution*: the resolution, *R*, between benzothiadiazine related compound A and chlorothiazide is not less than 3.5.

*Procedure*—[NOTE—The *Standard preparation* and the *Assay preparation* should be injected immediately upon preparation.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major chlorothiazide peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0 for chlorothiazide. Calculate the

quantity, in mg, of  $C_7H_6ClN_3O_4S_2$  in the portion of Chlorothiazide taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorothiazide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of chlorothiazide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorothiazide Oral Suspension

» Chlorothiazide Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorothiazide RS

**Identification**—The UV absorption spectrum of the solution of chlorothiazide prepared from Oral Suspension as directed in the *Assay* exhibits maxima and minima at the same wavelengths as that of a solution of USP Chlorothiazide RS, prepared as directed in the *Assay*, concomitantly measured.

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.2 and 4.0.

**Assay**—Transfer to a 250-mL volumetric flask an accurately measured volume of Oral Suspension, equivalent to about 250 mg of chlorothiazide, dilute with sodium hydroxide solution (1 in 250) to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 100) to volume, and mix. Transfer 50.0 mL of the resulting solution to a 125-mL separator, and wash with two 25-mL portions of chloroform, discarding the washings. Transfer 10.0 mL of the washed solution to a 100-mL volumetric flask, dilute with sodium hydroxide solution (1 in 250) to volume, and mix. Dissolve an accurately weighed quantity of USP Chlorothiazide RS in sodium hydroxide solution (1 in 250) to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using sodium hydroxide solution (1 in 250) as the blank. Calculate the quantity, in mg, of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ) in each mL of the Oral Suspension taken by the formula:

$$25(C/V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorothiazide RS in the Standard solution; V is the volume, in mL, of Oral Suspension taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Oral Suspension and the Standard solution, respectively.

## Chlorothiazide Tablets

» Chlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_7H_6ClN_3O_4S_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Chlorothiazide RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a peak for chlorothiazide, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** Powder 1 Tablet, and fuse it with a pellet of sodium hydroxide: the ammonia fumes produced turn moistened red litmus paper blue, and the residue responds to the test for Sulfite (191).

**Dissolution** (711)—

**Medium:** 0.05 M pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_7H_6ClN_3O_4S_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 294 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chlorothiazide RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_6ClN_3O_4S_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed solution of 0.08 M monobasic sodium phosphate (adjusted with phosphoric acid to a pH of  $2.9 \pm 0.1$ ) and methanol (95:5), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer an accurately weighed quantity of about 25 mg of USP Chlorothiazide RS to a 50-mL volumetric flask, add 5 mL of 0.05 M monobasic sodium phosphate solution, followed by 10 mL of acetonitrile to the flask, and sonicate with occasional shaking for about 3 minutes. Dilute with water to volume, mix, and filter to obtain a *Standard preparation* having a known concentration of about 0.5 mg of USP Chlorothiazide RS per mL.

**Assay preparation**—[NOTE—Prepare fresh daily.] Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 250 mg of chlorothiazide, and transfer to a 500-mL volumetric flask. Add 50 mL of 0.05 M monobasic sodium phosphate solution, and shake by mechanical means for about 15 minutes followed by sonication for about 2 minutes. Add 100 mL of acetonitrile, sonicate for about 3 minutes, dilute with water to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1 and is fitted with a guard column. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the capacity factor ( $k'$ ) is not less than 4.3; the tailing factor (T) for chlorothiazide is not more than 2.0; the theoretical plate count (N) for chlorothiazide is not less than 1300; and

the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_7H_6ClN_3O_4S_2$  in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorothiazide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Chlorothiazide Sodium for Injection

$C_7H_5ClN_3NaO_4S_2$  317.71

2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide, monosodium salt.

6-Chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide monosodium salt [7085-44-1].

» Chlorothiazide Sodium for Injection is a sterile, freeze-dried mixture of Chlorothiazide Sodium (prepared by the neutralization of Chlorothiazide with the aid of Sodium Hydroxide) and Mannitol. It contains chlorothiazide sodium ( $C_7H_5ClN_3NaO_4S_2$ ) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Chlorothiazide RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification, Ultraviolet Absorption** (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* sodium hydroxide solution (1 in 250).

**Bacterial endotoxins** (85)—It contains not more than 0.3 USP Endotoxin Unit per mg of chlorothiazide sodium.

**Uniformity of dosage units** (905): meets the requirements.

**pH** (791): between 9.2 and 10.0, in a solution prepared as directed in the labeling.

**Other requirements**—It meets the requirements under *Injections* (1).

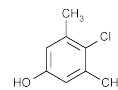
**Assay**—Transfer an accurately weighed portion of Chlorothiazide Sodium for Injection, equivalent to about 500 mg of chlorothiazide, to a 1000-mL volumetric flask, add sodium hydroxide solution (1 in 250) to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with sodium hydroxide solution (1 in 250) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorothiazide RS in the same medium having a known concentration of about 10  $\mu$ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using sodium hydroxide solution (1 in 250) as the blank. Calculate the quantity, in mg, of

$C_7H_6ClN_3O_4S_2$  in the portion of Chlorothiazide Sodium for Injection taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Chlorothiazide RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

### Chloroxylenol



$C_8H_9ClO$  156.61

Phenol, 4-chloro-3,5-dimethyl-

4-Chloro-3,5-xyleneol [88-04-0].

» Chloroxylenol contains not less than 98.5 percent of  $C_8H_9ClO$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Chloroxylenol RS

USP Chloroxylenol Related Compound A RS  
2-chloro-3,5-dimethylphenol.

**Identification, Infrared Absorption** (197K).

**Melting range** (741): between 114° and 116°.

**Water, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Iron** (241)—Transfer 0.10 g to a suitable crucible, add 5 drops of sulfuric acid, and ignite at a low heat until thoroughly ashed. Add to the carbonized mass 10 drops of sulfuric acid, and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Dilute with water to about 25 mL. Filter, if necessary, rinse the crucible and the filter with 10 mL of water, combining the filtrate and rinsing in a 50-mL color-comparison tube, add 2 mL of hydrochloric acid, dilute with water to 47 mL, and mix. The limit is 0.01%.

**Related compounds**—

**Standard solution**—Quantitatively dissolve accurately weighed quantities of 3,5-dimethylphenol and USP Chloroxylenol Related Compound A RS in chloroform to obtain a solution containing about 0.08 mg of each per mL.

**Test solution**—Quantitatively dissolve an accurately weighed quantity of Chloroxylenol in chloroform to obtain a solution containing about 40.0 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.8-m column packed with 3% phase G16 on support S1A. The carrier gas is dry nitrogen, flowing at a rate of about 30 mL per minute. The column temperature is maintained at 180°, and the injection port and detector block temperatures are maintained at 200°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between 3,5-dimethylphenol and chloroxylenol related compound A is not less than 4.5; and the relative standard deviation for replicate injections is not more than 10%.

**Procedure**—Separately inject equal volumes (about 1  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentages of 3,5-dimethylphenol ( $C_8H_{10}O$ ) and chloroxylenol related compound A ( $C_8H_9ClO$ ) in the portion of Chloroxylenol taken by the formula:

$$0.2(r_U / r_S)$$

in which  $r_U$  and  $r_S$  are the peak areas of the appropriate analyte obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.2% of 3,5-dimethylphenol or chloroxylenol related compound A is found. Calculate the percentage of each impurity in the portion of Chloroxylenol taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the area of each peak obtained from the *Test solution*, excluding that of the main chloroxylenol peak, the 3,5-dimethylphenol peak, and the chloroxylenol related compound A peak; and  $r_S$  is the sum of the areas of all the peaks: not more than 0.5% of any individual impurity is found. Calculate the percentage of total impurities in the portion of Chloroxylenol taken by the formula:

$$100(r_T / r_S)$$

in which  $r_T$  is the sum of the areas of all the peaks obtained from the *Test solution*, excluding that of the main chloroxylenol peak; and  $r_S$  is the sum of the areas of all the peaks obtained from the *Test solution*: not more than 1.5% of total impurities is found.

#### Assay—

**Internal standard solution**—Prepare a solution of *p*-chlorophenol in chloroform containing about 0.8 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chloroxylenol RS in *Internal standard solution* to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer about 100 mg of Chloroxylenol, accurately weighed, to a 100-mL volumetric flask, dissolve in *Internal standard solution*, dilute with *Internal standard solution* to volume, and mix.

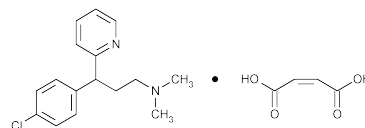
**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.8-m column packed with 3% phase G16 on support S1A. The injection port, column, and detector block are maintained at 210°. Dry nitrogen, flowing at a rate of about 30 mL per minute, is used as the carrier gas. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the *p*-chlorophenol peak and the chloroxylenol peak is not less than 2.0; the tailing factor for the chloroxylenol peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the gas chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of chloroxylenol ( $C_8H_9ClO$ ) in the portion of Chloroxylenol taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chloroxylenol RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the response of the chloroxylenol peak to that of the *p*-chlorophenol peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  390.86

2-Pyridinepropanamine,  $\gamma$ -(4-chlorophenyl)-*N,N*-dimethyl-, (*Z*)-2-butenedioate (1:1).

2-[*p*-Chloro- $\alpha$ -[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1) [113-92-8].

» Chlorpheniramine Maleate contains not less than 98.0 percent and not more than 100.5 percent of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Chlorpheniramine Maleate RS

**Identification**, *Infrared Absorption* <197K>.

**Melting range** <741>: between 130° and 135°.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.2%.

#### Related compounds—

**Test preparation**—Dissolve about 200 mg of Chlorpheniramine Maleate in 5 mL of methylene chloride, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 1.2-m  $\times$  4-mm glass column containing 3% phase G3 on support S1AB. The column temperature is maintained at about 190°, and the injection port and detector temperatures are both maintained at about 250°. The carrier gas is dry helium, flowing at a rate adjusted to obtain a retention time of 4 to 5 minutes for the main peak. Chromatograph the *Test preparation*, record the chromatogram, and determine the peak area as directed for *Procedure*: the tailing factor for the chlorpheniramine maleate peak is not more than 1.8.

**Procedure**—Inject a volume (about 1  $\mu$ L) of the *Test preparation* into the chromatograph. Record the chromatogram for a total time of not less than twice the retention time of the chlorpheniramine peak, and measure the areas of the peaks. The total relative area of all extraneous peaks (except that of the solvent peak and maleic acid, if observed) does not exceed 2.0%.

**Assay**—Dissolve about 500 mg of Chlorpheniramine Maleate, accurately weighed, in 20 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.54 mg of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

## Chlorpheniramine Maleate Extended-Release Capsules

» Chlorpheniramine Maleate Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled

amount of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Capsules to indicate the *Dissolution Test* with which the product complies.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

**Identification**—

**A:** The retention time of the chlorpheniramine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer the contents of 1 Capsule to a 10-mL volumetric flask, add 5 mL of methanol, and insert the stopper into the flask. Sonicate this solution for 10 minutes, dilute with water to volume, mix, and filter. Apply separately 10  $\mu$ L of this solution and 10  $\mu$ L of a solution of USP Chlorpheniramine Maleate RS in a mixture of methanol and water (1:1) containing about 1.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (100:5:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot observed in the chromatogram of the solution under test corresponds to that obtained from the Standard solution.

**Dissolution** (711)—

TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

*Medium:* water; 500 mL.

*Apparatus 1:* 100 rpm.

*Times:* 1.5, 6.0, and 10.0 hours.

*Procedure*—Determine the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved by employing the method set forth in the *Assay*, using a filtered portion of the solution under test in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same medium.

*Tolerances*—The percentages of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.5	between 15% and 40%
6.0	between 50% and 80%
10.0	not less than 70%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed for *Procedure for Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

*Medium*—Prepare as directed under *Method B*, except use 900 mL of media. Operate the apparatus for 1 hour in the *Acid Stage* and use the acceptance criteria given under *Tolerances*. Operate the apparatus for 6 hours in the *Buffer Stage*, except to use 900 mL of simulated intestinal fluid TS without enzyme, and use the acceptance criteria given under *Tolerances*.

*Apparatus 2:* 50 rpm.

*Times:* 1.0 hour, 3.0 hours, 7.0 hours.

*Procedure*—Proceed as directed in *Test 1*.

*Tolerances*—The percentages of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.0	between 30% and 60%
3.0	between 55% and 85%
7.0	not less than 70%

**Uniformity of dosage units** (905)—meet the requirements.

**Assay**—

*Mobile phase*—Dissolve 2.0 g of sodium perchlorate in 350 mL of water. Add 650 mL of methanol and 2.0 mL of triethylamine, and mix. Filter, and degas this solution prior to use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 0.12 mg per mL.

*Assay preparation*—Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 120 mg of chlorpheniramine maleate, to a 200-mL volumetric flask. Add about 100 mL of dilute hydrochloric acid (1 in 100), bring to a boil on a hot plate, and continue boiling moderately for 5 minutes. Cool, dilute with dilute hydrochloric acid (1 in 100) to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with dilute hydrochloric acid (1 in 100) to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 261-nm detector and a 3.9-mm  $\times$  15-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 900 theoretical plates, the tailing factor is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Capsules taken by the formula:

$$(1000C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpheniramine Maleate Injection

» Chlorpheniramine Maleate Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

USP Endotoxin RS

**Identification**—

**A:** Dilute a volume of Injection, equivalent to about 50 mg of chlorpheniramine maleate, with dilute hydrochlo-

ric acid (1 in 1000) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator." The Injection meets the requirements of the test.

**B:** Evaporate a volume of Injection, equivalent to about 25 mg of chlorpheniramine maleate, on a steam bath to dryness, and dry the residue at 105° for 1 hour: it melts between 128° and 135°.

**Bacterial endotoxins** (85)—It contains not more than 8.8 USP Endotoxin Units per mg of chlorpheniramine maleate.

**pH** (791): between 4.0 and 5.2.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* (501), to prepare the solution employed for the determination of the absorbance,  $A_U$ , at 264 nm. For the determination of  $A_S$ , dissolve about 25 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  in each mL of the Injection taken by the formula:

$$(C / V)(A_U / A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the *Standard preparation*, and V is the volume, in mL, of Injection taken.

## Chlorpheniramine Maleate Oral Solution

» Chlorpheniramine Maleate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

**Identification**—

**A:** Evaporate the remaining extract from the Assay on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, and mix: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is not more than +0.01° (*dissolution from dexchlorpheniramine maleate*).

**B:** *Ultraviolet Absorption* (197U)—

**Solution:** the solution employed for measurement of absorbance in the Assay.

**Alcohol content** (*if present*) (611): between 6.0% and 8.0% of  $C_2H_5OH$ .

**Assay**—Transfer 10 mL of Oral Solution, accurately measured, to a separator. Transfer about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, mix, and pipet 10 mL of this Standard solution into a separator similar to that containing the Oral Solution. Treat each solution as follows. Add 10 mL of sodium hydroxide solution (1 in 10), and extract with two 50-mL portions of solvent hexane. Combine the extracts in a second separator, wash with 10 mL of sodium hydroxide solution (1 in 250), and discard the washing. Extract the hexane solution with two 40-mL

portions of dilute hydrochloric acid (1 in 100), collect the extracts in a 100-mL volumetric flask, add the same dilute acid to volume, and mix. Wash 50-mL portions of each solution, and of dilute hydrochloric acid (1 in 100), respectively, with three 30-mL portions of chloroform and then with 50 mL of solvent hexane, and discard the washings. Filter the acid phases through paper, discarding the first few mL of each filtrate, and determine the absorbances of the solutions obtained from the Oral Solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using the extracted acid as the blank. Calculate the quantity, in  $\mu g$ , of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each mL of the Oral Solution taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in  $\mu g$  per mL, of USP Chlorpheniramine Maleate RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the Oral Solution and the Standard solution, respectively.

## Chlorpheniramine Maleate Tablets

» Chlorpheniramine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

**Identification**—Disperse a portion of powdered Tablets, equivalent to about 25 mg of chlorpheniramine maleate, in about 20 mL of dilute hydrochloric acid (1 in 100). Dissolve about 25 mg of USP Chlorpheniramine Maleate RS in 20 mL of dilute hydrochloric acid (1 in 100). Treat each solution as follows. Render alkaline, to a pH of about 11, with sodium hydroxide solution (1 in 10). Extract with two 50-mL portions of solvent hexane, collect the extracts in a beaker, and evaporate to dryness. Prepare a mineral oil dispersion of the residue so obtained and determine the IR absorption spectrum of the preparation in the region between 2  $\mu m$  and 12  $\mu m$ : the spectrum of the test preparation exhibits maxima only at the same wavelengths as that of the Standard preparation.

**Dissolution** (711)—

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Using a portion of powdered Tablets equivalent to 4 mg of chlorpheniramine maleate, proceed as directed under *Salts of Organic Nitrogenous Bases* (501), but using dilute hydrochloric acid (1 in 100) instead of the dilute sulfuric acid (1 in 350), and dilute sulfuric acid (1 in 70), and using solvent hexane instead of the ether, and diluting

10 mL of the *Assay preparation* with dilute hydrochloric acid (1 in 100) to 25.0 mL to prepare the solution employed for the determination of the absorbance,  $A_U$ , at 264 nm. For the determination of  $A_S$ , prepare a solution containing about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 200.0 mL of dilute hydrochloric acid (1 in 100), and treat 20.0 mL of this solution the same as the solution in dilute hydrochloric acid (1 in 100) of the portion of Tablets taken. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$C(A_U / A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the 20.0-mL portion of the *Standard preparation*.

### Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules

» Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Chlorpheniramine Maleate RS

USP Phenylpropanolamine Hydrochloride RS

#### Identification—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**B:** The retention time of the major peak for phenylpropanolamine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for phenylpropanolamine hydrochloride*.

#### Dissolution (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Times:* 3, 6, and 12 hours.

*Procedure*—Determine the amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_9H_{13}NO \cdot HCl$  dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for phenylpropanolamine hydrochloride*.

*Tolerances*—The percentages of the labeled amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_9H_{13}NO \cdot HCl$  dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 45% and 75%
12	not less than 75%

**Uniformity of dosage units (905):** meet the requirements.

#### Assay for chlorpheniramine maleate—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.05 g of triethylamine hydrochloride, 0.025 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with phosphoric acid solution (1 in 1000) to obtain a solution having a known concentration of about 8 µg per mL.

*Assay preparation*—Transfer not fewer than 10 Capsules to a suitable container, add 100 mL of water and 10 mL of phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 0.8 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

*System suitability solution*—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation* prepared as directed in the *Assay for phenylpropanolamine hydrochloride*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Separately inject about 20 µL of the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between phenylpropanolamine and chlorpheniramine is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the phenylpropanolamine peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for phenylpropanolamine hydrochloride—

*Mobile phase* and *Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Phenylpropanolamine Hydrochloride RS in water to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5 mL of methanol, dilute with phosphoric acid solution (1 in 1000) to volume, and mix.

*System suitability solution*—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation* prepared as directed in the *Assay for chlorpheniramine maleate*.

*Assay preparation*—Transfer not fewer than 10 Capsules to a suitable container, add 100 mL of water and 10 mL of phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution,



equivalent to about 5 mg of phenylpropanolamine hydrochloride, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the phenylpropanolamine peaks. Calculate the quantity, in mg, of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Phenylpropanolamine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the phenylpropanolamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Tablets

» Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Chlorpheniramine Maleate RS  
USP Phenylpropanolamine Hydrochloride RS

**Identification**—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**B:** The retention time of the major peak for phenylpropanolamine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for phenylpropanolamine hydrochloride*.

**Dissolution** <711>—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Times:* 3, 6, and 12 hours.

**Procedure**—Determine the amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_9H_{13}NO \cdot HCl$  dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for phenylpropanolamine hydrochloride*.

**Tolerances**—The percentages of the labeled amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_9H_{13}NO \cdot HCl$  dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 45% and 75%
12	not less than 75%

**Uniformity of dosage units** <905>: meet the requirements.

**Assay for chlorpheniramine maleate**—

*Mobile phase, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of chlorpheniramine maleate, to a suitable container, add 100 mL of water and 10 mL of phosphoric acid solution (1 in 20), and heat gently until the powder is fully dispersed. Cool to room temperature, and transfer an accurately measured volume of this solution, equivalent to about 0.8 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with phosphoric acid solution (1 in 1000) to obtain a solution having a concentration of about 8 µg of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for phenylpropanolamine hydrochloride**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Phenylpropanolamine Hydrochloride RS in water to obtain a solution having a known concentration of about 2.5 mg per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5 mL of methanol, dilute with phosphoric acid solution (1 in 1000) to volume, and mix.

**System suitability solution**—Proceed as directed in the *Assay for phenylpropanolamine hydrochloride* under *Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 500 mg of phenylpropanolamine hydrochloride, to a suitable container, add 100 mL of water and 10 mL of phosphoric acid solution (1 in 20), and heat gently until the powder is fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 5 mg of phenylpropanolamine hydrochloride, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the phenylpropanolamine peaks. Calculate the quantity, in mg, of phenylpropanolamine hydrochloride ( $C_9H_{13}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Phenylpropanolamine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the phenylpropanolamine peak

responses obtained from the Assay preparation and the Standard preparation, respectively.

**Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules**

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) and pseudoephedrine hydrochloride (C<sub>10</sub>H<sub>15</sub>NO · HCl).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**Labeling**—The labeling indicates the Dissolution Test with which the product complies.

**USP Reference standards** (11)—  
USP Chlorpheniramine Maleate RS  
USP Pseudoephedrine Hydrochloride RS

**Identification**—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay for chlorpheniramine maleate.

**B:** The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay for pseudoephedrine hydrochloride.

**Dissolution** (711)—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 1.

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Times: 3, 6, and 12 hours.

**Procedure**—Determine the amounts of chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) and pseudoephedrine hydrochloride (C<sub>10</sub>H<sub>15</sub>NO · HCl) dissolved by employing the methods set forth in the Assay for chlorpheniramine maleate and the Assay for pseudoephedrine hydrochloride, respectively.

**Tolerances**—The percentages of the labeled amounts of C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> and C<sub>10</sub>H<sub>15</sub>NO · HCl dissolved at the specified times conform to Acceptance Table 2.

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 45% and 75%
12	not less than 75%

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium 1: simulated gastric fluid TS, prepared without pepsin; 900 mL.

Medium 2: simulated intestinal fluid TS, prepared without pancreatin; 900 mL.

Apparatus 2: 50 rpm.

Time for Medium 1: 1.5 hours.

Times for Medium 2: 3 and 6 hours.

**Procedure**—Determine the amounts of chlorpheniramine maleate (C<sub>16</sub>N<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) and pseudoephedrine hydro-

chloride (C<sub>10</sub>H<sub>15</sub>NO · HCl) dissolved by employing the methods set forth in the Assay for chlorpheniramine maleate and the Assay for pseudoephedrine hydrochloride, respectively, using Standard solutions having known concentrations of the relevant USP Reference Standard in the appropriate Medium.

**Tolerances**—The percentages of the labeled amounts of C<sub>16</sub>N<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> and C<sub>10</sub>H<sub>15</sub>NO · HCl dissolved at the specified times conform to Acceptance Table 2.

Time (hours)	Amount dissolved (Medium 1)	Amount dissolved (Medium 2)
1.5	between 15% and 40%	
3.0		between 35% and 75%
6.0		not less than 50%

**Uniformity of dosage units** (905): meet the requirements.

**Assay for chlorpheniramine maleate**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with a phosphoric acid solution (1 in 1000) to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 0.8 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

**System suitability solution**—Mix 1 part of the Standard preparation prepared above with 1 part of the Standard preparation, prepared as directed in the Assay for pseudoephedrine hydrochloride.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Inject about 20 µL of the System suitability solution, and record the peak responses as directed for Procedure: the resolution, R<sub>s</sub>, between pseudoephedrine and chlorpheniramine is not less than 2.0. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the tailing factor for the chlorpheniramine peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Capsules taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in µg per mL, of USP Chlorpheniramine Maleate RS in the Standard preparation; and r<sub>u</sub> and r<sub>s</sub> are the chlorpheniramine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for pseudoephedrine hydrochloride—**

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay for chlorpheniramine maleate.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer about 1.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

*System suitability solution*—Mix 1 part of the Standard preparation prepared above with 1 part of the Standard preparation prepared as directed in the Assay for chlorpheniramine maleate.

*Assay preparation*—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 12 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the Standard preparation; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

USP Pseudoephedrine Hydrochloride RS

**Identification**—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the Assay preparation corresponds to that of the Standard preparation, as obtained in the Assay for chlorpheniramine maleate.

**B:** The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the Assay preparation corresponds to that of the Standard preparation in the Assay for pseudoephedrine hydrochloride.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay for chlorpheniramine maleate—**

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay for chlorpheniramine maleate under Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of Mobile phase, dilute with water to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add about 80 mL of Mobile phase, dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Oral Solution taken by the formula:

$$(100C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in that Standard preparation; V is the volume, in mL, of Oral Solution taken for the Assay preparation; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for pseudoephedrine hydrochloride—**

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay for chlorpheniramine maleate under Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer about 1.0 mL of this solution to a 10-mL volumetric flask, add 8 mL of Mobile phase, dilute with water to volume, and mix.

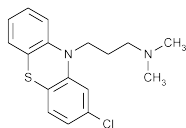
*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of Mobile phase, dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the Standard preparation; V is the volume, in mL, of Oral Solution taken for the Assay preparation; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Chlorpromazine



$C_{17}H_{19}ClN_2S$  318.86

10*H*-Phenothiazine-10-propanamine, 2-chloro-*N,N*-dimethyl-

2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine [50-53-3].

» Chlorpromazine contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{19}ClN_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** The IR absorption spectrum of a 1 in 100 solution in carbon disulfide, in a 1.0-mm cell between 7  $\mu$ m and 15  $\mu$ m, exhibits maxima only at the same wavelengths as that of a solution prepared by dissolving 55 mg of USP Chlorpromazine Hydrochloride RS in 3 mL of 1 N sodium hydroxide and extracting the resulting solution with 5.0 mL of carbon disulfide.

**B:** The principal spot found in the test for *Other alkylated phenothiazines* corresponds in  $R_f$  to the spot from the *Standard solution*.

**Loss on drying** (731)—Dry it in vacuum at room temperature for 3 hours: it loses not more than 1.0% of its weight.

**Other alkylated phenothiazines**—Dissolve 45.0 mg in 10 mL of methanol. Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a concentration of 5 mg per mL (*Standard solution*), and dilute it quantitatively and stepwise with methanol to obtain a concentration of 25  $\mu$ g per mL (*Diluted standard solution*). Apply separately 10  $\mu$ L of each of the three solutions to the starting line of a thin-layer chromatographic plate coated with chromatographic silica gel mixture. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 10 cm from the origin. Remove the plate from the chamber, and air-dry for 20 minutes. View under short-wavelength UV light: the area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Place about 750 mg of Chlorpromazine, accurately weighed, in a 250-mL conical flask, and dissolve in 25 mL of glacial acetic acid, warming gently on a steam bath to effect solution. Cool, add crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.89 mg of  $C_{17}H_{19}ClN_2S$ .

## Chlorpromazine Suppositories

» Chlorpromazine Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{19}ClN_2S$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers, at controlled room temperature.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—Suppositories respond to *Identification test B* under *Chlorpromazine*.

**Other alkylated phenothiazines**—Transfer a portion of Suppositories, equivalent to 45 mg of chlorpromazine, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously to disperse the solid, warming gently if necessary, and centrifuge. Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Suppositories are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Place not fewer than 10 Suppositories in a 250-mL beaker, reduce the mass to the consistency of a paste by crushing with a spatula, and mix. Weigh accurately a portion of the mass, equivalent to about 50 mg of chlorpromazine, place in a beaker, and dissolve in about 40 mL of ether. Transfer to a 250-mL separator with the aid of three 25-mL portions of ether, and extract with four 75-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 500-mL volumetric flask. Add 0.1 N hydrochloric acid to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a *Standard solution* having a known concentration of about 5.5  $\mu$ g of chlorpromazine hydrochloride per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of chlorpromazine ( $C_{17}H_{19}ClN_2S$ ) in the portion of Suppositories taken by the formula:

$$10(0.897C)(A_{254} - A_{277})_U / (A_{254} - A_{277})_S$$

in which 0.897 is the ratio of the molecular weight of chlorpromazine to that of chlorpromazine hydrochloride; C is the concentration, in  $\mu$ g per mL, of USP Chlorpromazine Hydrochloride RS in the *Standard solution*; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Suppositories (U) and the *Standard solution* (S), respectively.

## Chlorpromazine Hydrochloride

$C_{17}H_{19}ClN_2S \cdot HCl$  355.33  
 10*H*-Phenothiazine-10-propanamine, 2-chloro-*N,N*-dimethyl-, monohydrochloride.  
 2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine monohydrochloride [69-09-0].

» Chlorpromazine Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of  $C_{17}H_{19}ClN_2S \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** Infrared Absorption (197K).

**B:** The principal spot found in the test for *Other alkylated phenothiazines* corresponds in  $R_f$  to the spot from the *Standard solution*.

**C:** A solution (1 in 10) responds to the tests for *Chloride* (191).

**Melting range** (741): between 195° and 198°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Other alkylated phenothiazines**—Dissolve 50 mg, previously dried, in methanol to make 10 mL, and mix. Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine Hydrochloride are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Transfer to a beaker about 700 mg of Chlorpromazine Hydrochloride, accurately weighed, and dissolve in 75 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 35.53 mg of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

## Chlorpromazine Hydrochloride Oral Concentrate

» Chlorpromazine Hydrochloride Oral Concentrate contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it must be diluted prior to administration.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** It responds to *Identification test A* under *Chlorpromazine Hydrochloride Syrup*.

**B:** Dilute a portion of the Oral Concentrate with an equal volume of water: the resulting solution responds to the tests for *Chloride* (191).

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for the absence of *Escherichia coli*.

**pH** (791): between 2.3 and 4.1.

**Limit of chlorpromazine sulfoxide**—Proceed as directed in the test for *Chlorpromazine sulfoxide* under *Chlorpromazine Hydrochloride Syrup*.

**Assay**—Transfer an accurately measured volume of Oral Concentrate, previously diluted if necessary, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the *Assay* under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in each mL of the Oral Concentrate taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Chlorpromazine Hydrochloride RS in the *Standard solution*;  $V$  is the volume, in mL, of Oral Concentrate taken; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Oral Concentrate ( $U$ ) and the *Standard solution* ( $S$ ), respectively.

## Chlorpromazine Hydrochloride Injection

» Chlorpromazine Hydrochloride Injection is a sterile solution of Chlorpromazine Hydrochloride in Water for Injection. It contains, in each mL, not less than 23.75 mg and not more than 26.25 mg of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

USP Endotoxin RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** Transfer a volume of Injection, equivalent to about 25 mg of chlorpromazine hydrochloride, to a 10-mL volumetric flask, dilute with methanol to volume, and mix (test solution). Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in dilute methanol (9 in 10) to obtain a *Standard solution* having a known concentration of 2.5 mg per mL. Apply separately 5- $\mu\text{L}$  portions of each of the two solutions to the starting line of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide until the solvent front has moved about 10 cm from the origin. Remove the plate from the developing chamber, air-dry for 20 minutes, then view

under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B:** It responds to the tests for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 6.9 USP Endotoxin Units per mg of chlorpromazine hydrochloride.

**pH** (791): between 3.4 and 5.4.

**Limit of chlorpromazine sulfoxide**—[NOTE—Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.]

**Test preparation**—Pipet 4 mL of the test solution prepared with methanol as directed in *Identification* test A into a 10-mL volumetric flask, dilute with methanol to volume, and mix.

**Standard preparation**—Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a solution having a concentration of 50 µg per mL.

**Procedure**—Apply separate 10-µL portions of the *Standard preparation* and the *Test preparation* to the starting line of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the applied solutions with the aid of a stream of nitrogen. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 13 cm from the origin. Remove the plate from the chamber, and air-dry for 30 minutes. Examine under short-wavelength UV light: the area and intensity of the only other spot in the test specimen chromatogram, other than the principal spot, are not greater than those of the spot from the *Standard preparation* (5.0%).

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Pipet 10 mL of the solution into a 250-mL separator, add about 20 mL of water, render alkaline with ammonium hydroxide, and extract with four 25-mL portions of ether. Extract the combined ether extracts with four 25-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 250-mL volumetric flask. Aerate to remove residual ether, add 0.1 N hydrochloric acid to volume, and mix. Dissolve a suitable quantity, accurately weighed, of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same acid to obtain a Standard solution having a known concentration of about 8 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in each mL of the Injection taken by the formula:

$$12.5C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Injection (U) and the Standard solution (S), respectively.

## Chlorpromazine Hydrochloride Syrup

» Chlorpromazine Hydrochloride Syrup contains, in each 100 mL, not less than 190 mg and not more than 210 mg of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—

**A:** Transfer a volume of it, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water, 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 100 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a Standard solution, containing 0.2 mg of USP Chlorpromazine Hydrochloride RS per mL of methanol, to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with iodoplatinate reagent prepared by dissolving 100 mg of platinum chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25), 0.5 mL of formic acid, and diluting with water to 100 mL: the  $R_f$  value of the principal spot from the test solution corresponds to that obtained from the Standard solution.

**B:** Dilute a portion of the Syrup with an equal volume of water: the resulting solution responds to the tests for *Chloride* (191).

**Limit of chlorpromazine sulfoxide**—

**Chlorpromazine sulfoxide standard solution**—Transfer 5 mL of a solution in dilute hydrochloric acid (1 in 100) of USP Chlorpromazine Hydrochloride RS containing 10.6 mg per mL to a 50-mL volumetric flask. Add 2 mL of 30% hydrogen peroxide and heat at 60° for 10 minutes. Cool, dilute with 1 M sodium bisulfite to volume, and mix. Transfer 10.0 mL to a 60-mL separator, add 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the extracts through ether-wetted anhydrous sodium sulfate into a 250-mL conical flask. Cautiously evaporate the extracts to dryness. Dissolve the residue in 10.0 mL of methanol, and filter if necessary. Each mL of this solution contains 1 mg of chlorpromazine sulfoxide.

**Procedure**—Transfer an accurately measured volume of the Syrup, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water and 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 1.0 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a *Chlorpromazine sulfoxide standard solution* to a thin-layer chromatographic plate (see

*Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with iodoplatinate reagent prepared by dissolving 100 mg of platinum chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25) and 0.5 mL of formic acid, and diluting with water to 100 mL. The chromatogram from the Test solution may exhibit a secondary spot whose  $R_f$  value corresponds to, and whose size and intensity are not greater than, those of the spot from the *Chlorpromazine sulfoxide standard solution* (5.0%).

**Assay**—Transfer an accurately measured volume of Syrup, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the Assay under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in each mL of the Syrup taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in  $\mu g$  per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Syrup taken; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Syrup (U) and the Standard solution (S), respectively.

## Chlorpromazine Hydrochloride Tablets

» Chlorpromazine Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** Tablets respond to Identification test B under *Chlorpromazine Hydrochloride*.

**B:** Digest a quantity of powdered Tablets, equivalent to about 25 mg of chlorpromazine hydrochloride, with 25 mL of water, and filter: the solution so obtained responds to Identification test C under *Chlorpromazine Hydrochloride*.

### Dissolution (711)—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 1:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{19}ClN_2S \cdot HCl$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 254 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Chlorpromazine Hydrochloride RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{19}ClN_2S \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

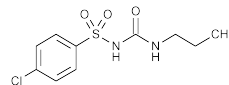
**Other alkylated phenothiazines**—Transfer a portion of finely powdered Tablets, equivalent to 50 mg of chlorpromazine hydrochloride, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously, and centrifuge (remove any sugar coating by prior washing with water). Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Tablets are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask. Add about 200 mL of water and 5 mL of hydrochloric acid, insert the stopper, and shake for about 10 minutes. Dilute with water to volume, and mix. Filter a portion of the solution, discarding the first 50 mL of the filtrate. Treat 10.0 mL of the filtrate as directed in the Assay under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in the portion of Tablets taken by the formula:

$$12.5C(A_{254} - A_{277})_U / (A_{254} - A_{277})_S$$

in which C is the concentration, in  $\mu g$  per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Tablets (U) and the Standard solution (S), respectively.

## Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$  276.74

Benzenesulfonamide, 4-chloro-N-[(propylamino)carbonyl]-1-[(p-chlorophenyl)sulfonyl]-3-propylurea [94-20-2].

» Chlorpropamide contains not less than 97.0 percent and not more than 103.0 percent of  $C_{10}H_{13}ClN_2O_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Chlorpropamide RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** It responds to the *Thin-Layer Chromatographic Identification Test* (201). Prepare the test solution by dissolving an accurately weighed quantity of Chlorpropamide in acetone to obtain a solution containing 1 mg per mL. Develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methanol, cyclohexane, and ammonium hydroxide (100:50:30:10).

**Melting range** (741): between 126° and 129°.

**Loss on drying** (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.4%.

**Selenium** (291): 0.003%.

**Heavy metals, Method II** (231): 0.003%.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of equal volumes of acetonitrile and dilute glacial acetic acid (1 in 100). [NOTE—Do not exceed 50% of acetonitrile.] Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorpropamide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

*Assay preparation*—Transfer about 50 mg of Chlorpropamide, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10 mL of this solution to a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>10</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S in the portion of Chlorpropamide taken by the formula:

$$1000C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation*, and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpropamide Tablets

» Chlorpropamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>10</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Chlorpropamide RS

**Identification**—Shake a quantity of finely powdered Tablets, equivalent to about 100 mg of chlorpropamide, with 20 mL of 1 N hydrochloric acid, and extract with 50 mL of chloroform. Filter the chloroform through chloroform-washed cotton into a suitable beaker, and evaporate the chloroform on a steam bath with the aid of a current of dry air to dryness. Dry the residue at 105° for 1 hour: the residue so obtained responds to the *Identification* tests under *Chlorpropamide*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 60 minutes.

*Procedure*—Determine the amount of C<sub>10</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S dissolved from UV absorbances at the wavelength of maximum absorbance at about 230 nm of filtered portions of the solu-

tion under test, suitably diluted with 0.1 N hydrochloric acid in comparison with a Standard solution having a known concentration of USP Chlorpropamide RS in 0.1 N hydrochloric acid. [NOTE—A volume of alcohol not exceeding 10% of the final volume of the Standard solution may be used to dissolve the USP Chlorpropamide RS.]

*Tolerances*—Not less than 75% (Q) of the labeled amount of C<sub>10</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Chlorpropamide*.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of chlorpropamide, to a 100-mL volumetric flask. Add *Mobile phase* to volume, mix, and filter, discarding the first 10 mL of the filtrate. Pipet 10 mL of the filtrate into a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chlorpropamide*. Calculate the quantity, in mg, of C<sub>10</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S in the portion of Tablets taken by the formula:

$$1000C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlortetracycline Bisulfate

» Chlortetracycline Bisulfate has a potency equivalent to not less than 760 µg of chlortetracycline hydrochloride (C<sub>22</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>8</sub> · HCl) per mg, calculated on the dried and butyl alcohol-free basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards** (11)—  
USP Chlortetracycline Hydrochloride RS

**Identification, Ultraviolet Absorption** (197U)—

*Solution*: 40 µg per mL.

*Medium*: 0.1 N hydrochloric acid.

Absorptivity at 368 nm, calculated on the dried and butyl alcohol-free basis, is not less than 83.0% and not more than 95.0% of that of the USP Chlortetracycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Sulfate content**—Transfer about 1 g, accurately weighed, to a 250-mL beaker, and dissolve in about 100 mL of water. Neutralize the solution with 7.5 N ammonium hydroxide to litmus paper, and warm. Filter, and wash the filter with warm water. Neutralize the filtrate with 6 N hydrochloric acid to litmus, and add an additional 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to precipitate all of the sulfate. Add an additional 2 mL of bar-



ium chloride TS, and digest on a steam bath for 1 hour. Filter the mixture through ashless filter paper, transferring the residue quantitatively to the filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Perform a blank determination concurrently with the test specimen determination, and subtract the weight of residue obtained from that obtained in the test specimen determination to obtain the weight of residue attributable to the sulfate content of the specimen: not less than 15.0% is found, calculated on the dried and butyl alcohol-free basis.

#### Butyl alcohol—

**Ceric ammonium nitrate solution**—Dissolve 20 g of ceric ammonium nitrate in 4 N nitric acid to obtain 100 mL of solution.

**Standard preparations**—Transfer about 3 g of butyl alcohol, accurately weighed, to a 1000-mL volumetric flask containing 800 mL of water, shake to dissolve, dilute with water to volume, and mix (*Standard preparation 1*). Transfer 10.0 mL of *Standard preparation 1* and 1 drop of dimethicone to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice-water bath. Distill slowly, and collect about 8 mL of distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix (*Standard preparation 2*).

**Test preparation**—Transfer an accurately weighed specimen, equivalent to about 30 mg of butyl alcohol, to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice bath. Add 25 mL of water and 1 drop of dimethicone to the distilling flask. Distill slowly, and collect about 8 mL of the distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—To four separate test tubes add, respectively, 5.0 mL of *Standard preparation 1*, 5.0 mL of *Standard preparation 2*, 5.0 mL of *Test preparation*, and 5.0 mL of water to provide a blank. To each add 2.0 mL of *Ceric ammonium nitrate solution*, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparations* and the *Test preparation* at the wavelength of maximum absorbance at about 475 nm, with a suitable spectrophotometer, using the blank to set the instrument to zero. In a suitable determination, the absorbance of the solution from *Standard preparation 2* is not less than 98.0% of the absorbance of the solution from *Standard preparation 1*. Calculate the percentage of butyl alcohol in the specimen taken by the formula:

$$1000(W_S / W_U)(A_U / A_S)$$

in which  $W_S$  is the weight, in g, of butyl alcohol taken to prepare *Standard preparation 1*,  $W_U$  is the weight, in mg, of specimen taken, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and *Standard preparation 2*, respectively: not more than 15.0% is found.

**Assay**—Proceed with Chlortetracycline Bisulfate as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81).

### Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder

» Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder is a dry mixture of Chlortetracy-

cline Bisulfate and Sulfamethazine Bisulfate and one or more suitable buffers and diluents. It contains the equivalent of not less than 85.0 percent and not more than 125.0 percent of the labeled amounts of chlortetracycline hydrochloride ( $C_{22}H_{24}N_2O_8 \cdot HCl$ ) and sulfamethazine ( $C_{12}H_{14}N_4O_2S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

#### USP Reference standards (11)—

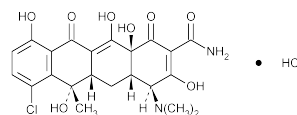
USP Chlortetracycline Hydrochloride RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Assay for chlortetracycline hydrochloride**—Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Powder, equivalent to about 100 mg of chlortetracycline hydrochloride, dissolved in an accurately measured volume of 0.01 N hydrochloric acid to obtain a stock solution having a convenient concentration. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for sulfamethazine**—Proceed as directed under *Nitrite Titration* (451), using an accurately weighed quantity of Powder, equivalent to about 500 mg of sulfamethazine. Each mL of 0.1 M sodium nitrite is equivalent to 27.83 mg of sulfamethazine ( $C_{12}H_{14}N_4O_2S$ ).

### Chlortetracycline Hydrochloride



$C_{22}H_{23}ClN_2O_8 \cdot HCl$  515.34

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride [4S-(4 $\alpha$ ,4a $\alpha$ ,5a $\alpha$ ,6 $\beta$ ,12a $\alpha$ )]-

7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-72-2].

» Chlortetracycline Hydrochloride has a potency of not less than 900  $\mu$ g of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  per mg.

**NOTE**—Chlortetracycline Hydrochloride labeled solely for use in preparing oral veterinary dosage forms has a potency of not less than 820  $\mu$ g of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  per mg.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

USP Oxytetracycline RS

USP Tetracycline Hydrochloride RS

**Identification**—

**A:** Proceed as directed for *Method II* under *Identification—Tetracyclines* (193), using a methanol solution containing 0.5 mg per mL as the *Test solution* and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*.

**B:** A solution (1 in 20) meets the requirements of the tests for *Chloride* (191).

**Specific rotation** (781S): between  $-235^\circ$  and  $-250^\circ$ .

*Test solution:* 5 mg per mL, in water, that has been allowed to stand in the dark for 30 minutes.

**Crystallinity** (695): meets the requirements.

**Sterility** (71)—Where the label states that Chlortetracycline Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen aseptically dissolved in 200 mL of *Fluid D* being used.

**pH** (791): between 2.3 and 3.3, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—Proceed with Chlortetracycline Hydrochloride as directed under *Antibiotics—Microbial Assays* (81).

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**Chlortetracycline Hydrochloride Ointment**


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» Chlortetracycline Hydrochloride Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Ointment, equivalent to about 30 mg of chlortetracycline hydrochloride, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the medium dose level of the Standard.

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**Chlortetracycline Hydrochloride Ophthalmic Ointment**


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» Chlortetracycline Hydrochloride Ophthalmic Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 10 mg of chlortetracycline hydrochloride, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

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**Chlortetracycline Hydrochloride Soluble Powder**


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» Chlortetracycline Hydrochloride Soluble Powder contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Label it to indicate that it is intended for oral veterinary use only.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—

*Assay preparation 1* (where it is labeled on a weight basis)—Dissolve about 3 g of Powder in an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000  $\mu$ g of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) per mL.

*Assay preparation 2* (where the label states the amount of chlortetracycline in the immediate container)—Transfer the contents of 1 container of Powder to an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000  $\mu$ g of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) per mL.

*Procedure*—Proceed with Powder as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with water to yield a *Test Dilu-*

tion having a concentration assumed to be equal to the median dose level of the Standard.

## Chlortetracycline Hydrochloride Tablets

» Chlortetracycline Hydrochloride Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Label the Tablets to indicate that they are intended for veterinary use only.

### USP Reference standards (11)—

USP Chlortetracycline Hydrochloride RS

USP Oxytetracycline RS

USP Tetracycline Hydrochloride RS

**Identification**—Shake a suitable quantity of finely ground Tablet powder with methanol to obtain a solution containing about 0.5 mg of chlortetracycline hydrochloride per mL, and filter. Using the filtrate so obtained as the *Test solution*, and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*, proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

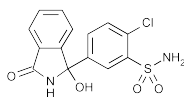
**Disintegration** (701): 1 hour, simulated gastric fluid TS being used as the test medium in place of water.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation*.

**Water, Method I** (921): not more than 3.0%, or where the Tablets have a diameter of greater than 15 mm, not more than 6.0%, a quantity of finely ground Tablet powder, accurately weighed, being used.

**Assay**—Transfer not less than 5 Tablets to a high-speed glass blender jar containing an accurately measured volume of 0.01 N hydrochloric acid, so that after blending for 3 to 5 minutes a solution of convenient concentration is obtained. Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Chlorthalidone



$C_{14}H_{11}ClN_2O_4S$  338.77

Benzenesulfonamide, 2-chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1H-indol-1-yl)-.

2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide [77-36-1].

» Chlorthalidone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{14}H_{11}ClN_2O_4S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Chlorthalidone RS

USP Chlorthalidone Related Compound A RS

4'-Chloro-3'-sulfamoyl-2-benzophenone carboxylic acid.

### Identification—

**A: Infrared Absorption** (197M).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 100 µg per mL.

*Medium:* 2 N hydrochloric acid in methanol (1 in 50).

Absorptivities at 275 nm, calculated on the dried basis, do not differ by more than 4.0%.

**C:** Dissolve about 50 mg in 3 mL of sulfuric acid: an intense yellow color develops.

**Loss on drying** (731)—Dry about 2 g, accurately weighed, at 105° for 4 hours: it loses not more than 0.4% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Shake 1.0 g with 40 mL of water for 5 minutes, and filter through chloride-free filter paper previously rinsed with water: the filtrate shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

**Heavy metals, Method II** (231): 0.001%.

**Limit of chlorthalidone related compound A**—Proceed as directed in the *Assay*, except to calculate the percentage of chlorthalidone related compound A in the portion of Chlorthalidone taken by the formula:

$$0.1(C_R / C_T)(R_U / R_S)$$

in which  $C_R$  is the concentration, in µg per mL, of USP Chlorthalidone Related Compound A RS in the *Standard preparation*;  $C_T$  is the concentration, in mg per mL, of Chlorthalidone in the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone related compound A to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 1.0% is present. [NOTE—USP Chlorthalidone Related Compound A RS is 4'-chloro-3'-sulfamoyl-2-benzophenone carboxylic acid (CCA).]

### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of 0.01 M dibasic ammonium phosphate and methanol (3:2), adjust dropwise with phosphoric acid to a pH of  $5.5 \pm 0.1$ , and filter.

**Internal standard solution**—Prepare a solution of 2,7-naphthalenediol in methanol having a concentration of about 1.0 mg per mL.

**Chlorthalidone related compound A solution**—Prepare a solution of USP Chlorthalidone Related Compound A RS in methanol having a known concentration of about 5 µg per mL.

**Standard preparation**—Prepare a solution of USP Chlorthalidone RS in methanol having a known concentration of about 1 mg per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of *Chlorthalidone related compound A solution*. Dilute with water to volume, and mix. This solution contains about 0.1 mg of chlorthalidone and about 1 µg of chlorthalidone related compound A per mL.

**Assay preparation**—Transfer about 50 mg of Chlorthalidone, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and re-

cord the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for chlorthalidone related compound A, 0.8 for chlorthalidone, and 1.0 for the internal standard; the resolution,  $R$ , between chlorthalidone and chlorthalidone related compound A, and between chlorthalidone and the internal standard is not less than 1.5; the tailing factor for chlorthalidone and chlorthalidone related compound A is not more than 2.0; and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{11}ClN_2O_4S$  in the portion of Chlorthalidone taken by the formula:

$$500C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorthalidone Tablets

» Chlorthalidone Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{14}H_{11}ClN_2O_4S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Chlorthalidone RS

### Identification—

**A:** Digest a quantity of powdered Tablets, equivalent to about 100 mg of chlorthalidone, with 10 mL of acetone on a steam bath for about 5 minutes. Filter the solution into a 50-mL beaker, add 20 mL of water, and boil on the steam bath for about 5 minutes, passing a gentle current of air above the solution to remove the acetone. Cool in an ice bath, filter, and dry the crystals at 105° for 4 hours: the crystals so obtained respond to *Identification test A* under *Chlorthalidone*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 60 minutes.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorthalidone RS in methanol to obtain a solution having a known concentration of about 5 mg per mL.

*Procedure*—Determine the amount of  $C_{14}H_{11}ClN_2O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 275 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a quantitative dilution in water of the *Standard preparation* having a known concentration of USP Chlorthalidone RS comparable to the concentration of the solution under test.

*Tolerances*—Not less than 70% (Q) of the labeled amount of  $C_{14}H_{11}ClN_2O_4S$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Mobile phase* and *Internal standard solution*—Prepare as directed in the *Assay* under *Chlorthalidone*.

*Standard preparation*—Prepare as directed in the *Assay* under *Chlorthalidone*, except to substitute 10.0 mL of methanol for the *CCA solution*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of Chlorthalidone, to a 100-mL volumetric flask. Dissolve in about 50 mL of methanol, shake for 30 minutes, dilute with methanol to volume, and mix. Transfer about 30 mL of this solution to a 50-mL centrifuge tube, and centrifuge for 10 minutes. Pipet 5 mL of the clear supernatant into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.

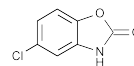
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%, and the resolution factor between chlorthalidone and the internal standard is not less than 1.5. The tailing factors for the chlorthalidone and internal standard peaks are not more than 2.0.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for chlorthalidone and 1.0 for the internal standard. Calculate the quantity, in mg, of  $C_{14}H_{11}ClN_2O_4S$  in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorzoxazone



$C_7H_4ClNO_2$  169.57

2(3*H*)-Benzoxazolinone, 5-chloro-

5-Chloro-2-benzoxazolinone [95-25-0].

» Chlorzoxazone contains not less than 98.0 percent and not more than 102.0 percent of  $C_7H_4ClNO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorzoxazone RS

USP Chlorzoxazone Related Compound A RS

2-Amino-4-chlorophenol.

$C_6H_6ClNO$  143.57

**Identification—**

**A:** *Infrared Absorption* (197K): previously dried.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 20 µg per mL.

*Medium:* methanol.

**Melting range** (741): between 189° and 194°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Heavy metals, Method II** (231): 0.002%.

**Residue on ignition** (281): not more than 0.15%.

**Chromatographic purity**—Prepare a *Test solution* in methanol containing 20 mg per mL. Dissolve a suitable quantity of USP Chlorzoxazone Related Compound A RS (2-Amino-4-chlorophenol) in methanol to obtain a solution containing 100 µg per mL (*Standard solution A*). Dissolve a suitable quantity of *p*-chlorophenol in methanol to obtain a solution containing 50 µg per mL (*Standard solution B*). Apply separate 10 µL portions of the three solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of hexane and dioxane (63:37) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution A*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapors in a closed chamber, and locate the spots: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.25% of any individual impurity.

**Chlorine content**—Dissolve about 300 mg, accurately weighed, in 10 mL of alcohol in a suitable flask. Add 3.5 g of Raney's nickel-aluminum catalyst, and connect to a suitable reflux condenser. Chill the flask in an ice bath, and add through the condenser 75 mL of 2.5 N sodium hydroxide. When the reaction has subsided, remove the ice bath. After 10 minutes, heat the flask gently, gradually increasing the heat until the mixture refluxes rapidly. After 90 minutes from the time of the addition of the alkali, discontinue heating, cool, and rinse the condenser with water, collecting the rinsings in the flask. Transfer the liquid to a 200-mL volumetric flask, wash the residue with water, and add the washing to the volumetric flask. Dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a beaker, neutralize, then acidify (using congo red as the indicator) by adding nitric acid dropwise with mixing. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using silver and calomel electrodes. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl, calculated on the dried basis, is between 20.6% and 21.2%.

**Assay**—Transfer about 50 mg of Chlorzoxazone, accurately weighed, to a 100-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorzoxazone RS in methanol at a concentration of about 20 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 282 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate

the quantity, in mg, of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> in the Chlorzoxazone taken by the formula:

$$2.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorzoxazone RS in the Standard solution; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solution of Chlorzoxazone and the Standard solution, respectively.

## Chlorzoxazone Tablets

» Chlorzoxazone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub>.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorzoxazone RS

**Identification—**

**A:** Disperse a portion of powdered Tablets, equivalent to about 100 mg of chlorzoxazone, in 100 mL of methanol, shake for 15 minutes, and filter. Transfer 2.0 mL of the filtrate to a 100-mL volumetric flask, dilute with methanol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Chlorzoxazone RS, concomitantly measured.

**B:** The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for chlorzoxazone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained in the *Assay*.

**Dissolution** (711)—[NOTE—Use 2-liter vessels for this test.]

*Medium:* pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1800 mL.

*Apparatus 2:* 75 rpm.

*Time:* 60 minutes.

*Procedure*—Determine the amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a Standard solution of USP Chlorzoxazone RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*1% Acetic acid solution*—Dilute 10 mL of glacial acetic acid with water to make 1000 mL of solution.

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of phenacetin in acetonitrile containing about 1.25 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chlorzoxazone RS quantitatively in *Mobile phase* to obtain a stock solution having a known concentration of about 1.25 mg per mL. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with *1% Acetic acid solution* to volume, and mix.

*Resolution solution*—Prepare a solution of *p*-chlorophenol in acetonitrile containing about 8.5 mg per mL. Transfer

1 mL of this solution to a 50-mL volumetric flask containing 4 mL of the stock solution used to prepare the *Standard preparation* and 10 mL of *Internal standard solution*, dilute with 1% *Acetic acid solution* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of chlorzoxazone, to a 100-mL volumetric flask, add about 70 mL of acetonitrile, and shake by mechanical means for about 30 minutes. Dilute with acetonitrile to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with 1% *Acetic acid solution* to volume, and mix.

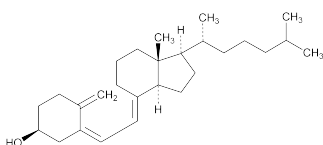
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.7 for phenacetin, 1.0 for chlorzoxazone, and 1.2 for *p*-chlorophenol; and the resolution, *R*, between the chlorzoxazone peak and the *p*-chlorophenol peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorzoxazone RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the chlorzoxazone peak to the phenacetin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cholecalciferol



C<sub>27</sub>H<sub>44</sub>O 384.64  
9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3β,5Z,7E)-;  
Cholecalciferol [67-97-0].

### DEFINITION

Cholecalciferol contains NLT 97.0% and NMT 103.0% of cholecalciferol (C<sub>27</sub>H<sub>44</sub>O).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>  
Wavelength range: 2–12 µm
- **B. ULTRAVIOLET ABSORPTION** <197U>  
Analytical wavelength: 265 nm  
Sample solution: 10 µg/mL in alcohol  
Acceptance criteria: Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.

- **C.**  
**Sample solution:** 0.5 mg in 5 mL of chloroform  
**Analysis:** Add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid to the *Sample solution*, and shake vigorously.  
**Acceptance criteria:** A bright red color is produced, and it rapidly changes through violet and blue to green.
- **D. THIN-LAYER CHROMATOGRAPHY**  
[NOTE—For the *Standard solution* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]  
**Diluent:** 10 mg/mL of squalane in chloroform  
**Standard solution:** 50 mg/mL of USP Cholecalciferol RS in *Diluent*  
**Sample solution:** 50 mg/mL of Cholecalciferol in *Diluent*  
**Chromatographic system**  
(See *Chromatography* <621>, *Thin-Layer Chromatography*.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 10 µL  
**Developing solvent system:** Cyclohexane and diethyl ether (1:1)  
**Spray reagent:** 20 mg/mL of acetyl chloride in anti-mony trichloride TS  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
[NOTE—Perform the development and subsequent operations in the dark.]  
Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.  
**Acceptance criteria:** The *Sample solution* shows a yellowish-orange area (cholecalciferol) having the same *R<sub>F</sub>* value as the area of the *Standard solution* and may show below the cholecalciferol area a violet area, attributed to 7-dehydrocholesterol.

### ASSAY

- **PROCEDURE**  
**Dehydrated hexane:** Prepare a chromatographic column by packing a chromatographic tube, 8 cm × 60 cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* <621>, *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.  
**Mobile phase:** *n*-Amyl alcohol in *Dehydrated hexane* (3 in 1000)  
**System suitability solution:** 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]  
[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]  
**Standard stock solution:** 0.6 mg/mL of USP Cholecalciferol RS in toluene  
**Standard solution:** 120 µg/mL of USP Cholecalciferol RS in *Mobile phase*, prepared from *Standard stock solution*  
**Sample stock solution:** 0.6 mg/mL of Cholecalciferol in toluene  
**Sample solution:** 120 µg/mL of Cholecalciferol in *Mobile phase*, prepared from *Sample stock solution*  
**Chromatographic system**  
(See *Chromatography* <621>, *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Injection size:** 5–10 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between *trans*-cholecalciferol and precholecalciferol

**Relative standard deviation:** NMT 2.0% for the peak response of cholecalciferol

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cholecalciferol ( $C_{27}H_{44}O$ ) in the portion of Cholecalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Cholecalciferol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–103.0%

**SPECIFIC TESTS**

• **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 5 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Cholecalciferol from a container opened not longer than 30 min.]

**Acceptance criteria:** +105° to +112°

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.
- **USP REFERENCE STANDARDS (11)**  
USP Cholecalciferol RS  
USP Vitamin D Assay System Suitability RS

## Cholecalciferol Solution

**DEFINITION**

Cholecalciferol Solution is a solution of Cholecalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin D as cholecalciferol ( $C_{27}H_{44}O$ ).

**ASSAY**

• **PROCEDURE**

**Mobile phase:** Hexane and pentanol (997:3)

**Standard stock solution:** Dissolve USP Cholecalciferol RS in toluene, and dilute with *Mobile phase* to 50 µg/mL. [NOTE—Prepare this solution fresh daily.]

**Standard solution A:** 5 µg/mL from *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above 0°.]

**Standard solution B:** Transfer 5.0 mL of *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** Equivalent to 5 µg/mL of cholecalciferol in *Mobile phase* from an accurately measured volume of Cholecalciferol Solution

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution B*

[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

**Cholecalciferol response factor**

Calculate the cholecalciferol response factor,  $F_C$ :

$$F_C = C_S/r_S$$

$C_S$  = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)

$r_S$  = peak area of cholecalciferol from *Standard solution A*

**Pre-cholecalciferol response factor**

Calculate the concentration,  $C'_S$ , in µg/mL, of cholecalciferol in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

$F_C$  = response factor for cholecalciferol

$r'_S$  = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration,  $C'_{pre}$ , in µg/mL, of pre-cholecalciferol:

$$C'_{pre} = C_S - C'_S$$

$C_S$  = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)

$C'_S$  = concentration of cholecalciferol in *Standard solution B* (µg/mL)

Calculate the response factor,  $F_{pre}$ , for pre-cholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

$C'_{pre}$  = concentration of pre-cholecalciferol (µg/mL)

$r_p$  = peak response of pre-cholecalciferol from *Standard solution B*

**Content of vitamin D**

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ( $C_{27}H_{44}O$ ) in the portion of the Cholecalciferol Solution taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

$F_C$  = response factor for cholecalciferol

$r_C$  = peak area of cholecalciferol from the *Sample solution*

$F_{pre}$  = response factor for pre-cholecalciferol

$r_{pre}$  = peak area of pre-cholecalciferol from the *Sample solution*

$C_U$  = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–120.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **LABELING:** Label it to indicate the concentration, in mg/mL, of cholecalciferol. Label it also to state that it is to be used for manufacturing only.
- **USP REFERENCE STANDARDS** (11)  
USP Cholecalciferol RS

## Cholestyramine Resin

Cholestyramine.

Cholestyramine [11041-12-6].

» Cholestyramine Resin is a strongly basic anion-exchange resin in the chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium functional groups. Each g exchanges not less than 1.8 g and not more than 2.2 g of sodium glycocholate, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cholestyramine Resin RS

**Identification**—*Infrared Absorption* (197K).

**pH** (791): between 4.0 and 6.0, in a slurry (1 in 100).

**Loss on drying** (731)—Dry over phosphorus pentoxide at a pressure not exceeding 50 mm of mercury at 70° for 16 hours: it loses not more than 12.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Dialyzable quaternary amines**—

*pH 9.2 Buffer*—Transfer 3.80 g of sodium borate decahydrate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Bromothymol blue solution*—Transfer 150 mg of bromothymol blue and 405 mg of sodium carbonate to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution*—Take 1 mL of 60% benzyltrimethylammonium chloride solution, accurately pipeted, and dilute quantitatively, and stepwise, with water to obtain a stock solution having a concentration of  $0.2 \pm 0.01$  mg per mL [NOTE—Prepare this solution fresh]. Cut a 20- to 25-cm piece of cellulose dialysis tubing\* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Pipet 5 mL of the stock solution into the tubing, add 5 mL of water, appropriately seal the open end, place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water, and stir the fluid for 16 hours to effect dialysis.

*Test solution*—Cut a 20- to 25-cm piece of cellulose dialysis tubing\* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Weigh  $2 \pm 0.01$  g of Cholestyramine Resin, and carefully transfer the specimen into the tubing, taking care to ensure that none adheres to the upper walls of the tubing. Add 10 mL of water to the contents of the tube, appropriately seal the open end, and place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water. Stir the fluid for 16 hours to effect dialysis.

*Procedure*—Pipet the following into each of three separators: Separator 1—5 mL of *Standard solution*, 5 mL of *pH 9.2 Buffer*, 1 mL of *Bromothymol blue solution*, and 10 mL

of chloroform; Separator 2—5 mL of *Test solution*, 5 mL of *pH 9.2 Buffer*, 1 mL of *Bromothymol blue solution*, and 10 mL of chloroform; Separator 3—5 mL of water, 5 mL of *pH 9.2 Buffer*, 1 mL of *Bromothymol blue solution*, and 10 mL of chloroform. Shake each separator vigorously for 1 minute, allow the phases to separate until the chloroform phase is clear, and collect the chloroform extracts in separate 25-mL volumetric flasks. Repeat the extraction process with a second 10-mL portion of chloroform, and combine with the previous extracts. Dilute each solution with chloroform to volume, if necessary, and mix. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using the solution from Separator 3 as the blank: the absorbance of the *Test solution* does not exceed that of the *Standard solution* (0.05% as benzyltrimethylammonium chloride).

**Chloride content**—To about 750 mg of Cholestyramine Resin, accurately weighed, add 100 mL of water and 50 mg of potassium nitrate. Add, with stirring, 2 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, and using a silver-glass electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not less than 13.0% and not more than 17.0% of Cl, calculated on the dried basis, is found.

**Exchange capacity**—

*Mobile phase*—Prepare a filtered and degassed mixture of 0.08 M monobasic potassium phosphate and acetonitrile (65:35). Adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Potassium phosphate buffer*—Transfer about 4 g of monobasic potassium phosphate and about 12 g of dibasic potassium phosphate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume, and mix.

*Sodium glycocholate solution*—Transfer about 15 g of sodium glycocholate to a 500-mL volumetric flask, and dissolve in and dilute with *Potassium phosphate buffer* to volume.

*Reference solution*—Pipet 4.0 mL of *Sodium glycocholate solution* into a 100-mL volumetric flask, and dilute with water to volume.

*Standard solution*—Transfer about 100 mg of USP Cholestyramine Resin RS, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

*System suitability solution*—Prepare a solution in water containing, in each mL, about 0.6 mg of sodium glycocholate and about 0.3 mg of taurodeoxycholic acid.

*Test solution*—Transfer about 100 mg of anhydrous Cholestyramine Resin, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between sodium glycocholate and taurodeoxycholic acid is not less than 1.5. Chromatograph the *Reference solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Reference solution*, the *Standard solution*, and the *Test*

\*A suitable tubing is Spectra/Por 1, Item # 132 665, available from Spectrum Laboratories, Inc. (www.spectrum.com), or equivalent.



solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sodium glycocholate absorbed on each g of the Resin taken by the formula:

$$M(2.5r_R - r_U)W_S / (2.5r_R - r_S)W_U$$

in which *M* is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS; *r<sub>R</sub>*, *r<sub>U</sub>*, and *r<sub>S</sub>* are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively; *W<sub>U</sub>* is the weight, in mg, of Cholestyramine Resin, calculated on the dried basis, taken to prepare the *Test solution*; and *W<sub>S</sub>* is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution*.

## Cholestyramine for Oral Suspension

» Cholestyramine for Oral Suspension is a mixture of Cholestyramine Resin with suitable excipients and coloring and flavoring agents. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of dried cholestyramine resin.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Cholestyramine Resin RS

**Identification**—Transfer a quantity of Cholestyramine for Oral Suspension, equivalent to about 500 mg of dried cholestyramine resin, to a suitable flask, add 100 mL of 0.1 N hydrochloric acid, stir to suspend the solid, and heat on a steam bath for 10 minutes. Filter, wash the residue with three 50-mL portions of water, and dry at 70° and at a pressure not exceeding 50 mm of mercury for 16 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cholestyramine Resin RS.

**Uniformity of dosage units** (905): meets the requirements for *Weight Variation*.

**Assay**—

*Mobile phase, Potassium phosphate buffer, Sodium glycocholate solution, Reference solution, Standard solution, System suitability solution, and Chromatographic system*—Proceed as directed in the test for *Exchange capacity* under *Cholestyramine Resin*.

*Test solution*—Transfer an accurately weighed portion of Cholestyramine for Oral Suspension, equivalent to about 100 mg of cholestyramine resin, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Reference solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cholestyramine resin per mg of Cholestyramine for Oral Suspension taken by the formula:

$$[M(2.5r_R - r_U)W_S] / [(2.5r_R - r_S)W_UQ]$$

in which *M* is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS; *r<sub>R</sub>*, *r<sub>U</sub>*, and *r<sub>S</sub>* are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively; *W<sub>S</sub>* is the weight, in mg, of USP Cholestyramine Resin

RS taken to prepare the *Standard solution*; *W<sub>U</sub>* is the weight, in mg, of Cholestyramine for Oral Suspension taken to prepare the *Test solution*; and *Q* is the quantity of sodium glycocholate absorbed per g of dried cholestyramine resin, as obtained in the test for *Exchange capacity* under *Cholestyramine Resin*.

## Chromic Chloride

CrCl<sub>3</sub> · 6H<sub>2</sub>O 266.45

Chromium chloride (CrCl<sub>3</sub>) hexahydrate;  
Chromium(3+) chloride hexahydrate [10060-12-5].

CrCl<sub>3</sub> 158.36  
Anhydrous [10025-73-7].

### DEFINITION

Chromic Chloride contains NLT 98.0% and NMT 101.0% of CrCl<sub>3</sub> · 6H<sub>2</sub>O.

### IDENTIFICATION

- **A.**  
**Analysis:** To 5 mL of a solution (1 in 250) in a test tube, add 1 mL of 5 N sodium hydroxide and 10 drops of 30% hydrogen peroxide, and heat gently for about 2 min.  
**Acceptance criteria:** A yellow color develops.
- **B.**  
**Analysis:** To 5 mL of a solution (1 in 250) in a test tube, add 5 drops of silver nitrate TS.  
**Acceptance criteria:** A white, curdy precipitate that is insoluble in nitric acid is formed.

### ASSAY

#### PROCEDURE

**Sample solution:** Dissolve 0.4 g of Chromic Chloride in 100 mL of water contained in a glass-stoppered, 500-mL conical flask. Add 5 mL of 5 N sodium hydroxide, and mix. Pipet slowly 4 mL of 30% hydrogen peroxide into the flask, and boil the solution for 5 min. Cool the solution slightly, and add 5 mL of nickel sulfate solution (1 in 20). Boil the solution until no more oxygen is evolved. Cool, and add 2 N sulfuric acid dropwise until the color of the solution changes from yellow to orange. Add to the flask a freshly prepared solution of 4 g of potassium iodide and 2 g of sodium bicarbonate in 100 mL of water, then add 6 mL of hydrochloric acid. Immediately insert the stopper in the flask, and allow to stand in the dark for 10 min. Rinse the stopper and the sides of the flask with a few mL of water.

**Analysis:** Titrate the liberated iodine with 0.1 N sodium thiosulfate VS to an orange color. Add 3 mL of starch TS, and continue the titration to a blue-green endpoint. Each mL of 0.1 N sodium thiosulfate is equivalent to 8.882 mg of chromium chloride hexahydrate (CrCl<sub>3</sub> · 6H<sub>2</sub>O).

**Acceptance criteria:** 98.0%–101.0%

### IMPURITIES

#### CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** Dissolve 2.0 g of Chromic Chloride in 10 mL of water. Add 1 mL of 3 N hydrochloric acid, and filter if necessary to obtain a clear solution. Wash the filter with two 5-mL portions of water, and dilute with water to 40 mL.

**Control solution:** Prepare as directed in the *Sample solution*, but use 1.0 g of the substance under test. After the filtration step, add 0.10 mL of 0.020 N sulfuric acid.

**Analysis:** To each solution add 3 mL of barium chloride TS, mix, and allow to stand overnight. Decant most of the supernatants, without disturbing the precipitates, but leaving twice the volume of liquid in the *Control*

solution as in the *Sample solution*. Dilute each solution with water to 25 mL, and sonicate for 1 min.

**Acceptance criteria:** Any turbidity in the *Sample solution* does not exceed that in the *Control solution* (0.01%).

• **IRON (241)**

**Test preparation:** Dissolve 1.0 g of Chromic Chloride in 100 mL of water. Transfer 10 mL of this solution to a 100-mL color comparison tube. Dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

**Analysis:** Proceed as directed for *Procedure*, except add 15 mL of butyl alcohol to the *Test preparation* and the *Standard Preparation* at the same time that the *Ammonium Thiocyanate Solution* is added. Shake for 30 s, and allow the layers to separate.

**Acceptance criteria:** The color in the upper butyl alcohol layer from the *Test preparation* is not darker than that from the *Standard Preparation* (NMT 0.01%).

**SPECIFIC TESTS**

• **INSOLUBLE MATTER**

**Sample:** 10 g

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water, cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 30 min, and filter through a tared filtering crucible of fine porosity. Rinse the beaker with hot water, passing the rinsings through the filter, and wash the filter with hot water until the last washing is colorless. Dry the filter at 105°.

**Acceptance criteria:** The weight of the residue does not exceed 1 mg (0.01%).

• **SUBSTANCES NOT PRECIPITATED BY AMMONIUM HYDROXIDE**

**Sample:** 2.0 g

**Analysis:** Dissolve the *Sample* in 80 mL of water, heat the solution to boiling, and add a slight excess of ammonium hydroxide. Continue heating to remove the excess ammonia. Cool, dilute with water to 100.0 mL, and mix. Pass through a retentive filter, and transfer 50.0 mL of the clear filtrate to an evaporating dish that previously has been ignited and tared. Add 0.5 mL of sulfuric acid to the filtrate, and evaporate on a steam bath to dryness. Heat gently to remove the excess acid, and ignite gently.

**Acceptance criteria:** The weight of the residue does not exceed 2.0 mg (0.20% as sulfate).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Chromic Chloride Injection

» Chromic Chloride Injection is a sterile solution of Chromic Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chromium (Cr).

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling—**Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

**USP Reference standards (11)—**

USP Endotoxin RS

**Identification—**The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 360 nm when tested as directed for *Procedure* in the *Assay*.

**Bacterial endotoxins (85)—**It contains not more than 16.70 USP Endotoxin Units per µg of chromium.

**pH (791):** between 1.5 and 2.5.

**Other requirements—**It meets the requirements under *Injections* (1).

**Assay—**

**Sodium chloride solution—**Dissolve 54 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

**Chromium stock solution—**Transfer 2.829 g of potassium dichromate, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. This solution contains 1000 µg of chromium per mL. Store in a polyethylene bottle.

**Standard preparations—**Pipet 10 mL of the *Chromium stock solution* into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL and 20.0 mL, respectively, of this stock solution to separate 100-mL volumetric flasks, and transfer 15.0 mL and 20.0 mL, respectively, of the stock solution to separate 50-mL volumetric flasks. Add 20 mL of *Sodium chloride solution* to each 100-mL volumetric flask, and 10 mL of *Sodium chloride solution* to each 50-mL volumetric flask, dilute the contents of each flask with water to volume, and mix. These *Standard preparations* contain, respectively, 1.0, 2.0, 3.0, and 4.0 µg of chromium per mL.

**Assay preparation—**Transfer an accurately measured volume of Injection, equivalent to about 60 µg of chromium, to a 25-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the volume of Injection taken, and add sufficient *Sodium chloride solution* to bring the total sodium chloride content of the flask to 135 mg. Dilute with water to volume, and mix.

**Procedure—**Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a chromium hollow-cathode lamp and an air-acetylene flame, using a 1:5 dilution of the *Sodium chloride solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, in µg per mL, of chromium in the *Assay preparation*. Calculate the quantity, in µg, of chromium in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

## Sodium Chromate Cr 51 Injection

Chromic acid (H<sub>2</sub><sup>51</sup>CrO<sub>4</sub>), disodium salt.

Disodium chromate (Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub>) [7775-11-3].

» Sodium Chromate Cr 51 Injection is a sterile solution of radioactive chromium (<sup>51</sup>Cr) processed in the form of sodium chromate in Water for Injection. For those uses where an isotonic solution is required, Sodium Chloride may be added in appropriate amounts as provided under *Injections* (1). Chromium 51 is produced by the neutron bombardment of enriched chromium 50.

Sodium Chromate Cr 51 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>51</sup>Cr as

sodium chromate expressed in megabecquerels (millicuries) per mL at the time indicated in the labeling. The sodium chromate content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. The specific activity is not less than 370 megabecquerels (10 millicuries) per mg of sodium chromate at the end of the expiry period. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of sodium chromate expressed in  $\mu\text{g}$  per mL; the amount of  $^{51}\text{Cr}$  as sodium chromate expressed as total megabecquerels (millicuries) and as megabecquerels (millicuries) per mL at the time of calibration; a statement to indicate whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of  $^{51}\text{Cr}$  is 27.8 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{51}\text{Cr}$  of known purity that exhibits a photopeak having an energy of 0.320 MeV.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.5 and 8.5.

**Radiochemical purity**—Place a volume of Injection, appropriately diluted so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* (621)), and immediately develop with a mixture of 5 parts of water, 2 parts of dilute alcohol (9.5 in 10), and 1 part of ammonium hydroxide. Air-dry the chromatogram, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the chromate band is not less than 90.0% of the total radioactivity. The  $R_f$  value for the chromate band falls within  $\pm 10\%$  of the value found for a known sodium chromate specimen when determined under identical conditions.

**Other requirements**—It meets the requirements under *Injections* (1), except that it is not subject to the recommendation on *Container Content*.

**Assay for sodium chromate**—

*Standard stock preparation*—Dissolve 3.735 g of potassium chromate in 1000 mL of water to obtain a solution having a known concentration of 1.0 mg per mL of chromium.

*Standard preparation*—Pipet 0.25, 0.50, 0.75, 0.100, 0.125, and 0.150 mL of the *Standard stock preparation*, accurately measured, into separate 100-mL volumetric flasks. To each flask add 0.42 mL of 0.1 N sodium bicarbonate, and dilute with water to volume to obtain solutions having final concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50  $\mu\text{g}$  of chromium per mL.

*Assay preparation*—Use the Injection.

*Blank preparation*—Transfer 0.42 mL of 0.1 N sodium bicarbonate to a 100-mL volumetric flask, and dilute with water to volume.

*Procedure*—Concomitantly determine the absorbances of the *Assay preparation*, the *Standard preparations*, and the *Blank preparation* at the chromium emission line at 357.7 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with a chromium hollow-cathode lamp and an air-acetylene (fuel-rich) flame using water to set the instrument to zero. Plot the absorbances of the *Standard preparations* and the *Blank preparation* versus concentration, in  $\mu\text{g}$  per mL, of chromium, and perform a regression analysis. A suitable standard curve will have an intercept between  $-0.002$  and  $+0.002$ , and a regression coefficient of not less than 0.99. Using the standard curve so obtained, determine the concentration, C, in  $\mu\text{g}$  per mL, of chromium in the Injection taken. Calculate the quantity of sodium chromate, in  $\mu\text{g}$  per mL, by the formula:

$$3.115C$$

in which 3.115 is the conversion factor.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly under Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Chromium Cr 51 Edetate Injection

Glycine, *N,N'*-1,2-ethanedithylbis[*N*-(carboxymethyl)]-, chromium-51 complex.  
(Ethylenedinitrilo)tetraacetic acid, chromium-51 complex [27849-89-4].

» Chromium Cr 51 Edetate Injection is a sterile solution containing radioactive chromium ( $^{51}\text{Cr}$ ) in the form of a complex of chromium (III) with edetic acid, present in excess. It is made isotonic by the addition of Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{51}\text{Cr}$  as edetate complex expressed in megabecquerels (or microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5.0 percent of the total radioactivity. It may contain a suitable preservative. It contains not more than 1 mg of chromium (Cr) per mL.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, at a temperature between  $2^\circ$  and  $8^\circ$ .

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{51}\text{Cr}$  as edetate complex expressed as total MBq (or mCi) and as MBq (or mCi) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of  $^{51}\text{Cr}$  is 27.8 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of

$^{51}\text{Cr}$  of known purity that exhibits a photopeak having an energy of 0.320 MeV.

**Bacterial endotoxins** (85): not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 3.5 and 6.5.

**Radiochemical purity—**

*Electrolyte solution*—Dissolve 0.2 g of barbital sodium and 10 g of sodium nitrate in water to make 1000 mL.

*Procedure* (see *Electrophoresis* (726))—Soak a 2.5-cm  $\times$  17.0-cm  $\times$  0.22-mm cellulose strip in 100 mL of *Electrolyte solution* for 10 to 60 minutes. Remove the strip with forceps, and blot to remove excess solution. Attach the strip to the support bridge of an electrophoresis chamber containing *Electrolyte solution*. Apply to the strip about 10  $\mu\text{L}$  of Injection as a 3-mm band at a position 10 cm from the cathode. Attach the chamber cover, and perform the electrophoresis at 30 V per cm, using a stabilized current. Remove the strip from the chamber, and blot the ends. Using a suitable scanner and counting assembly, determine the radioactivity distribution: chromium  $^{51}\text{Cr}$  edetate moves about 5 cm towards the anode; and  $^{51}\text{Cr}$  chromic ion moves about 7 cm towards the cathode. The radioactivity of the chromium  $^{51}\text{Cr}$  edetate band is not less than 95% of the total radioactivity.

**Radionuclidic purity**—Using a suitable gamma-ray spectrometer (see *Selection of a Counting Assembly* in the Assay section under *Radioactivity* (821)), determine the radioactivity of each radionuclidic impurity observed in the gamma-ray spectrum: not more than 0.1% of any individual impurity is found; and not more than 0.3% of total impurities is found.

**Chemical purity**—Using a validated limit test and a known analytical technique, demonstrate the absence of any ingredients and reagents employed in the synthetic process.

**Limit of free chromium—**

*Standard solution*—Dissolve 0.96 g of chromium potassium sulfate dodecahydrate and 2.87 g of edetate disodium in 50 mL of water, boil for 10 minutes, cool, adjust with 0.2 M sodium hydroxide to a pH between 3.5 and 6.5, and dilute with water to 100.0 mL to obtain a solution having a known concentration of about 1 mg of chromium per mL.

*Test solution*—Use the Injection.

*Procedure*—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the wavelength of maximum absorbance at about 560 nm, with a suitable spectrophotometer, using water as the blank: the absorbance of the *Test solution* is not more than that of the *Standard solution*.

**Other requirements**—It meets the requirements under *Injections* (1), except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system.

110.0 percent of the labeled potency, as determined by the Assay.

**Packaging and storage**—Preserve in tight containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Chymotrypsin RS

USP Trypsin Crystallized RS

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Pseudomonas aeruginosa* and *Salmonella* species and *Staphylococcus aureus*.

**Loss on drying** (731)—Dry it in a vacuum oven at 60° for 4 hours: it loses not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 2.5%.

**Limit of trypsin—**

*Chymotrypsin solution*—Dissolve 100 mg in 10.0 mL of water.

*pH 8.1 Tris(hydroxymethyl)aminomethane buffer, 0.08 M*—Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane, adjust with 1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

*Substrate solution*—Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *pH 8.1 Tris(hydroxymethyl)aminomethane buffer, 0.08 M*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red-methylene blue TS, and dilute with water to volume.

*Procedure*—[NOTE—Determine the suitability of the substrate by performing the *Procedure* using the appropriate amount of USP Trypsin Crystallized RS in place of the test specimen.] By means of a micropipet, transfer 50  $\mu\text{L}$  of *Chymotrypsin solution* to a depression on a white spot plate. Add 0.2 mL of *Substrate solution*: no purple color develops within 3 minutes (not more than 1% of trypsin).

**Assay—**

*pH 7.0 phosphate buffer, fifteenth-molar*—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of dibasic sodium phosphate solution. If necessary, adjust to a pH of 7.0 by the dropwise addition of dibasic sodium phosphate solution.

*Substrate solution*—Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying Chymotrypsin, in about 50 mL of *pH 7.0 phosphate buffer, fifteenth-molar*, with warming. When the solution is cool, dilute with additional pH 7.0 buffer to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

*Chymotrypsin solution*—Dissolve a sufficient quantity of Chymotrypsin, accurately weighed, in 0.0012 N hydrochloric acid to yield a solution containing between 12 and 16 USP Chymotrypsin Units per mL. The dilution is correct if, during the conduct of the assay, there is a change in absorbance of between 0.008 and 0.012 in each 30-second interval.

*Procedure*—[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Procedure* using USP Chymotrypsin RS in place of the assay specimen.] Conduct the assay in a suitable spectrophotometer equipped to maintain a temperature of  $25 \pm 0.1^\circ$  in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance in order to ensure that the temperature does not change by more than  $0.5^\circ$ . Pipet 0.2 mL of 0.0012 N hydrochloric acid and 3.0 mL of *Substrate solution* into a 1-cm cell. Place this cell in the spectrophotometer, and ad-

## Chymotrypsin

Chymotrypsin.

Chymotrypsin [9004-07-3].

» Chymotrypsin is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus* Linné (Fam. Bovidae). It contains not less than 1000 USP Chymotrypsin Units in each mg, calculated on the dried basis, and not less than 90.0 percent and not more than

just the instrument so that the absorbance will read 0.200 at 237 nm. Pipet 0.2 mL of *Chymotrypsin solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for not less than 3 minutes, repeat the test and, if necessary, use a lower concentration. The duplicate determination at the same dilution matches the first determination in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this assay. Calculate the number of USP Chymotrypsin Units per mg taken by the formula:

$$(A_2 - A_1) / (0.0075TW)$$

in which  $A_2$  is the absorbance straight-line initial reading,  $A_1$  is the absorbance straight-line final reading,  $T$  is the elapsed time, in minutes, between the initial and final readings, and  $W$  is the weight, in mg, of Chymotrypsin in the volume of solution used in determining the absorbance.

### Chymotrypsin for Ophthalmic Solution

» Chymotrypsin for Ophthalmic Solution is sterile Chymotrypsin. When constituted as directed in the labeling, it yields a solution containing not less than 80.0 percent and not more than 120.0 percent of the labeled potency.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.

**Completeness of solution** (641)—It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

**Identification**—Prepare a *Substrate solution* as follows. Transfer 237.0 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, to a 100-mL volumetric flask, add 2 mL of alcohol, and swirl until solution is effected. Add 20 mL of *pH 7.0 phosphate buffer, fifteenth-molar*, prepared as directed in the *Assay* under *Chymotrypsin*, add 1 mL of methyl red–methylene blue TS, and dilute with water to volume. If necessary, adjust to a pH of 7.0 by the dropwise addition of monobasic potassium phosphate solution, prepared by dissolving 4.54 g of monobasic potassium phosphate in sufficient water to yield 500 mL of solution. Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 1 mL of saline TS, transfer 0.2 mL to a suitable dish, and add 0.2 mL of *Substrate solution*: a purple color is produced within 3 minutes. [NOTE—distinction from trypsin, which produces no purple color within 3 minutes.]

**Uniformity of dosage units** (905): meets the requirements.

*Procedure for content uniformity*—Assay 10 individual units as directed in the *Assay*, and calculate the average of the 10 results. The average is not less than 80.0% and not more than 120.0% of the labeled amount. The contents of not more than 2 vials deviate by more than 10% from the average content. The contents of none of the vials deviate by more than 15% from the average.

**pH** (791): between 4.3 and 8.7, in the solution constituted as directed in the labeling.

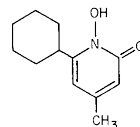
**Other requirements**—It meets the requirements of the test for *Limit of trypsin* under *Chymotrypsin*. It also meets the requirements for *Sterility Tests* (71).

**Assay**—Proceed with Chymotrypsin for Ophthalmic Solution as directed in the *Assay* under *Chymotrypsin*, but use the following as the *Chymotrypsin solution*: dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 5.0 mL of 0.0012 N hydrochloric acid. Dilute an accurately measured volume ( $V$ , in mL) of this solution, equivalent to about 300 USP Chymotrypsin Units, with 0.0012 N hydrochloric acid to 25.0 mL. Calculate the number of USP Chymotrypsin Units per vial taken by the formula:

$$300(5 / V)(A_2 - A_1) / [T(2.4)(0.0075)]$$

in which  $A_2$  is the absorbance straight-line initial reading,  $A_1$  is the absorbance straight-line final reading,  $T$  is the elapsed time in minutes between the initial and final readings, and 2.4 is the number of USP Chymotrypsin Units in the solution on which the absorbance was determined.

### Ciclopirox



$C_{12}H_{17}NO_2$  207.27  
2(1*H*)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-  
6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone  
[29342-05-0].

» Ciclopirox contains not less than 98.0 percent and not more than 101.0 percent of  $C_{12}H_{17}NO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at a temperature between 15° and 30°.

**USP Reference standards** (11)—

USP Ciclopirox RS

USP Ciclopirox Related Compound A RS

3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.

USP Ciclopirox Related Compound B RS

6-Cyclohexyl-4-methyl-2-pyrone.

**Identification, Infrared Absorption** (197K).

**Loss on drying** (731)—Dry it in vacuum to constant weight: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 0.001%.

**Related compounds**—[NOTE—Carry out the operations avoiding exposure to actinic light. All materials in direct connection with Ciclopirox, like column materials, reagents, solvents, and others should contain only very low amounts of extractable metal cations.]

*Mobile phase*—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770:230:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Rinsing solution*—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500 : 500 : 1:1).

**Standard stock solution**—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and *Mobile phase* solution (approximate ratio, 1:7). Further dilute with *Mobile phase* to obtain a solution having a known final concentration of about 1.5 mg each per mL.

**Standard solution A**—Dilute 1.0 mL of *Standard stock solution* to 200.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Standard solution B**—Dilute 2.0 mL of *Standard solution A* to 10.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Test solution**—Dissolve 30 mg of Ciclopirox, accurately weighed, in a mixture of 2 mL of acetonitrile and 15 mL of *Mobile phase*. If necessary, use an ultrasonic bath. Dilute with *Mobile phase* to 20.0 mL.

**Resolution solution**—Mix 5 mL of *Standard stock solution* with 5 mL of the *Test solution*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm × 8-cm column that contains packing L10. [NOTE—Ciclopirox related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1H)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure* at 298 nm: the resolution, *R*, between the ciclopirox related compound B peak and ciclopirox peak is not less than 2.0. Chromatograph the *Standard solution B*, and record the peak responses as directed for *Procedure* at 298 nm: the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure* at 298 nm: the tailing factor for the ciclopirox peak is less than 2.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with the *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1H)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5%). The sum of responses at 298 nm of the peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5%). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1%).

**Assay**—Dissolve 150 mg of Ciclopirox, accurately weighed, in 20 mL of methanol. Add 20 mL of water, mix, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Carry out a blank test. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 20.73 mg of C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>.

## Ciclopirox Topical Solution

### DEFINITION

Ciclopirox Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ciclopirox (C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

**Buffer:** Transfer 5.25 g of citric acid and 25 mL of 0.1 M edetate disodium to a 1-L volumetric flask, and dilute with water to volume. Adjust with 8.5% diluted sodium hydroxide solution to a pH of 6.5.

**Mobile phase:** Acetonitrile and *Buffer* (35:65)

**Standard solution:** 0.2 mg/mL of USP Ciclopirox RS and 1 µg/mL each of USP Ciclopirox Related Compound B RS and USP Ciclopirox Related Compound C RS in methanol

**Sample solution:** Equivalent to 0.2 mg/mL of ciclopirox in methanol from Topical Solution. Pass through a filter of 0.45-µm pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 303 nm

**Column:** 4-mm × 12.5-cm; 5-µm packing L1

**Column temperature:** 30 ± 5°

**Flow rate:** 0.9 mL/min

**Run time:** 5 times the retention time of the major peak

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—For information only, see *Table 1* for relative retention times of impurities.]

#### Suitability requirements

**Resolution:** NLT 3.0 between ciclopirox and ciclopirox related compound B; and NLT 3.0 between ciclopirox related compound C and ciclopirox

**Tailing factor:** NMT 2.0 for the ciclopirox peak

**Relative standard deviation:** NMT 2.0% for the ciclopirox peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ciclopirox (C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Ciclopirox RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of ciclopirox in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- MINIMUM FILL <755>:** Meets the requirement

### IMPURITIES

#### ORGANIC IMPURITIES

**Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_T$  = sum of responses of all the peaks in the *Sample solution* $F$  = relative response factor (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

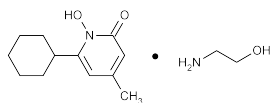
Compound	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ciclopirox related compound C	0.54	1.3	0.5
Ciclopirox	1.0	—	—
Ciclopirox related compound B <sup>a</sup>	1.87	—	—
Any unspecified individual impurity	—	1.0	0.2
Total impurities	—	—	1.2

<sup>a</sup> Process impurity already monitored in the drug substance.**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^2$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^1$  cfu/g.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Ciclopirox RS
  - USP Ciclopirox Related Compound B RS  
6-Cyclohexyl-4-methyl-2-pyrone.  
 $C_{12}H_{16}O_2$  192.25
  - USP Ciclopirox Related Compound C RS  
6-Cyclohexyl-4-methylpyridin-2(1*H*)-one.  
 $C_{12}H_{17}NO$  191.27

**Ciclopirox Olamine** $C_{12}H_{17}NO_2 \cdot C_2H_7NO$  268.352(1*H*)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-, compound with 2-aminoethanol (1:1).6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone compound with 2-aminoethanol (1:1) [41621-49-2].

» Ciclopirox Olamine contains not less than 97.5 percent and not more than 101.5 percent of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in tight containers, protected from light. Store between 5° and 25°.

**USP Reference standards** (11)—

USP Ciclopirox Olamine RS

USP Ciclopirox Related Compound A RS

3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.

USP Ciclopirox Related Compound B RS

6-Cyclohexyl-4-methyl-2-pyrone.

**Identification,** *Infrared Absorption* (197K).**pH** (791): between 8.0 and 9.0, in a mixture with water (1:100).**Residue on ignition** (281): not more than 0.1%.**Heavy metals,** *Method II* (231): not more than 0.001%.

**Monoethanolamine content**—Dissolve about 300 mg, accurately weighed, in 25 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 6.108 mg of  $C_2H_7NO$ . The content of monoethanolamine ( $C_2H_7NO$ ) is not less than 223 mg and not more than 230 mg per g of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ) found in the Assay.

**Related compounds**—[NOTE—Carry out the operations avoiding exposure to actinic light. All materials that are in direct contact with Ciclopirox Olamine (e.g., column materials, reagents, solvents, etc.) should contain only very low amounts of extractable metal cations.]

**Mobile phase**—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770:230:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Rinsing solution**—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500:500:1:1).

**Standard stock solution**—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and *Mobile phase* solution (approximate ratio, 1:7). Further dilute with *Mobile phase* to obtain a solution having a known final concentration of about 1.5 mg of each per mL.

**Standard solution A**—Dilute 1.0 mL of *Standard stock solution* to 200.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Standard solution B**—Dilute 2.0 mL of *Standard solution A* to 10.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Test solution**—Dissolve 40 mg of Ciclopirox Olamine, accurately weighed, in a mixture of 2 mL of acetonitrile, 20  $\mu$ L of glacial acetic acid, and 15 mL of *Mobile phase*. If necessary, use an ultrasonic bath to dissolve. Dilute with *Mobile phase* to 20.0 mL, and mix.

**Resolution solution**—Mix 5 mL of *Standard stock solution* with 5 mL of the *Test solution*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm  $\times$  8-cm column that contains packing L10. [NOTE—Ciclopirox related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution* at 298 nm, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the ciclopirox related compound B peak and the ciclopirox peak is not less than 2.0. Chromatograph *Standard solution B* at 298 nm, and record the peak responses as directed for *Procedure*: the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution* at 298 nm, and record the peak responses as directed for *Procedure*: the tailing factor for the ciclopirox peak is less than 2.0.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). The sum of responses at 298 nm of the impurity peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1% with reference to ciclopirox).

**Assay**—Dissolve 200 mg of Ciclopirox Olamine, accurately weighed, in 2 mL of methanol. Add 38 mL of water, mix, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 26.84 mg of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

## Ciclopirox Olamine Cream

» Ciclopirox Olamine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in collapsible tubes, and store at controlled room temperature.

### USP Reference standards (11)—

USP Benzyl Alcohol RS

USP Ciclopirox Olamine RS

**Identification**—Dilute 4 mL of the *Assay preparation* obtained as directed in the *Assay* with a mixture of methanol and 6.25 N sodium hydroxide (123:2) to make 100 mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution prepared from the *Standard preparation* obtained as directed in the *Assay*, concomitantly measured.

**Minimum fill** (755): meets the requirements.

**pH** (791)—Add 15 mL of boiling water, previously adjusted with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6 to 7, to 3.5 g of Cream in a 50-mL centrifuge tube. Place a cap on the tube, and shake vigorously until an emulsion is formed. Loosen the cap, and heat the tube on a steam bath for 10 minutes. Allow to cool, centrifuge, and determine the pH of the aqueous phase: the pH is between 5.0 and 8.0.

### Content of benzyl alcohol (if present)—

**Solvent mixture**—Mix chloroform and methanol (4:1).

**Internal standard solution**—Prepare a solution of 1-nonyl alcohol in *Solvent mixture* containing about 1.75 mg per mL.

**Standard preparation**—Dilute an accurately weighed quantity of USP Benzyl Alcohol RS, quantitatively and stepwise, with *Solvent mixture* to obtain a solution having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Test preparation**—Transfer 1.0 g of Cream to a 50-mL volumetric flask, add about 30 mL of *Solvent mixture*, and mix. Add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix to obtain a clear solution.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and contains a 4-mm  $\times$  2-m glass column packed with 3% phase G3 on 100- to 120-mesh support S1AB. The column is maintained at a temperature of about 100°, the injection port and detector temperatures are maintained at about 315°, and nitrogen is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the peaks is not less than 1.6; the tailing factor for the benzyl alcohol peak and the internal standard peak is not greater than 3.5; and the relative standard deviation for replicate injections is not more than 3%.

**Procedure**—Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—After six injections, raise the column temperature to about 300° for about 5 minutes, then cool to 100°.] Calculate the percentage of benzyl alcohol in the Cream taken by the formula:

$$C(R_U / R_S),$$

in which  $C$  is the concentration, in mg per mL, of benzyl alcohol in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the benzyl alcohol peak to the internal standard peak obtained from the *Test preparation* and the *Standard preparation*, respectively: between 90.0% and 110.0% of the claimed amount is present.

### Assay—

**Ferrous sulfate solution**—Transfer 600 mg of ferrous sulfate to a 25-mL volumetric flask. Add 0.6 mL of glacial acetic acid, dilute with water to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ciclopirox Olamine RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of ciclopirox olamine, to a 50-mL volumetric flask, add 25 mL of methanol, and shake by mechanical means for about 10 minutes. Dilute with methanol to volume, mix, centrifuge, and use the supernatant.

**Procedure**—Transfer 4.0 mL of the *Standard preparation*, 4.0 mL of the *Assay preparation*, and 4.0 mL of methanol to provide a blank, to separate 25-mL volumetric flasks. Add 15 mL of methanol to each flask, and mix. Then to each flask add 1.0 mL of *Ferrous sulfate solution*, mix, dilute with methanol to volume, and mix. Store the flasks in the dark for 1 hour. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* against the blank in 1-cm cells at the wavelength of maximum absorbance at about 440 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of



ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ) in each g of the Cream taken by the formula:

$$50(C/W)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Ciclopirox Olamine RS in the *Standard preparation*; W is the weight, in g, of Cream taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ciclopirox Olamine Topical Suspension

» Ciclopirox Olamine Topical Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Benzyl Alcohol RS

USP Ciclopirox Olamine RS

**Identification**—It responds to the *Identification* test under *Ciclopirox Olamine Cream*.

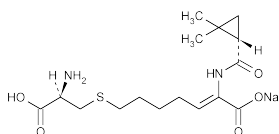
**Minimum fill** (755): meets the requirements.

**pH** (791)—Proceed as directed for pH under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

**Content of benzyl alcohol**—Proceed as directed for *Content of benzyl alcohol* under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

**Assay**—Proceed as directed in the *Assay* under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

## Cilastatin Sodium



$C_{16}H_{25}N_2NaO_5S$  380.43

2-Heptenoic acid, 7-[(2-amino-2-carboxyethyl)thio]-2-[[[(2,2-dimethylcyclopropyl)carbonyl]amino]-, monosodium salt, [R-[R\*,S\*-(Z)]]-

Sodium (Z)-7-[[[(R)-2-amino-2-carboxyethyl]thio]-2-[(S)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoate [81129-83-1].

» Cilastatin Sodium contains not less than 98.0 percent and not more than 101.5 percent of  $C_{16}H_{25}N_2NaO_5S$ , calculated on the anhydrous and solvent-free basis.

**Packaging and storage**—Preserve in *Containers for Sterile Solutions* as described under *Injections* (1), and store in a cold place.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile.

**USP Reference standards** (11)—

USP Cilastatin Ammonium Salt RS

USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak for cilastatin in the chromatogram of the *Test solution*, as obtained in the test for *Chromatographic purity*, corresponds to that in the chromatogram of a similar preparation of USP Cilastatin Ammonium Salt RS.

**B:** Ignite a small portion of it on a platinum wire in a nonluminous flame: an intense yellow color is imparted to the flame.

**Specific rotation** (781S): between +41.5° and +44.5°, on the anhydrous and solvent-free basis.

*Test solution:* 10 mg per mL, in a mixture of methanol and hydrochloric acid (120:1).

**Bacterial endotoxins** (85)—Where the label states that Cilastatin Sodium is sterile, it contains not more than 0.17 USP Endotoxin Unit per mg of cilastatin.

**Sterility** (71)—Where the label states that Cilastatin Sodium is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen dissolved in 200 mL of *Fluid A* being used.

**pH** (791): between 6.5 and 7.5, in a solution (1 in 100).

**Water**, *Method I* (921): not more than 2.0%.

**Heavy metals**, *Method II* (231): 0.002%.

**Limit of solvents**—

*Internal standard solution*—Transfer 0.5 mL of *n*-propyl alcohol to a 1000-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution*—Transfer 2.0 mL of acetone, 0.50 mL of methanol, and 0.50 mL of mesityl oxide to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 10-mL volumetric flask, dilute with water to volume, and mix. This solution contains 316 µg of acetone, 79 µg of methanol, and 86 µg of mesityl oxide per mL.

*Test solution*—Transfer about 200 mg of Cilastatin Sodium, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution* and about 5 mL of water, and dissolve by shaking. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column, the internal wall of which is coated with a 1.0-µm film of liquid phase G16. The column temperature is maintained at 50° for 2.5 minutes, then increased at a rate of 8° per minute to 70°, and maintained at 70° for 0.5 minute; the injection port temperature is maintained at 160°; the detector temperature is maintained at 250°; and helium is used as the carrier gas at a flow rate of about 9 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.26 for acetone, 0.35 for methanol, 0.67 for *n*-propyl alcohol, and 1.0 for mesityl oxide; and the relative standard deviation for replicate injections, determined from peak area ratios of each analyte to *n*-propyl alcohol, is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, using the solvent (water) flush technique; record the chromatograms; and measure the areas for the acetone, methanol, *n*-propyl alcohol, and mesityl oxide peaks. Calculate the percentages of acetone, methanol, and mesityl

oxide in the portion of Cilastatin Sodium taken by the formula:

$$(C/W)(R_U / R_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of the appropriate analyte in the *Standard solution*;  $W$  is the quantity, in mg, of Cilastatin Sodium taken to prepare the *Test solution*; and  $R_U$  and  $R_S$  are the peak area ratios of the corresponding analyte to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 1.0% of acetone is found; not more than 0.5% of methanol is found; and not more than 0.4% of mesityl oxide is found.

#### Chromatographic purity—

**Solvent**—Use water.

**Solution A**—Prepare a mixture of dilute phosphoric acid (1 in 1000) and acetonitrile (700:300), pass through a filter having a 0.5- $\mu\text{m}$  or finer porosity, and degas.

**Solution B**—Use dilute phosphoric acid (1 in 1000). Pass through a filter having a 0.5- $\mu\text{m}$  or finer porosity, and degas.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Test solution**—Prepare a solution of Cilastatin Sodium in *Solvent* having a concentration of about 1.6 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.5-mm  $\times$  25-cm column containing packing L1. The column is maintained at a constant temperature of about 50°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	15	85	equilibration
0–30	15→100	85→0	linear gradient

Chromatograph the *Test solution*, and measure the peak responses as directed for *Procedure*: the capacity factor,  $K'$ , is not less than 10; the column efficiency determined from the cilastatin peak is not less than 3000 theoretical plates; and the tailing factor is not more than 4.5.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Test solution* and the *Solvent* into the chromatograph, record the chromatograms, and measure the areas of the peaks. Calculate the chromatographic purity, in percentage, of the portion of Cilastatin Sodium taken by the formula:

$$100r_C / (r_T - r_B - r_A)$$

in which  $r_C$  is the area of the cilastatin peak obtained from the *Test solution*;  $r_T$  is the sum of the areas of all the peaks obtained from the *Test solution*;  $r_B$  is the sum of the areas of all the peaks obtained from the *Solvent*; and  $r_A$  is the response of the peak, if any, of nonretained substances, such as acetone, at the solvent front obtained from the *Test solution*: not less than 98.5% is found. Calculate the percentage of each impurity in the portion of Cilastatin Sodium taken by the formula:

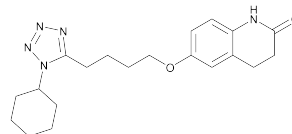
$$100r_i / (r_T - r_B - r_A)$$

in which  $r_i$  is the peak area for each impurity in the chromatogram obtained from the *Test solution* and the other terms are as defined above: not more than 0.5% of any individual impurity is found.

**Assay**—Transfer about 300 mg of Cilastatin Sodium, accurately weighed, to a suitable beaker, add 30 mL of methanol, and dissolve by swirling. Add 5 mL of water, and titrate potentiometrically with 0.1 N hydrochloric acid to a pH of

about 3. Then titrate with 0.1 N sodium hydroxide until three inflection points have been observed. Calculate the titer difference, in mL, between the first and third inflection points. Each mL of 0.1 N sodium hydroxide is equivalent to 19.022 mg of  $\text{C}_{16}\text{H}_{25}\text{N}_2\text{NaO}_5\text{S}$ .

## Cilostazol



$\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$  369.46

2(1*H*)-Quinolinone, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-carbostyryl [73963-72-1].

» Cilostazol contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store at room temperature.

#### USP Reference standards <11>—

USP Cilostazol RS

USP Cilostazol Related Compound A RS

6-Hydroxy-3,4-dihydro-1*H*-quinolin-2-one.

$\text{C}_9\text{H}_9\text{NO}_2$  163.17

USP Cilostazol Related Compound B RS

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1*H*-quinolin-2-one.

$\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_2$  367.45

USP Cilostazol Related Compound C RS

1-(4-(5-Cyclohexyl-1*H*-tetrazol-1-yl)butyl)-

6-(4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one.

$\text{C}_{31}\text{H}_{43}\text{N}_9\text{O}_3$  589.73

#### Identification—

**A: Infrared Absorption** <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** <731>—Dry it at 110° for 3 hours: it loses not more than 0.3% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

#### Chloride <221>—

**Test solution**—Dissolve 0.5 g of Cilostazol in 40 mL of dimethylformamide, add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

**Control solution**—To 0.25 mL of 0.01 M hydrochloric acid add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

**Procedure**—Add 1 mL of silver nitrate TS to the *Test solution* and to the *Control solution*, mix well, and allow to stand for 5 minutes, protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the *Test solution* is not more than that of the *Control solution* (0.018%).

**Heavy metals, Method II** <231>: 0.001%.

#### Related compounds—

*Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve accurately weighed quantities of USP Cilostazol RS and USP Cilostazol Related Compound C RS in acetonitrile, with sonication if necessary, to obtain a solution having known concentrations of about 0.5 mg per mL of each component. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution, stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 0.4 µg per mL of each component.

**Test solution**—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, with sonication if necessary. Dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of cilostazol related compound C by the formula:

$$0.1(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in µg per mL, of cilostazol related compound C in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Cilostazol in the *Test solution*;  $r_U$  is the peak response for cilostazol related compound C obtained from the *Test solution*; and  $r_S$  is the peak response for cilostazol related compound C obtained from the *Standard solution*. Calculate the percentage of other impurities by the formula:

$$0.1(1/F)(C_S / C_T)(r_U / r_S)$$

in which  $F$  is the relative response factor from Table 1;  $C_S$  is the concentration, in µg per mL, of cilostazol in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of cilostazol in the *Test solution*;  $r_U$  is the peak response for any other impurity obtained from the *Test solution*; and  $r_S$  is the peak response for cilostazol obtained from the *Standard solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor ( $F$ )	Limit (%)
Cilostazol related compound A <sup>1</sup>	0.2	1.7	0.1
Cilostazol related compound B <sup>2</sup>	0.9	0.58	0.1
Cilostazol	1.0	1.0	—
Cilostazol related compound C <sup>3</sup>	1.9	—	0.1
Any other individual impurity	—	1.0	0.1

<sup>1</sup> 6-Hydroxy-3,4-dihydro-1H-quinolin-2-one

<sup>2</sup> 6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)butoxy]-1H-quinolin-2-one

<sup>3</sup> 1-(4-(5-Cyclohexyl-1H-tetrazol-1-yl)butyl)-6-(4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one

In addition to not exceeding the limits for impurities in Table 1, not more than 0.4% of total impurities is found.

#### Assay—

**Diluent**—Use a mixture of water and acetonitrile (60:40).

**Solution A**—Use a mixture of water and acetonitrile (70:30).

**Solution B**—Use a mixture of water and acetonitrile (50:50).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Diluent* having known concentrations of about 0.05 mg per mL each of USP Cilostazol RS, USP Cilostazol Related Compound A RS, and USP Cilostazol Related Compound B RS.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cilostazol RS in acetonitrile, with sonication if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

**Assay preparation**—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, sonicate if necessary, dilute with water to volume, and mix. Transfer 1 mL of this solution to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 3.5-µm packing L7. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6.5	100→50	0→50	linear gradient
6.5–10	50→0	50→100	linear gradient
10–20	0	100	isocratic
20–20.1	0→100	100→0	linear gradient
20.1–28	100	0	re-equilibration

Chromatograph the *System suitability solution*, identify the components using Table 1, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cilostazol related compound B and cilostazol is not less than 3.0; the tailing factor for the cilostazol peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{27}N_5O_2$  in the portion of Cilostazol taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of cilostazol in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cilostazol Tablets

### DEFINITION

Cilostazol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cilostazol ( $C_{20}H_{27}N_5O_2$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197S)

**Standard solution:** 100 mg/mL of USP Cilostazol RS in chloroform

**Sample solution:** Transfer the equivalent of 100 mg of cilostazol from finely powdered Tablets into a glass container. Add 1 mL of chloroform, shake for 1 min, and pass through a suitable filter of 0.5-µm or finer pore size.

- **B.** The retention time of the cilostazol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile, methanol, and water (7:3:10)

**Internal standard solution:** 4 mg/mL of benzophenone in methanol

**Standard solution:** 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of *Internal standard solution* in methanol

**Sample solution:** Transfer the equivalent of 50 mg of cilostazol from powdered Tablets (NLT 20) into a suitable volumetric flask and add an appropriate quantity of *Internal standard solution*. Dilute with methanol to obtain a solution of 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of the internal standard. Pass a portion of this solution through a membrane filter of 0.5-μm or finer pore size, and use the filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 9.0 between the cilostazol and benzophenone peaks, eluted in this order

**Relative standard deviation:** NMT 1.5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cilostazol (C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cilostazol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cilostazol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cilostazol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cilostazol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****Change to read:**• **DISSOLUTION** <711>**Test 1**

**Medium:** 0.30% sodium lauryl sulfate in water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution:** 0.28 mg/mL (ERR 1-Jul-2012) of USP Cilostazol RS in methanol. Dilute this solution with *Medium* to obtain a solution with a final concentration of about 5.6 μg/mL (ERR 1-Jul-2012)

**Sample solution:** Pass NLT 20 mL of the solution under test through a suitable filter of 0.45-μm pore size, discarding the first 10 mL. Dilute with *Medium* to obtain a final theoretical concentration of about 5.6 μg/mL (ERR 1-Jul-2012) considering complete dissolution of the label claim.

**Wavelength:** UV 257 nm

**Path length:** 1 cm

**Blank:** *Medium*

Calculate the percentage of cilostazol dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of cilostazol is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.3% sodium lauryl sulfate in water; 900 mL, deaerated

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Standard solution:** Prepare a solution containing 1.1 mg/mL of USP Cilostazol RS in methanol. Dilute this solution with 0.5% sodium lauryl sulfate in water to obtain a final concentration of (L/900) mg/mL, where L is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Wavelength:** UV 258 nm

**Path length:** 0.2 cm

**Blank:** *Medium*

**Tolerances:** NLT 75% (Q) of the labeled amount of cilostazol is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** 0.3% sodium lauryl sulfate in water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution, Sample solution, Wavelength,**

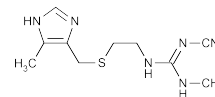
**Path length, and Blank:** Proceed as directed for *Test 1*.

**Tolerances:** NLT 70% (Q) of the labeled amount of cilostazol is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** <11>  
USP Cilostazol RS

**Cimetidine**

C<sub>10</sub>H<sub>16</sub>N<sub>6</sub>S 252.34

Guanidine, N'-cyano-N-methyl-N'-[2-[[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]-2-cyano-1-methyl-3-[[[(5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine [51481-61-9].

» Cimetidine contains not less than 98.0 percent and not more than 102.0 percent of C<sub>10</sub>H<sub>16</sub>N<sub>6</sub>S, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Cimetidine RS

**Identification**—**A:** *Infrared Absorption* (197K).**B:** The UV absorption spectrum of a solution (1 in 80,000) in 0.1 N sulfuric acid exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Cimetidine RS, concomitantly measured.**Melting range** (741): between 139° and 144°.**Loss on drying** (731)—Dry it at 110° for 2 hours: it loses not more than 1.0% of its weight.**Residue on ignition** (281): not more than 0.2%.**Heavy metals, Method II** (231): 0.002%.**Chromatographic purity**—**Mobile phase**—Mix 240 mL of methanol, 0.3 mL of phosphoric acid (85%), 940 mg of sodium 1-hexanesulfonate, and sufficient water to make 1 L. Filter before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).**Standard solution**—Prepare a solution of USP Cimetidine RS in *Mobile phase* having a concentration of 0.80 µg per mL.**Test solution**—Transfer 100.0 mg of Cimetidine, accurately weighed, to a 250-mL volumetric flask, dissolve in about 50 mL of *Mobile phase*, and dilute with *Mobile phase* to volume. Mix, sonicate for 15 minutes, and mix again.**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak response as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 3.0; the number of theoretical plates,  $n$ , is not less than 2000; and the relative standard deviation of the response for replicate injections is not more than 2.0%.**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Cimetidine taken by the formula:

$$100(0.001 C_s / C_u)(r_u / r_s)$$

in which  $C_s$  is the concentration, in µg per mL, of cimetidine in the *Standard solution*, the multiplier of 0.001 is for conversion of µg per mL to mg per mL;  $C_u$  is the concentration, in mg per mL, of Cimetidine in the *Test solution*;  $r_u$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the response of the cimetidine peak obtained from the *Standard solution*: not more than 0.2% of any single impurity is found, and not more than 1.0% of total impurities is found.**Assay**—**Mobile phase**—Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).**Standard preparation**—Dissolve an accurately weighed quantity of USP Cimetidine RS in a mixture of water and methanol (4:1) to obtain a stock solution having a known concentration of about 0.4 mg per mL by initially dissolving the Reference Standard in one part of methanol and diluting the methanolic solution quantitatively with about 4 parts of water to volume in a volumetric flask. Transfer 5.0 mL of this stock solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 10 µg per mL.**Assay preparation**—Transfer an accurately weighed quantity of about 100 mg of Cimetidine to a 250-mL volumetric flask, add 50 mL of methanol to dissolve the specimen, di-lute with water to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 0.6; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; and the relative standard deviation of the response for replicate injections is not more than 2.0%.**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_{10}H_{16}N_6S$  in the portion of Cimetidine taken by the formula:

$$10C(r_u / r_s)$$

in which  $C$  is the concentration, in µg per mL, of USP Cimetidine RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the Cimetidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Cimetidine Tablets**


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» Cimetidine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ).**Packaging and storage**—Preserve in tight, light-resistant containers, at controlled room temperature.**USP Reference standards** (11)—

USP Cimetidine RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Dissolution** (711)—**Medium:** 0.01 N hydrochloric acid; 900 mL.**Apparatus 1:** 100 rpm. A 20-mesh basket may be used for 800-mg strength Tablets.**Time:** 15 minutes.**Procedure**—Determine the amount of  $C_{10}H_{16}N_6S$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 218 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a *Standard solution* having a known concentration of USP Cimetidine RS in the same *Medium*.**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{10}H_{16}N_6S$  is dissolved in 15 minutes.**Uniformity of dosage units** (905): meet the requirements.**Assay**—**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Cimetidine*.**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cimetidine, to a 250-mL volumetric flask. Add 50 mL of methanol, shake for 2 minutes, add 40 mL of water, sonicate for 15 minutes, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cimetidine ( $\text{C}_{10}\text{H}_{16}\text{N}_6\text{S}$ ) in the portion of Tablets taken by the formula:

$$10C(r_U / r_S)$$

in which the terms are as defined in the *Assay* under *Cimetidine*.

## Cimetidine Hydrochloride

$\text{C}_{10}\text{H}_{16}\text{N}_6\text{S} \cdot \text{HCl}$  288.80

Guanidine, *N''*-cyano-*N*-methyl-*N'*-[2-[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-, monohydrochloride.  
2-Cyano-1-methyl-3-[2-[(5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine monohydrochloride [70059-30-2].

» Cimetidine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{10}\text{H}_{16}\text{N}_6\text{S} \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Cimetidine Hydrochloride RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 14  $\mu\text{g}$  per mL.

*Medium:* 0.1 N sulfuric acid.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

### Chromatographic purity—

**Mobile phase**—Transfer about 940 mg of sodium 1-hexanesulfonate to a 1000-mL volumetric flask, add 240 mL of methanol followed by 0.3 mL of phosphoric acid, and dilute with water to volume. Mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Test solution 1**—Transfer about 100 mg of Cimetidine Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Test solution 2**—Transfer 1.0 mL of *Test solution 1* to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Resolution solution**—Dissolve about 50 mg of Cimetidine Hydrochloride in 10 mL of 1 N hydrochloric acid, and heat on a steam bath for about 10 minutes (or boil on a hot plate for about 2 minutes), and allow to cool. Dilute a suitable volume of this solution with *Mobile phase* to obtain a solution having a concentration of about 2  $\mu\text{g}$  per mL. [NOTE—Use this solution within 24 hours of its preparation. Adjustment of the heating step may be necessary to achieve a satisfactory amide analog peak response for the measurement of the resolution between the cimetidine and the amide analog peaks.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as di-

rected for *Procedure*: the resolution,  $R$ , between the cimetidine and the amide analog peaks is not less than 4.0. Chromatograph *Test solution 2*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 3.0; the column efficiency is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections is not more than 7.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of *Test solution 1* and *Test solution 2* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Cimetidine Hydrochloride taken by the formula:

$$0.2(r_i / r_S)$$

in which  $r_i$  is the peak response for an individual impurity observed in the chromatogram obtained from *Test solution 1*, and  $r_S$  is the peak response of cimetidine in the chromatogram obtained from *Test solution 2*: no single impurity is greater than 0.2%, and the sum of all impurities is not more than 1.0%.

### Assay—

**Mobile phase**—Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cimetidine Hydrochloride RS in a mixture of water and methanol (80:20) to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer about 115 mg of Cimetidine Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in about 50 mL of water, add 50 mL of methanol, and dilute with water to volume. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 0.6; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $\text{C}_{10}\text{H}_{16}\text{N}_6\text{S} \cdot \text{HCl}$  in the portion of Cimetidine Hydrochloride taken by the formula:

$$10C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Cimetidine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cimetidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cimetidine Injection

» Cimetidine Injection is a sterile solution of Cimetidine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not

more than 110.0 percent of the labeled amount of  $C_{10}H_{16}N_6S$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**USP Reference standards** (11)—

USP Cimetidine Hydrochloride RS

USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* exhibits a major peak for cimetidine, the retention time of which corresponds to that of the cimetidine peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of cimetidine hydrochloride.

**pH** (791): between 3.8 and 6.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cimetidine Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of cimetidine, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_{10}H_{16}N_6S$  in each mL of the Injection taken by the formula:

$$200(252.34/288.81)(C/V)(r_U/r_S)$$

in which 252.34 and 288.81 are the molecular weights of cimetidine and cimetidine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Cimetidine Hydrochloride RS in the *Standard preparation*; V is the volume of Injection taken; and  $r_U$  and  $r_S$  are the cimetidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cimetidine in Sodium Chloride Injection

» Cimetidine in Sodium Chloride Injection is a sterile solution of Cimetidine Hydrochloride and Sodium Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) and not less than 95.0 percent and not more than 110.0 percent of the labeled amount of sodium chloride (NaCl).

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**USP Reference standards** (11)—

USP Cimetidine Hydrochloride RS

USP Endotoxin RS

**Identification**—

A: The chromatogram obtained from the *Assay preparation* exhibits a major peak for cimetidine, the retention time

of which corresponds to that of the cimetidine peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: It responds to the tests for *Sodium* (191) and for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of cimetidine hydrochloride.

**pH** (791): between 5.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for cimetidine**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cimetidine Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to 2 mg of cimetidine, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

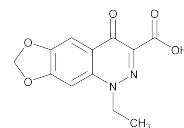
*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of cimetidine ( $C_{10}H_{16}N_6S$ ) in each mL of the Injection taken by the formula:

$$200(252.34/288.81)(C/V)(r_U/r_S)$$

in which 252.34 and 288.81 are the molecular weights of cimetidine and cimetidine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Cimetidine Hydrochloride RS in the *Standard preparation*; V is the volume of Injection taken; and  $r_U$  and  $r_S$  are the cimetidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for sodium chloride**—Dilute an accurately measured volume of Injection quantitatively, and stepwise if necessary, with water to obtain a solution having a concentration of about 0.5 mg of sodium chloride per mL. Titrate with 0.1 N silver nitrate VS, using a silver-silver chloride electrode. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride. From the determined chloride concentration per mL, subtract the quantity,  $(35.453/252.35)W$ , to correct for the chloride present as cimetidine hydrochloride, where W is the quantity, in mg per mL, of cimetidine in the Injection, as determined in the *Assay for cimetidine*. Multiply the corrected value by 1.648 to obtain the quantity, in mg per mL, of sodium chloride in the volume of Injection taken.

## Cinoxacin



$C_{12}H_{10}N_2O_5$  262.22  
[1,3]Dioxolo[4,5-g]cinnoline-3-carboxylic acid, 1-ethyl-1,4-dihydro-4-oxo-;  
1-Ethyl-1,4-dihydro-4-oxo[1,3]dioxolo[4,5-g]cinnoline-3-carboxylic acid [28657-80-9].

**DEFINITION**

Cinoxacin contains NLT 97.0% and NMT 102.0% of cinoxacin ( $C_{12}H_{10}N_2O_5$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>
- **B.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

**ASSAY**• **PROCEDURE**

**Solution A:** 38.1 mg/mL of sodium borate in water

**Internal standard solution:** An aqueous solution containing 2 mg/mL of sulfanilic acid and 5.0 mL of *Solution A* in each 100 mL

**Mobile phase:** Dilute 100.0 mL of *Solution A* and 0.426 g of sodium sulfate with water to 1000 mL. The quantity of sodium sulfate may be varied to meet *System suitability* requirements and to provide a suitable elution time.

**Standard stock solution:** 1 mg/mL of USP Cinoxacin RS in *Solution A*

**Standard solution:** 50 µg/mL of USP Cinoxacin RS prepared as follows. Dilute 5.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* with water to 100 mL.

**Sample stock solution:** 1 mg/mL of Cinoxacin in *Solution A*

**Sample solution:** Dilute 5.0 mL of *Sample stock solution* and 5.0 mL of *Internal standard solution* with water to 100 mL.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 1.8-mm × 1-m; packing L12

**Flow rate:** 1 mL/min

**Injection volume:** 1.0 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for cinoxacin and sulfanilic acid are 1.0 and 2.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 4.4 between cinoxacin and sulfanilic acid

**Tailing factor:** NMT 2.1 for cinoxacin

**Relative standard deviation:** NMT 2.0% from five replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cinoxacin ( $C_{12}H_{10}N_2O_5$ ) in the portion of Cinoxacin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cinoxacin to sulfanilic acid from the *Sample solution*

$R_S$  = peak response ratio of cinoxacin to sulfanilic acid from the *Standard solution*

$C_S$  = concentration of USP Cinoxacin RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Cinoxacin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

**IMPURITIES**• **ORGANIC IMPURITIES**

**Diluent:** Chloroform, dimethylformamide, dimethyl sulfide, and nitromethane (1:1:1:1)

**Standard solution A:** 5 mg/mL of USP Cinoxacin RS in *Diluent*

**Standard solution B:** 0.05 mg/mL from *Standard solution A* in *Diluent*

**Sample solution:** 5 mg/mL of Cinoxacin in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Acetonitrile, ammonium hydroxide, and water (105: 7.5: 30)

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

In a suitable chromatographic chamber lined with paper, place a volume of the *Developing solvent system* sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 min. Apply the *Samples*, dry the plate, and apply each sample three additional times at the corresponding initial locations. Dry the plate thoroughly after each application, and develop the chromatogram until the solvent front has moved to the top of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. View the plate under short- and long-wavelength UV light.

**Acceptance criteria:** 1.0%; the  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, and no spot of the *Sample solution* other than the principal spot is larger or more intense than the principal spot of *Standard solution B*.

**SPECIFIC TESTS**• **LOSS ON DRYING** <731>

**Analysis:** Dry a sample under vacuum at 60° for 3 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** <11>

USP Cinoxacin RS

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**Cinoxacin Capsules**


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**DEFINITION**

Cinoxacin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cinoxacin ( $C_{12}H_{10}N_2O_5$ ).

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Diluent:** Chloroform, dimethylformamide, dimethyl sulfide, and nitromethane (1:1:1:1)

**Standard solution:** 5 mg/mL of USP Cinoxacin RS in *Diluent*

**Sample solution:** Equivalent to 5 mg/mL of cinoxacin from Capsules in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Acetonitrile, ammonium hydroxide, and water (105: 7.5: 30)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

In a suitable chromatographic chamber lined with paper, place a volume of the *Developing solvent system* sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 min. Apply the *Samples*, dry the plate, and apply each sample three additional times at the corresponding initial locations. Dry the plate thoroughly after each application, and develop the chromatogram until the solvent front has moved to the top of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. View the plate under short- and long-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.



**ASSAY****• PROCEDURE**

**Sample solution:** Nominally 10 µg/mL prepared as follows. Transfer an equivalent to 250 mg of cinoxacin to a 100-mL volumetric solution from the contents of NLT 20 Capsules. Dilute with 0.1 M sodium borate to volume. Filter the solution, discarding the first 20 mL of the filtrate, and dilute with water to 10 µg/mL.

**Standard stock solution:** 2.5 mg/mL of USP Cinoxacin RS in 0.1 M sodium borate

**Standard solution:** 10 µg/mL of USP Cinoxacin RS in water, prepared from *Standard stock solution*

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 352 nm

**Cell:** 1 cm

**Blank:** 0.1 M sodium borate and water (2 in 500)

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of cinoxacin (C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>O<sub>5</sub>) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Buffer:** pH 6.5 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

**Medium:** *Buffer*, 500 mL for Capsules containing 250 mg or less of cinoxacin; 1000 mL for Capsules containing more than 250 mg of cinoxacin

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution:** 0.35 mg/mL of USP Cinoxacin RS in *Medium*

**Sample solutions:** Filter, and dilute with 0.1 N sodium hydroxide solution as needed.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 270 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 60% (Q) of the labeled amount of cinoxacin (C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>O<sub>5</sub>) is dissolved.

**• UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in well-closed containers.**• USP REFERENCE STANDARDS <11>**  
USP Cinoxacin RS

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-;  
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [85721-33-1].

**DEFINITION**

Ciprofloxacin contains NLT 98.0% and NMT 102.0% of C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>, calculated on the dried basis.

**IDENTIFICATION**

**• A. INFRARED ABSORPTION:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima at the same wavelengths as that of a similar preparation of USP Ciprofloxacin RS.

**• B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.

**Mobile phase:** Acetonitrile and *Solution A* (13:87)

**Standard solution:** Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask. Add 0.1 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

**System suitability stock solution:** 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

**System suitability solution:** Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with the *Standard solution* to volume.

**Sample solution:** Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography <621>*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 30 ± 1°

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

**Tailing factor:** NMT 2.5 for the ciprofloxacin peak, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub> in the portion of Ciprofloxacin taken:

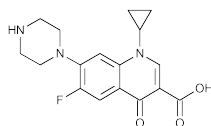
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

**Ciprofloxacin**

Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%, except that where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is NMT 0.2%.

- **CHLORIDE**

**Standard solution:** 8.2 µg/mL of sodium chloride (5 µg/mL of chloride)

**Sample solution:** Add 30.0 mL of water to 0.5 g of Ciprofloxacin, shake for 5 min, and pass through chloride-free filter paper. Use the filtrate as the *Sample solution*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Transfer 15.0 mL of the *Sample solution* to a 50-mL color-comparison tube. Transfer 10.0 mL of the *Standard solution* to a second matched 50-mL color-comparison tube, add 5.0 mL of water, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix.

**Acceptance criteria:** The turbidity exhibited by the *Sample solution* does not exceed that of the *Standard solution* (0.02%).

- **SULFATE**

**Standard solution:** 18.1 µg/mL of potassium sulfate in 30% alcohol (10 µg/mL of sulfate)

**Sample solution:** Dissolve 0.5 g of Ciprofloxacin in 5.0 mL of 2 N acetic acid and 15.0 mL of water.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
To each of two 50-mL matched color-comparison tubes transfer 1.50 mL of the *Standard solution*. To each tube add, successively and with continuous shaking, 1.0 mL of 250 mg/mL barium chloride solution, and allow to stand for 1 min. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Sample solution* and 0.5 mL of 30% acetic acid, and mix.

**Acceptance criteria:** The turbidity exhibited in the tube containing the *Sample solution* does not exceed that of the tube containing the *Standard solution* (0.04%).

- **HEAVY METALS, Method II** (231): NMT 20 ppm

### Organic Impurities

- **PROCEDURE 1: LIMIT OF FLUOROQUINOLONIC ACID**

**Standard stock solution:** Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide, and dilute with water to volume.

**Standard solution:** Dilute 2.0 mL of the *Standard stock solution* with water to 10.0 mL.

**Sample solution:** 10.0 mg/mL of Ciprofloxacin in 0.1 N acetic acid

**Developing solvent system:** Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of silica gel mixture

**Application volume:** 5 µL

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Place the plate in a suitable chamber in which is placed a beaker containing 50 mL of ammonium hydroxide. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for

about 15 min. Examine the plate under short-wave-length UV light.

**Acceptance criteria:** Any spot from the *Sample solution*, at an  $R_f$  value corresponding to the principal spot from the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%).

- **PROCEDURE 2**

**Solution A, Mobile phase, System suitability stock solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each impurity peak

$r_T$  = sum of the responses of all the peaks

#### Acceptance criteria

**Ciprofloxacin ethylenediamine analog or any other individual impurity peak:** NMT 0.2%

**Total impurities:** NMT 0.5%

### SPECIFIC TESTS

- **CLARITY OF SOLUTION:** Dissolve 0.25 g in 10 mL of 0.1 N hydrochloric acid: a clear to slightly opalescent solution is obtained.

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, the total microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. It also meets the requirement for absence of *Salmonella* species and *Escherichia coli*.

- **LOSS ON DRYING** (731): Dry a sample in a vacuum at 120° for 6 h: it loses NMT 1.0% of its weight, except that where it is labeled as intended for use in preparing Ciprofloxacin for Oral Suspension, it loses between 10% and 20% of its weight.

- **STERILITY TESTS** (71): Where the label states that it is sterile, it meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that it is sterile or where the label states that Ciprofloxacin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursion permitted between 15° and 30°, and avoid excessive heat.

- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is so labeled.

- **USP REFERENCE STANDARDS** (11)

USP Ciprofloxacin RS

USP Ciprofloxacin Ethylenediamine Analog RS

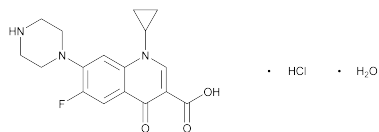
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.

$C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77

USP Endotoxin RS

USP Fluoroquinolonic Acid RS

## Ciprofloxacin Hydrochloride



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$  385.82

3-Quinolonecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-, monohydrochloride, monohydrate;

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride, monohydrate [86393-32-0].

### DEFINITION

Ciprofloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{18}FN_3O_3 \cdot HCl$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** <191>

### ASSAY

#### • PROCEDURE

**Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Solution A* (13:87)

**Standard solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

**System suitability solution:** 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** 0.5 mg/mL of Ciprofloxacin Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:**  $30 \pm 1^\circ$

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

**Tailing factor:** NMT 2.5 for the ciprofloxacin peak, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{18}FN_3O_3 \cdot HCl$  in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area response from the *Sample solution*  
 $r_S$  = peak area response from the *Standard solution*  
 $C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **CHLORIDE AND SULFATE, Sulfate** <221>: A 375-mg portion shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.04%).
- **HEAVY METALS, Method II** <231>: NMT 20 ppm

#### Organic Impurities

##### • PROCEDURE 1: LIMIT OF FLUOROQUINOLONIC ACID

**Standard solution:** Transfer 5.0 mg of USP Fluoroquinolonic Acid RS to a 50-mL volumetric flask containing 0.05 mL of 6 N ammonium hydroxide, add water to volume, and mix. Transfer 2.0 mL of this solution to a 10.0-mL volumetric flask, and dilute with water to volume.

**Sample solution:** 10 mg/mL of Ciprofloxacin Hydrochloride in water

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Methylene chloride, methanol, acetonitrile, and ammonium hydroxide (4:4:1:2)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Proceed as directed in the chapter. Place a beaker containing 50 mL of ammonium hydroxide in a suitable chamber, and then place the plate in the chamber. After 15 min, transfer the plate to a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 15 min. Examine the plate under short-wavelength UV light.

**Acceptance criteria:** Any spot from the *Sample solution*, at an  $R_f$  value corresponding to the principal spot from the *Standard solution*, is not greater in size or intensity than the principal spot from the *Standard solution* (0.2%).

##### • PROCEDURE 2

**Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity

$r_T$  = sum of the responses of all the peaks

#### Acceptance criteria

**Individual impurities:** NMT 0.2% for the ciprofloxacin ethylenediamine analog or any other individual impurity peak

Total impurities: NMT 0.5%

#### SPECIFIC TESTS

- **pH** (791): 3.0–4.5, in a 25 mg/mL solution
- **WATER DETERMINATION, Method I** (921): 4.7%–6.7%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)
  - USP Ciprofloxacin Ethylenediamine Analog RS
  - 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  - $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77
  - USP Ciprofloxacin Hydrochloride RS
  - USP Fluoroquinolonic Acid RS

### Ciprofloxacin and Dexamethasone Otic Suspension

» Ciprofloxacin and Dexamethasone Otic Suspension is a sterile aqueous suspension containing ciprofloxacin hydrochloride and dexamethasone. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight containers, protected from light. Avoid freezing.

#### USP Reference standards (11)—

- USP Ciprofloxacin Ethylenediamine Analog RS
- 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
- $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77
- USP Ciprofloxacin Formamide RS
- USP Ciprofloxacin Hydrochloride RS
- USP Dexamethasone RS
- USP Dexamethasone Acetate RS

#### Identification—

**A:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay for ciprofloxacin*, exhibits a major peak for ciprofloxacin, the retention time of which corresponds to that obtained in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay for ciprofloxacin*.

**B:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dexamethasone*, exhibits a major peak for dexamethasone, the retention time of which corresponds to that obtained in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay for dexamethasone*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 3.8 and 4.8.

#### Particle size—

**Carrier fluid**—Heat Purified Water to a temperature of 40° to 50°, add 100 mg of dexamethasone per L while stirring, cool to room temperature while stirring, pass through a 0.2- $\mu$ m filter, and store in a clean, covered container.

**Test preparation**—Dilute a volume of about 10  $\mu$ L of Otic Suspension with *Carrier fluid* to 25 mL.

**Procedure**—(see *Light Obscuration Particle Count Test* under *Particulate Matter in Injections* (788)). Analyze the *Test preparation* using an electronic, liquid-borne particle counting system that employs a light obscuration sensor with a suitable sample feeding device. Not less than 99.5% of the particles are  $\leq 25$   $\mu$ m, not less than 99.95% are  $\leq 50$   $\mu$ m, and not less than 99.995% are  $\leq 100$   $\mu$ m.

**Osmolality** (785): between 270 and 330 mOsmol per kg.

#### Limit of ciprofloxacin formamide—

**Buffer**—Add 6.0 mL of phosphoric acid to 2.0 L of water. Adjust with 50% sodium hydroxide to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (73:27). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 25 mg of USP Ciprofloxacin Formamide RS, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a known concentration of about 0.015 mg per mL.

**System suitability solution**—Transfer about 2.5 mg of USP Dexamethasone RS and about 2.5 mg of USP Ciprofloxacin Formamide RS to a 100-mL volumetric flask. Dissolve in 15 mL of methanol, then dilute with *Mobile phase* to volume.

**Test solution**—Transfer an accurately measured volume of freshly mixed Otic Suspension, equivalent to about 6 mg of ciprofloxacin, to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the column efficiency for ciprofloxacin formamide is not less than 2000 theoretical plates; the resolution,  $R$ , between ciprofloxacin formamide and dexamethasone is not less than 8; and the tailing factor for ciprofloxacin formamide is not more than 2.0. The relative standard deviation for replicate injections of the *Standard solution* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks at the retention time of ciprofloxacin formamide. Calculate the percentage of ciprofloxacin formamide in the portion of the Otic Suspension taken by the formula:

$$10(C/VL)(r_U / r_S)100$$

in which  $C$  is the concentration, in mg per mL, of USP Ciprofloxacin Formamide RS in the *Standard solution*;  $V$  is the volume, in mL, of Otic Suspension taken;  $L$  is the labeled amount, in mg per mL, of ciprofloxacin; and  $r_U$  and  $r_S$  are the ciprofloxacin formamide peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Ciprofloxacin formamide is not more than 0.5% of the labeled amount of ciprofloxacin.

#### Ciprofloxacin related compounds—

**Procedure**—From the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for ciprofloxacin*, measure the responses for the ciprofloxacin ethylenediamine analog and the other minor peaks. Calculate the percentage

of each related compound in the portion of Otic Suspension taken by the formula:

$$(331.34/367.81)25(C/V)(r_U/r_S)100/FL$$

in which 331.34 and 367.81 are the molecular weights of ciprofloxacin and anhydrous ciprofloxacin hydrochloride, respectively;  $C$  is the concentration, in mg per mL, of USP Ciprofloxacin Hydrochloride RS in the *Dilute standard preparation*, calculated on the anhydrous basis;  $V$  is the volume, in mL, of Otic Suspension taken;  $r_U$  and  $r_S$  are the related compound peak responses obtained from the *Assay preparation* and the ciprofloxacin peak response obtained from the *Dilute standard preparation*, respectively;  $F$  is the relative response factor (1.3 for ciprofloxacin ethylenediamine analog and 1.0 assumed for all other degradation products); and  $L$  is the labeled amount, in mg per mL, of ciprofloxacin. The ciprofloxacin ethylenediamine analog is not more than 0.4% of the labeled amount of ciprofloxacin. No other single related compound is greater than 0.2%, and the sum of all related compounds found is not more than 0.8%.

#### Dexamethasone related compounds—

**Procedure**—From the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dexamethasone*, measure the responses for the 21-dehydro-17-deoxy related compound, the 20-carboxy-17-deoxy related compound, and other minor peaks. Calculate the percentage of each related compound in the portion of the Otic Suspension taken by the formula:

$$10(C/VL)(r_U/r_S)100$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Dilute standard preparation*;  $V$  is the volume, in mL, of Otic Suspension taken;  $L$  is the labeled amount, in mg per mL, of dexamethasone; and  $r_U$  and  $r_S$  are the related compound peak responses obtained from the *Assay preparation* and the dexamethasone peak response obtained from the *Dilute standard preparation*, respectively. The 21-dehydro-17-deoxy related compound is not more than 1.0%, the 20-carboxy-17-deoxy related compound is not more than 2.6%, no other related compound is greater than 0.3%, and the sum of all related compounds found is not more than 3.5%. [NOTE—Identification of known related compounds is accomplished by measuring relative retention times versus dexamethasone. The relative retention times are about 1.4 to 1.6 for the 21-dehydro-17-deoxy related compound and about 2.8 to 3.2 for the 20-carboxy-17-deoxy related compound.]

#### Assay for ciprofloxacin—

**Buffer**—Add 6.0 mL of phosphoric acid and 8 g of diethylamine phosphate to 2.0 L of water. Adjust with 50% sodium hydroxide to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (89:11). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Accurately weigh about 37 mg of USP Ciprofloxacin Hydrochloride RS into a 25-mL volumetric flask, and dissolve in and dilute with 0.1 N hydrochloric acid to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume to obtain a solution having a known concentration of about 0.13 mg of ciprofloxacin per mL.

**Dilute standard preparation**—Transfer 2.0 mL of the *Standard preparation* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a known concentration of about 0.0025 mg of ciprofloxacin per mL.

**System suitability solution**—Weigh about 1 mg of USP Ciprofloxacin Hydrochloride RS and 1 mg of USP Ciprofloxacin Ethylenediamine Analog RS into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer

2.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Assay preparation**—Transfer an accurately measured volume of freshly mixed Otic Suspension, equivalent to about 3 mg of ciprofloxacin, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between ciprofloxacin and the ciprofloxacin ethylenediamine analog is not less than 3.0; the column efficiency for ciprofloxacin is not less than 2500 theoretical plates; and the tailing factor for ciprofloxacin is not more than 2.0. The relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%; and the relative standard deviation for replicate injections of the *Dilute standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in each mL of the Otic Suspension taken by the formula:

$$(331.34/367.81)(25C/V)(r_U/r_S)$$

in which 331.34 and 367.81 are the molecular weights of ciprofloxacin and anhydrous ciprofloxacin hydrochloride, respectively;  $C$  is the concentration, in mg per mL, of USP Ciprofloxacin Hydrochloride RS in the *Standard preparation*, calculated on the anhydrous basis;  $V$  is the volume, in mL, of Otic Suspension taken; and  $r_U$  and  $r_S$  are the ciprofloxacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for dexamethasone—

**Buffer and Mobile phase**—Prepare as directed under *Limit of ciprofloxacin formamide*.

**Standard preparation**—Transfer about 50 mg of USP Dexamethasone RS, accurately weighed, to a 25-mL volumetric flask, dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. This solution contains about 0.2 mg of USP Dexamethasone RS per mL.

**Dilute standard preparation**—Transfer 2.0 mL of the *Standard preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.004 mg of USP Dexamethasone RS per mL.

**System suitability solution**—Transfer about 2 mg of USP Dexamethasone RS and about 2 mg of USP Dexamethasone Acetate RS to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of freshly mixed Otic Suspension, equivalent to about 2 mg of dexamethasone, to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the column efficiency for dexamethasone is not less than 2000 theoretical plates; the resolution,  $R$ , between dexamethasone and dexamethasone acetate is not less than 12; the tailing factor for dexamethasone is not more than 2.0; the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%; and the relative standard deviation for replicate injections of the *Dilute standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dexamethasone ( $C_{22}H_{29}FO_5$ ) in each mL of the Otic Suspension taken by the formula:

$$10(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; V is the volume, in mL, of Otic Suspension taken; and  $r_U$  and  $r_S$  are the dexamethasone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ciprofloxacin Injection

### DEFINITION

Ciprofloxacin Injection is a sterile solution of Ciprofloxacin or Ciprofloxacin Hydrochloride in Water for Injection, in 5% Dextrose Injection, or in 0.9% Sodium Chloride Injection prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and Solution A (13:87)

**Standard solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

**System suitability solution:** 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** Equivalent to 0.5 mg/mL of Ciprofloxacin from Injection diluted with *Mobile phase*

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Temperature:**  $30 \pm 1^\circ$

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak

**Column efficiency:** NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

**Tailing factor:** NMT 2.5 for the ciprofloxacin peak, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of  $C_{17}H_{18}FN_3O_3$  from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### LACTIC ACID CONTENT

**Mobile phase:** Acetonitrile and 0.005 N sulfuric acid (3:17)

**Standard solution:** 0.8 mg/mL of USP Sodium Lactate RS in water or 4 mg/mL where the Injection is labeled as being a concentrated form

**Sample solution:** Use the undiluted Injection.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 208 nm

**Column:** 7.8-mm  $\times$  30-cm; packing L17

**Temperature:**  $40 \pm 1^\circ$

**Flow rate:** 0.6 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for the analyte peak

**Relative standard deviation:** NMT 2.0%

[NOTE—After each analysis, rinse the column with a mixture of 0.01 N sulfuric acid and acetonitrile to elute the ciprofloxacin from the column. Promptly regenerate the column with 0.01 N sulfuric acid, and the column may be reused or stored.]

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration of lactic acid ( $C_3H_6O_3$ ) in mg/mg of ciprofloxacin:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

$r_U$  = peak response of lactic acid from the *Sample solution*

$r_S$  = peak response of lactic acid from the *Standard solution*

$C_S$  = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of lactic acid, 90.08

$M_{r2}$  = molecular weight of sodium lactate, 112.07

**Acceptance criteria:** 0.288–0.352 mg of lactic acid for each mg of ciprofloxacin claimed on the label, except that where the Injection is labeled as being a concentrated form, it contains 0.335–0.409 mg of lactic acid for each mg of ciprofloxacin claimed on the label

#### DEXTROSE CONTENT (if present)

**Sample solution:** Undiluted Injection

**Analysis:** Determine the angular rotation in a suitable polarimeter tube (see Optical Rotation <781>).

Calculate the percentage (g/100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken:

$$\text{Result} = A \times R \times (M_{r1}/M_{r2}) \times (100/F)$$

- A = 100 mm divided by the length of the polarimeter tube (mm)  
 R = observed rotation (degrees)  
 $M_{r1}$  = molecular weight of dextrose monohydrate, 198.17  
 $M_{r2}$  = molecular weight of anhydrous dextrose, 180.16  
 F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

Acceptance criteria: 4.75–5.25 g/100 mL

• **SODIUM CHLORIDE CONTENT** (if present)

Sample solution: Injection

Analysis: Transfer 10.0 mL of *Sample solution* to a suitable container, dilute with water to 150 mL, add 1.5 mL of potassium chromate TS, and titrate with 0.1 N silver nitrate TS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Acceptance criteria: 85.5–94.5 mg

**IMPURITIES**

**Organic Impurities**

• **PROCEDURE: LIMIT OF CIPROFLOXACIN ETHYLENEDIAMINE ANALOG**

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Analysis**

Sample: *Sample solution*

Calculate the percentage of ciprofloxacin ethylenediamine analog from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = [F \times r_A / (F \times r_A + r_C)] \times 100$$

- F = correction factor for ciprofloxacin ethylenediamine analog, 0.7  
 $r_A$  = ciprofloxacin ethylenediamine analog peak response  
 $r_C$  = peak response of ciprofloxacin

Acceptance criteria: NMT 0.5%

**SPECIFIC TESTS**

- **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements
- **PH** <791>: 3.5–4.6, except that where the Injection is labeled as being a concentrated form, its pH is 3.3–3.9
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.
- **STERILITY TESTS** <71>: It meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **COLOR AND ACHROMICITY** <631> (where it is labeled as being a concentrated form): It has no more color than a solution prepared by diluting 5.0 mL of *Matching Fluid O* with 95.0 mL of 0.12 N hydrochloric acid.
- **OTHER REQUIREMENTS**: It meets the requirements for *Injections* <1>, *Container Content*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in single-dose containers, preferably of Type I glass, and store in a cool place or at controlled room temperature. Avoid freezing and exposure to light.
- **LABELING**: The label indicates whether the vehicle is Sterile Water for Injection, 5% Dextrose Injection, or 0.9% Sodium Chloride Injection. Label the Injection that has Sterile Water for Injection as the vehicle to indicate that it is a concentrated form that must be diluted to appropriate strength (1–2 mg/mL) with 5% Dextrose Injection or

0.9% Sodium Chloride Injection before administration, and that the resulting solution is stable for up to 14 days when stored in a cool place or at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

- USP Ciprofloxacin Ethylenediamine Analog RS  
 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.  
 $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77  
 USP Ciprofloxacin Hydrochloride RS  
 USP Endotoxin RS  
 USP Sodium Lactate RS

## Ciprofloxacin Ophthalmic Ointment

**DEFINITION**

Ciprofloxacin Ophthalmic Ointment contains an amount of Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

Buffer: 0.005 M tetrabutylammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Methanol and Buffer (250:750)

Standard solution: 0.033 mg/mL of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid

System suitability solution: 0.005 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*

Sample solution: Transfer an amount nominally equivalent to 750 µg of ciprofloxacin from Ophthalmic Ointment to a screw-capped tube. Add 15 mL of solvent hexane, and shake vigorously until the Ophthalmic Ointment is dispersed. Loosen the cap, and heat in a water bath at 60° for 30 min, with occasional swirling. Remove from the bath, tighten the cap, and shake for 1.5 min while still hot. Add 25.0 mL of 0.1 N hydrochloric acid, and shake vigorously for 1.5 min. Allow the layers to separate, and use the lower, aqueous layer.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

**System suitability**

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 2.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Column efficiency: NLT 500 theoretical plates, *Standard solution*

Tailing factor: 0.9–2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the label claim of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of ciprofloxacin, 331.34  
 $M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81  
**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL <755>:** Meets the requirements

**SPECIFIC TESTS**

- **STERILITY TESTS <71>:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **METAL PARTICLES IN OPHTHALMIC OINTMENTS <751>:** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at a temperature between 2° and 25°.
- **USP REFERENCE STANDARDS <11>**  
 USP Ciprofloxacin Ethylenediamine Analog RS  
 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.  
 $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77  
 USP Ciprofloxacin Hydrochloride RS

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**Ciprofloxacin Ophthalmic Solution**


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**DEFINITION**

Ciprofloxacin Ophthalmic Solution is a sterile, aqueous solution of Ciprofloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 0.005 M tetrabutylammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.0.  
**Mobile phase:** Methanol and *Solution A* (1:3)  
**Standard solution:** 0.14 mg/mL of USP Ciprofloxacin Hydrochloride RS in water  
**System suitability solution:** 0.01 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*  
**Sample solution:** Equivalent to 0.12 mg/mL of ciprofloxacin from Ophthalmic Solution, in water  
**Chromatographic system**  
 (See *Chromatography <621>*, *System Suitability*.)

**Mode:** LC**Detector:** UV 280 nm**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1.5 mL/min**Injection size:** 20 µL**System suitability****Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between the ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

**Capacity factor:** 1.5–6 for the ciprofloxacin peak, *Standard solution*

**Column efficiency:** NLT 500 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–2.0, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of  $C_{17}H_{18}FN_3O_3$  in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of ciprofloxacin, 331.34  
 $M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81  
**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **pH <791>:** 3.5–5.5
- **STERILITY TESTS <71>:** It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at room temperature.
- **USP REFERENCE STANDARDS <11>**  
 USP Ciprofloxacin Ethylenediamine Analog RS  
 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.  
 $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77  
 USP Ciprofloxacin Hydrochloride RS

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**Ciprofloxacin Tablets**


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**DEFINITION**

Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $2.0 \pm 0.1$ .

**Solution B:** Acetonitrile and *Solution A* (13:87)

**Solution C:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Solution C* (13:87)

**Standard solution:** 0.2 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Solution B*

**System suitability solution:** 0.05 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in the *Standard solution*

**Sample solution:** Transfer 5 Tablets to a 500-mL volumetric flask, add 400 mL of *Solution B*, and sonicate for about 20 min. Dilute with *Solution B* to volume, mix, and pass through a membrane filter of 0.45- $\mu$ m pore size. Prepare the equivalent of 0.20 mg/mL of ciprofloxacin from the filtrate with *Solution B*.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:**  $30 \pm 1^\circ$

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The retention time for ciprofloxacin is 6.4–10.8 min. The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak, *System suitability requirements*

**Column efficiency:** NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

**Tailing factor:** NMT 2.0 for the ciprofloxacin peak, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{18}FN_3O_3$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL), calculated on the anhydrous basis

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Standard solution:** USP Ciprofloxacin Hydrochloride RS in *Medium*

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 276 nm

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

**Tolerances:** An amount of ciprofloxacin hydrochloride ( $C_{17}H_{18}FN_3O_3 \cdot HCl$ ) equivalent to NLT 80% (Q) of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

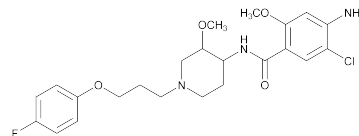
- **USP REFERENCE STANDARDS <11>**

USP Ciprofloxacin Ethylenediamine Analog RS

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.

$C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77

USP Ciprofloxacin Hydrochloride RS

**Cisapride**

$C_{23}H_{29}ClFN_3O_4$  465.95

Benzamide, 4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxy-, *cis-cis*-4-Amino-5-chloro-N-[1-[3-(p-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-o-anisamide [81098-60-4].

Monohydrate 484.0 [260779-88-2].

» Cisapride contains not less than 99.0 percent and not more than 101.0 percent of  $C_{23}H_{29}ClFN_3O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store at room temperature.

**USP Reference standards <11>**

USP Cisapride RS

USP Haloperidol RS

**Completeness of solution <641>**—A solution, 10 mg per mL in methylene chloride, meets the requirements.

**Identification, Infrared Absorption <197K>.**

**Specific rotation <781S>:** between  $-10^\circ$  and  $+10^\circ$ , measured at  $20^\circ$ .

*Test solution:* 10 mg per mL, in methylene chloride.

**Water, Method I <921>:** between 3.4% and 4.0%.

**Residue on ignition <281>:** not more than 0.1%.

**Chromatographic purity**

*Solution A*—Prepare a 20 g per L solution of tetrabutylammonium hydrogen sulfate in water.

*Solution B*—Use methanol.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Blank solution**—Use methanol.

**System suitability solution**—Prepare a solution of USP Cisapride RS and USP Haloperidol RS in methanol containing about 0.05 mg per mL and 0.4 mg per mL, respectively.

**Test solution 1**—Dissolve an accurately weighed quantity of Cisapride, in methanol to obtain a solution having a known concentration of about 10 mg per mL.

**Test solution 2**—Dilute quantitatively and stepwise *Test solution 1* in methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

**Chromatographic system** (see *Chromatography* <621>).—The liquid chromatograph is equipped with a 275-nm detector and a 4.0-mm × 10-cm column that contains 3-μm base-deactivated packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–20	80→55	20→45	linear gradient
20–21	55→5	45→95	linear gradient
21–25	5	95	isocratic
25–26	5→80	95→20	return to initial conditions
26–30	80	20	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the order of elution is cisapride followed by haloperidol, the resolution,  $R$ , between these two peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0% for the cisapride peak.

**Procedure**—Inject a volume (about 10 μL) of the *Blank solution*, *Test solution 1*, and *Test solution 2* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of cisapride impurities in the portion of Cisapride taken by the formula:

$$100(C_s / C_i)(r_i / r_s)$$

in which  $C_s$  and  $C_i$  are the concentration of cisapride, in mg per mL, of *Test solution 2* and *Test solution 1*, respectively;  $r_i$  is the individual peak response of cisapride impurities in *Test solution 1*; and  $r_s$  is cisapride peak area in *Test solution 2*: not more than 0.5% of any cisapride impurity is found, and not more than 1.0% of total impurities is found. Disregard any peak also found in the *Blank solution* and any peak with an area less than 0.1 times the area of the principal peak in the *Test solution 2* chromatogram.

**Assay**—Dissolve about 0.350 g of Cisapride, accurately weighed, in 70 mL of a mixture of methyl ethyl ketone and acetic acid (7:1). Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 N perchloric acid is equivalent to 46.60 mg of  $C_{23}H_{29}ClFN_3O_4$ .

## Cisplatin



$Cl_2H_6N_2Pt$  300.05

Platinum, diamminedichloro-, (SP-4-2)-  
cis-Diamminedichloroplatinum [15663-27-1].

» Cisplatin contains not less than 98.0 percent and not more than 102.0 percent of  $Cl_2H_6N_2Pt$ , calculated on the anhydrous basis.

**Caution**—Cisplatin is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers. Protect from light.

**USP Reference standards** <11>—

USP Cisplatin RS

USP Transplatin RS

USP Potassium Trichloroammineplatinate RS

$Cl_3H_3KNPt$  357.58

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**B:** *Infrared Absorption* <197K>.

**C:** *Spray reagent*—Add 5.6 g of stannous chloride to 10 mL of hydrochloric acid, and stir for 5 minutes. [NOTE—It is not necessary that all of the solids dissolve.] Dissolve 0.2 g of potassium iodide in 90 mL of water. Mix the two solutions together. Disregard any precipitate that is formed. Store in the dark. The solution is usable for at least 1 week.

**Procedure**—Prepare a test solution containing 1 mg of Cisplatin per mL and a Standard solution containing 1 mg of USP Cisplatin RS per mL, both in dimethylformamide. Apply separately 5-μL quantities of each solution to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* <621>). Place the plate in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 minutes with a developer consisting of a mixture of acetone and 1 N nitric acid (180:20). Develop the plate for a distance of about 8 cm from the origin. Remove the plate, and allow it to air-dry. Complete the drying by heating in a forced-air oven at about 100° for 1 minute. Spray the plate with *Spray reagent*, heat it in an oven at about 100° for 5 minutes, cool, and spray with a 1 in 50 solution of potassium iodide in water, to bring out the full color of the spots: the principal spot from the test solution corresponds in appearance and  $R_f$  value to that produced by the Standard solution.

**Crystallinity** <695>: meets the requirements.

**Water, Method I** <921>: not more than 1.0%.

**UV purity ratio**—[NOTE—Cleanse all glassware with a mixture of hydrochloric acid and nitric acid (3:1), rinse thoroughly with water, and dry before use. Do not use dichromate for cleaning. Do not use acetone or pressurized air for drying. Protect the test solution from light, and use within 1 hour after its preparation.] Transfer  $98.5 \pm 0.5$  mg of ground Cisplatin to a 100-mL volumetric flask, and add 0.1 N hydrochloric acid to volume. Using a clean magnetic stir bar, alternately stir at a high speed for 5 minutes and sonicate for 10 seconds until complete solution is effected, inverting the flask frequently to remove particles that may cling to the neck. Obtain the UV absorption spectrum, using thoroughly rinsed 2-cm cells, with 0.1 N hydrochloric acid in the reference cell: the ratio of the absorbance at the maximum near 301 nm to that at the minimum near 246 nm is not less than 4.5.

**Limit of trichloroammineplatinate**—

**Mobile phase**—Transfer 0.8 g of ammonium sulfate to a 2-liter volumetric flask, dissolve in water, and dilute with water to volume. Degas, and filter through a membrane filter prior to use. The pH of this solution is  $5.9 \pm 0.1$ . Make adjustments to the ionic strength of the *Mobile phase*, if necessary, to meet the system suitability requirements.

**Standard preparation**—[NOTE—Use low-actinic glassware.] Dissolve a suitable quantity of USP Potassium Trichloroammineplatinate RS, accurately weighed, in saline TS, and dilute quantitatively with saline TS to obtain a solution having

a known concentration of about 6 µg per mL. Use within 4 hours.

**Test preparation**—[NOTE—Use low-actinic glassware.] Transfer about 50 mg of Cisplatin, accurately weighed, to a 100-mL volumetric flask, and dilute with saline TS to volume. Completely dissolve by stirring by mechanical means for 30 minutes. Use within 4 hours.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 209-nm detector and a 4.6-mm × 25-cm column that contains packing L14. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the saline TS peak and the trichloroamineplatininate peak is not less than 2.0, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the areas for the peaks due to trichloroamineplatininate. The relative retention times are about 1.0 for cisplatin (in the void volume) and 5.0 for trichloroamineplatininate. Calculate the percentage of trichloroamineplatininate taken by the formula:

$$10(318.48 / 357.58)(r_U / r_S)(C / W)$$

in which 318.48 and 357.58 are the formula weights of trichloroamineplatininate and potassium trichloroamineplatininate, respectively;  $r_U$  and  $r_S$  are the peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively;  $C$  is the concentration, in µg per mL, of the *Standard preparation*; and  $W$  is the weight, in mg, of Cisplatin taken: not more than 1.0% is found.

#### Limit of transplatin—

**Mobile phase**—Prepare a 0.18 M solution in water of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.2, and filter.

**Stock standard solution**—Transfer about 10 mg of USP Transplatin RS, accurately weighed, to a 200-mL volumetric flask, dilute with saline TS to volume, and dissolve by stirring by mechanical means for 30 minutes.

**Working standard solution**—Pipet 5 mL of *Stock standard solution* into a 25-mL volumetric flask containing about 12 mg of USP Cisplatin RS. Dilute with saline TS to volume, and stir by mechanical means for 30 minutes to dissolve.

**Standard preparation**—Pipet 10 mL of *Working standard solution* into a 50-mL volumetric flask. Add 5.0 mL of a 1 in 200 solution of thiourea, prepared fresh daily, and 5.0 mL of 1 N hydrochloric acid. Dilute with saline TS to volume, and mix. Place about 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 minutes. Remove, and cool to room temperature.

**Test solution**—Transfer about 50 mg of Cisplatin, accurately weighed, to a 100-mL volumetric flask, dilute with saline TS to volume, and dissolve by stirring by mechanical means for 30 minutes.

**Test preparation**—Pipet 10 mL of *Test solution* into a 50-mL volumetric flask, and proceed as directed for *Standard preparation*, beginning with "add 5.0 mL of a 1 in 200 solution of thiourea."

**Resolution solution**—Place about 10 mg of USP Cisplatin RS in a 200-mL volumetric flask, dilute with saline TS to volume, and stir by mechanical means for 30 minutes to dissolve. Pipet 10 mL of this solution and 10 mL of *Stock standard solution* into a 50-mL volumetric flask, and proceed as directed for *Standard preparation*, beginning with "add 5.0 mL of a 1 in 200 solution of thiourea."

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L9.

The column is maintained throughout at a temperature of 45°. The flow rate is about 2.0 mL per minute. Condition the column by pumping *Mobile phase* at a flow rate of 2.0 mL per minute for 30 minutes, then at 0.5 mL per minute for 30 minutes, and then again at 2.0 mL per minute for 30 minutes. Chromatograph the *Standard preparation*. The retention time of the derivatized transplatin is between 5.0 and 9.0 minutes; or, if it is not, modify the *Mobile phase* as necessary, and recondition the column. The column efficiency,  $n$ , is not less than 2500. Chromatograph the *Resolution solution*. The resolution,  $R$ , is not less than 1.7. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas of the transplatin peaks. The relative retention times are about 1.0 for cisplatin and 1.3 for transplatin. Calculate the percentage of transplatin taken by the formula:

$$10(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Transplatin RS in the *Working standard solution*;  $W$  is the weight, in mg, of Cisplatin taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively. Not more than 2.0% is found.

**Platinum content**—[NOTE—Thoroughly cleanse all glassware with nitric acid, and rinse with *Purified Water*, to prevent "mirroring" of the platinum precipitate.] Transfer about 0.5 g of Cisplatin, accurately weighed, to a 600-mL beaker. Add 300 mL of 0.1 N hydrochloric acid, and slowly dissolve by heating nearly to boiling on a hot plate covered with an insulating pad, and stirring frequently with a glass stirring rod. When solution is complete, remove the insulating pad, and boil for about 10 minutes. Remove the beaker from the hot plate, allow to cool for 1 minute without stirring, and filter through quantitative, fine-porosity, smooth, dense, ashless filter paper, collecting the filtrate in a 600-mL beaker, completing the transfer to the filter with hot water. Wash the filter with hot water. Place the beaker containing the combined filtrate and washings on a hot plate, and evaporate to a volume of about 300 mL. Place a glass stirring rod in the beaker, and heat the solution to boiling. Slowly add to the center of the beaker, by dropwise additions, 10.0 mL of hydrazine hydrate, 85%. [Caution—Hydrazine is toxic.] Add 2 drops of 10 N sodium hydroxide, boil for 10 minutes to coagulate the precipitate for ease of filtration, cool, and filter through quantitative, medium-porosity, smooth, ashless filter paper. Rinse the beaker with hot water, and pour the rinsings onto the filter. Wipe the beaker and the stirring rod with small pieces of the same kind of paper used for this filtration, and place these and the filter containing the precipitate in a No. 1 porcelain crucible, previously ignited to constant weight. Dry on a hot plate covered with an insulating pad, slowly increase the heat to char, and ignite for 1 hour at 800°. Cool in a desiccator, and again weigh: the weight of the platinum so obtained is between 64.42% and 65.22% of the weight of Cisplatin taken, on the anhydrous basis.

#### Assay—

**Mobile phase**—Prepare a suitable solution by mixing ethyl acetate, methanol, dimethylformamide, and degassed water (25:16:5:5), and degas.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cisplatin RS quantitatively in dimethylformamide to obtain a solution having a known concentration of about 1 mg per mL. Use within 1 hour.

**Assay preparation**—Dissolve about 100 mg of Cisplatin, accurately weighed, in dimethylformamide in a 100-mL vol-

umetric flask, dilute with dimethylformamide to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 310-nm detector and a 4.0-mm × 30-cm column that contains packing L8. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 40 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$  in the portion of Cisplatin taken by the formula:

$$100C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cisplatin RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cisplatin for Injection

» Cisplatin for Injection is a sterile, lyophilized mixture of Cisplatin, Mannitol, and Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cisplatin ( $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ ).

**Caution**—Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under *Injections* <1>. Protect from light.

**USP Reference standards** <11>—

USP Cisplatin RS

USP Endotoxin RS

USP Transplatin RS

USP Potassium Trichloroamineplatinate RS

$\text{Cl}_3\text{H}_3\text{KNPt}$  357.58

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Identification**—

**Spray reagent**—Prepare as directed for *Spray reagent* in *Identification* test C under *Cisplatin*.

**Standard preparation**—Prepare a solution containing 1.0 mg of USP Cisplatin RS per mL, 9 mg of sodium chloride per mL, and 10 mg of D-mannitol per mL, in water.

**Test preparation**—Dissolve the contents of 1 container in water to provide a Cisplatin concentration of 1.0 mg per mL, based on label claim.

**Procedure**—Proceed as directed for *Procedure* in *Identification* test C under *Cisplatin*, beginning with “Apply separately 5-µL quantities.” The principal spot from the *Test preparation* corresponds in appearance and  $R_f$  value to that from the *Standard preparation*.

**Bacterial endotoxins** <85>—It contains not more than 2.0 USP Endotoxin Units per mg of cisplatin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** <905>: meets the requirements.

**pH** <791>: between 3.5 and 6.2, in the solution constituted as directed in the labeling, using Sterile Water for Injection.

**Water, Method I** <921>—Use anhydrous formamide as the extraction solvent, and use the following procedure. Introduce about 50 mL of anhydrous formamide into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with a 22-gauge needle, about 8 cm long. Add the rinse back to the titration vessel, and again titrate the vessel contents, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated, and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting, to avoid over-titration. The amount of water found is not more than 2.0%.

**Limit of trichloroamineplatinate**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the test for *Limit of trichloroamineplatinate* under *Cisplatin*.

**Test preparation**—Using low-actinic volumetric glassware, quantitatively dissolve with water the contents of 1 container to yield a 0.5 mg per mL solution of Cisplatin.

**Procedure**—Proceed as directed for *Procedure* in the test for *Limit of trichloroamineplatinate* under *Cisplatin*. Calculate the percentage of trichloroamineplatinate taken by the formula:

$$0.1(318.48/357.58)(r_u / r_s)(CV/W)$$

in which 318.48 and 357.58 are the formula weights of trichloroamineplatinate and potassium trichloroamineplatinate, respectively;  $r_u$  and  $r_s$  are the peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively; *C* is the concentration, in µg per mL, of the *Standard preparation*; *V* is the volume, in mL, of the constituted container contents; and *W* is the labeled amount, in mg, of Cisplatin per container. Not more than 1.0% is found.

**Limit of transplatin**—

**Mobile phase, Stock standard solution, Working standard solution, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the test for *Limit of transplatin* under *Cisplatin*.

**Test solution**—Quantitatively dissolve the contents of 1 container with water to yield a 0.5 mg per mL solution of Cisplatin.

**Test preparation**—Prepare as directed for *Test preparation* in the test for *Transplatin* under *Cisplatin*.

**Procedure**—Proceed as directed for *Procedure* in the test for *Limit of transplatin* under *Cisplatin*. Calculate the percentage of transplatin taken by the formula:

$$0.1(CV/W)(r_u / r_s)$$

in which *C* is the concentration, in µg per mL, of the *Standard preparation*; *V* is the volume, in mL, of the constituted container contents; *W* is the labeled amount, in mg, of Cisplatin per container; and  $r_u$  and  $r_s$  are the peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively. Not more than 2.0% is found.

**Other requirements**—It meets the requirements for *Labeling* under *Injections* <1>.

**Assay**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Cisplatin*.

**Assay preparation**—Quantitatively dissolve the Cisplatin in 1 container by sonicating for 5 minutes with dimethylformamide to yield a Cisplatin concentration of about 1.0 mg

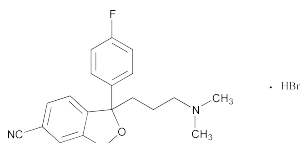
per mL. Filter 5 mL through a suitable membrane filter, and collect the filtrate after discarding the first mL passing through the filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cisplatin*. Calculate the quantity, in mg, of  $\text{C}_{20}\text{H}_{21}\text{FN}_2\text{O}$  in the container taken by the formula:

$$CV(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cisplatin RS in the *Standard preparation*; V is the volume, in mL, of the constituted container contents; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Citalopram Hydrobromide



$\text{C}_{20}\text{H}_{21}\text{FN}_2\text{O} \cdot \text{HBr}$  405.30

5-Isobenzofurancarbonitrile, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-, monohydrobromide.

1-[3-(Dimethylamino)propyl]-1-(p-fluorophenyl)-5-phthalanocarbonitrile monohydrobromide [59729-32-7].

» Citalopram Hydrobromide contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{20}\text{H}_{21}\text{FN}_2\text{O} \cdot \text{HBr}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

**Labeling**—If a test for *Related compounds* other than *Test 1* is used, then the labeling states with which *Related compounds* test the article complies.

### USP Reference standards (11)—

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound A RS

1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

$\text{C}_{20}\text{H}_{23}\text{FN}_2\text{O}_2$  342.22

USP Citalopram Related Compound C RS

3-[3-(Dimethylamino)-1-propyl]-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.

$\text{C}_{20}\text{H}_{19}\text{FN}_2\text{O}_2$  338.22

USP Citalopram Related Compound D RS

1-(4-Fluorophenyl)-1-(3-(methylamino)propyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.

$\text{C}_{19}\text{H}_{19}\text{FN}_2\text{O} \cdot \text{HCl}$  346.83

USP Citalopram Related Compound G RS

1-(4'-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-chlorophthalane hydrobromide.

$\text{C}_{19}\text{H}_{21}\text{FNOCl} \cdot \text{HBr}$  414.74

USP Citalopram Related Compound H RS

1-(4'-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-bromophthalane hydrobromide.

$\text{C}_{19}\text{H}_{21}\text{FNOBr} \cdot \text{HBr}$  459.1

**Completeness of solution**—The absorbance at 410 nm of a 2.5% w/v solution, in 96% alcohol, against a sample solvent in a 1-cm quartz cell is not more than 0.040.

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the

chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** A solution of 10 mg per mL meets the requirement of the silver nitrate precipitate test for *Bromide* (191).

**Specific rotation** (781S): between  $-0.2^\circ$  and  $+0.2^\circ$  at  $20^\circ$ .

**Test solution:** 25 mg per mL, in methanol.

**pH** (791): between 5.5 and 6.5, in a solution (0.5 in 100).

**Water, Method I** (921): not more than 0.5%, using about 250 mg of sample.

**Residue on ignition** (281): not more than 0.1%. The sample is moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Heavy metals, Method II** (231): 0.002%.

### Related compounds—

**NOTE**—On the basis of the synthetic route used, perform either *Test 1* or *Test 2*. However, if the chloro and bromo analogs are potential related compounds in the synthetic route used, *Test 2* is recommended.

#### TEST 1—

**Buffer, Mobile phase, Diluent, and Chromatographic system**—Proceed as directed in the *Assay*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Use the *Standard preparation*, prepared as directed in the *Assay*.

**Working standard solution**—Dilute the *Standard solution* with *Diluent*, quantitatively and stepwise if necessary, to obtain a solution having a concentration of 0.625  $\mu\text{g}$  per mL of citalopram hydrobromide.

**System suitability solution**—Dissolve an accurately weighed quantity of USP Citalopram Hydrobromide RS and USP Citalopram Related Compound D RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.001 mg per mL.

**Sensitivity solution**—Dilute 5.0 mL of the *Working standard solution* with *Diluent* to 50 mL to obtain a solution having 0.0625  $\mu\text{g}$  of citalopram hydrobromide per mL.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—Inject the *Diluent* as directed for *Procedure* to verify that there are no interfering peaks. Chromatograph the *Sensitivity solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio is at least 3. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between citalopram related compound D and citalopram is not less than 1.8; the tailing factor for the citalopram hydrobromide peak is not less than 0.8 and not more than 1.5; and the relative standard deviation for replicate injections, based on the citalopram peak, is not more than 5%.

**NOTE**—For the purpose of identification, the approximate relative retention times are 0.90 for citalopram related compound D and 1.0 for citalopram hydrobromide.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms for about 40 minutes, and measure the responses for the major peaks. Calculate the percentage of related compounds in the portion of Citalopram Hydrobromide taken by the formula:

$$100(C_S / C_T)(r_i / r_S)(324.39/405.30)(1/F)$$

in which  $C_S$  and  $C_T$  are the concentrations, in mg per mL, of Citalopram Hydrobromide in the *Working standard solution* and the *Test solution*, respectively;  $r_i$  is the peak response for each impurity obtained from the *Test solution*;  $r_S$  is the peak response for the citalopram peak, obtained from the *Working standard solution*; 324.39 and 405.30 are the molecular

Table 1

Related Compound	Relative Retention Time	Relative Response Factor ( <i>F</i> )	Limit (%)
1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-5-(4-dimethylaminobutyl)-1,3-dihydrobenzofuran	0.13	0.34	NMT* 0.1
Citalopram related compound A	0.18	0.77	NMT 0.1
4-[4-Dimethylamino-1-(4'-fluorophenyl)-1-hydroxy-1-butyl]-3-hydroxymethyl benzonitrile	0.26	0.99	NMT 0.1
Citalopram related compound B	0.40	0.98	NMT 0.1
Citalopram related compound C	0.67	0.69	NMT 0.1
Citalopram related compound D	0.90	1.04	NMT 0.1
Citalopram hydrobromide	1.0	1.0	—
Citalopram related compound E	1.29	0.91	NMT 0.1
Individual unknown impurity	—	1.0	NMT 0.1 each
Total impurities	—	—	NMT 0.5

\*NMT = not more than.

weights for citalopram and citalopram hydrobromide, respectively; and *F* is the relative response factor for each impurity relative to citalopram (free base), as presented in *Table 1*.

#### TEST 2—

**Buffer**—Dissolve about 2.7 g of monobasic potassium phosphate in 1000 mL of water, add 1 mL of *N,N*-dimethyloctylamine, stir, and adjust with phosphoric acid to a pH of 3.0.

**Diluent**—Prepare a mixture of *Buffer* and acetonitrile (70:30).

**Solution A**—Prepare a mixture of *Buffer*, methanol, and tetrahydrofuran (70:24:6).

**Solution B**—Prepare a mixture of acetonitrile and *Buffer* (80:20).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed in *Table 2* for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve accurately weighed quantities of USP Citalopram Hydrobromide RS, USP Citalopram Related Compound A RS, USP Citalopram Related Compound C RS, USP Citalopram Related Compound D RS, USP Citalopram Related Compound G RS, and USP Citalopram Related Compound H RS in *Diluent* to obtain a final solution having a concentration of 1.5 µg per mL of each compound.

**Test solution**—Dissolve an accurately weighed quantity of Citalopram Hydrobromide in a suitable volume of *Diluent* to obtain a solution having a final concentration of 1.5 mg per mL of citalopram hydrobromide.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 224-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.8 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–18	100	0	isocratic
18–40	100→10	0→90	linear gradient
40–45	10	90	isocratic
45–46	10→100	90→0	linear gradient
46–55	100	0	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between citalopram and citalopram related compound D is not less than 2.0, and that between citalopram related compound G and citalopram related compound H is not less than 4.0; and the relative standard deviation for the

citalopram peak in replicate injections is not more than 2.0%.

NOTE—For the purpose of identification, the approximate relative retention times of citalopram related compounds are provided in *Table 2*.

Table 2

Related Compound	Relative Retention Time	Limit (%)
Citalopram related compound A	0.40	NMT* 0.15
Citalopram related compound C	0.88	NMT 0.15
Citalopram	1.0	—
Citalopram related compound D	1.09	NMT 0.15
Citalopram related compound G	2.20	NMT 0.15
Citalopram related compound H	2.30	NMT 0.15
Individual unspecified impurity	—	NMT 0.1
Total specified and unspecified impurities	—	NMT 0.75

\*NMT = not more than.

**Procedure**—Inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each citalopram related compound in the portion of Citalopram Hydrobromide taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which *C<sub>S</sub>* is the concentration, in mg per mL, of each citalopram related compound in the *Standard solution*; *C<sub>T</sub>* is the concentration of citalopram hydrobromide in the *Test solution*; *r<sub>i</sub>* is the peak area of each impurity obtained from the *Test solution*; and *r<sub>S</sub>* is the peak area of each corresponding impurity obtained from the *Standard solution*. Calculate the percentage of each unspecified impurity using the same formula above, but using the concentration, in mg per mL, of citalopram free base in the *Standard solution* for *C<sub>S</sub>* and the response for the citalopram peak obtained from the *Standard solution* for *r<sub>S</sub>*. [NOTE—Disregard the peak due to bromide which may appear at a relative retention time of about 0.24.]

#### Assay—

**Buffer**—In a 1-L volumetric flask, dissolve about 1 g of sodium acetate in 800 mL of water, and add 6 mL of triethylamine. Adjust with acetic acid to a pH of 4.6, and dilute with water to volume.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (80:20). The apparent pH is 5.0 ± 0.1.

Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluent**—Prepare a mixture of methanol and water (1:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Citalopram Hydrobromide RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.625 mg per mL.

**Assay preparation**—Transfer about 62.5 mg of Citalopram Hydrobromide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix to obtain a solution containing 0.625 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 239-nm detector and a 150-mm × 4.6-mm column that contains 5-μm packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 50°. Inject the *Diluent* to verify that there are no interfering peaks. Inject the *Standard preparation*, and record the peak responses as directed for *Procedure*; the column efficiency is not less than 3000 theoretical plates; the tailing factor is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for about 30 minutes, and measure the responses for the major peaks. Calculate the quantity, in percentage of C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O · HBr, in the portion of Citalopram Hydrobromide taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which  $C_s$  and  $C_u$  are the concentrations, in mg per mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Citalopram Oral Solution

### DEFINITION

Citalopram Oral Solution contains an amount of citalopram hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O). It may contain a suitable preservative.

### IDENTIFICATION

- **PROCEDURE:** The retention time of the citalopram peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** Methanol and acetonitrile (1:9)

**Buffer:** 6.1 g/L of monobasic potassium phosphate in water. Add 1.5 mL of triethylamine per L of the solution. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** *Solution A* and *Buffer* (7:18)

**Diluent:** Acetonitrile and *Buffer* (1:3)

**Standard solution:** 0.25 mg/mL of USP Citalopram Hydrobromide RS

**Sample solution:** Transfer a suitable volume of Oral Solution to a suitable volumetric flask to obtain 0.2 mg/mL final concentration of citalopram free base. Add 50% of the flask volume of *Diluent*, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with *Diluent* to volume. [NOTE—The *Sample solution* may be filtered through either a PVDF or nylon membrane filter of suitable pore size.]

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

**Run time:** 2 times the retention time of citalopram

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O in the portion of Oral Solution taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times (M_{r1} / M_{r2}) \times 100$$

$r_u$  = peak response of citalopram from the *Sample solution*

$r_s$  = peak response of citalopram from the *Standard solution*

$C_s$  = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of citalopram in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of citalopram free base, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

**Acceptance criteria:** 90.0%–110.0% of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O)

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**Solution A:** Acetonitrile, methanol, and tetrahydrofuran (17:1:2)

**Buffer:** Dissolve 3.0 g of 1-octane sulphonic acid sodium salt in 1 L of water. Add 2 mL of triethylamine and 5 mL of tetra-*n*-butyl ammonium hydroxide, 40 percent in water. Mix and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** *Solution A* and *Buffer* (1:3)

**Diluent:** Acetonitrile and water (1:3)

**System suitability solution:** 6 μg/mL of USP Citalopram Related Compound D RS and 1.3 mg/mL of USP Citalopram Hydrobromide RS in *Diluent*

**Standard solution:** 6.3 μg/mL of USP Citalopram Hydrobromide RS in *Diluent*

**Sample solution:** Transfer a suitable volume of the Oral Solution to a suitable volumetric flask to obtain 1 mg/mL final concentration of citalopram. Add 60% of the flask volume of *Diluent*, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with *Diluent* to volume. [NOTE—The *Sample solution* may be filtered through either a PVDF or nylon membrane filter of suitable pore size.]

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL

**Injection size:** 20 μL

**Run time:** 2.6 times the retention time of citalopram for the *System suitability solution* and *Standard solution*; 5.7 times the retention time of citalopram for the *Sample solution*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.8 between citalopram and citalopram related compound D, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of citalopram from the *Standard solution*

$C_S$  = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of citalopram in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity as listed in *Impurity Table 1*

$M_{r1}$  = molecular weight of citalopram free base, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.50%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A <sup>a</sup>	0.29	0.86	0.20
Carboxyl citalopram <sup>b</sup>	0.47	0.72	0.20
Desfluoro citalopram <sup>c,h</sup>	0.71	—	—
Citalopram related compound C <sup>d</sup>	0.83	1.85	0.20
Citalopram	1.0	—	—
Citalopram related compound D <sup>e</sup>	1.11	0.95	0.20
Citalopram related compound G <sup>f,h</sup>	3.13	—	—

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

<sup>b</sup> 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid.

<sup>c</sup> 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>d</sup> 3-[3-(Dimethylamino)-1-propyl](4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.

<sup>e</sup> 1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>f</sup> 1-(4-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-chlorophthalane hydrobromide.

<sup>g</sup> 1-(4-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-bromophthalane hydrobromide.

<sup>h</sup> This process impurity is listed for information only.

**Impurity Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound H <sup>g,h</sup>	3.75	—	—
Any individual unspecified degradation product	—	—	0.15

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

<sup>b</sup> 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid.

<sup>c</sup> 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>d</sup> 3-[3-(Dimethylamino)-1-propyl](4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.

<sup>e</sup> 1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>f</sup> 1-(4-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-chlorophthalane hydrobromide.

<sup>g</sup> 1-(4-Fluorophenyl)-1-(3-dimethylaminopropyl)-5-bromophthalane hydrobromide.

<sup>h</sup> This process impurity is listed for information only.

**SPECIFIC TESTS**

• **DELIVERABLE VOLUME** <698>: Meets the requirements

• **PH** <791>: 3.5–7.0

• **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed 100 cfu/mL. The total yeasts and molds count does not exceed 50 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound D RS

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride

C<sub>19</sub>H<sub>19</sub>FN<sub>2</sub>O · HCl 346.83

**Citalopram Tablets****DEFINITION**

Citalopram Tablets contain an amount of Citalopram Hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O).

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** <197K>

**Sample:** Extract finely ground Tablet powder containing 200 mg of citalopram with 30 mL of water, and filter. Add 1 mL of 1 N sodium hydroxide to the filtrate, and extract with 50 mL of cyclohexane by shaking for 10 min. Pass the cyclohexane layer through a silicone-treated filter paper into a beaker. Reduce the filtrate down to 3 mL, using gentle heat as necessary. Transfer the hot solution to a small centrifuge tube. Induce crystallization while cooling by scratching the side of the test tube with a spatula. Centrifuge the mixture, and decant off the cyclohexane. Dry the residue under vacuum in a desiccator. [NOTE—If crystallization fails to occur in the above procedure, use the following alternative procedure. Extract finely ground Tablet powder containing about 50 mg of citalopram with 10 mL of



chloroform in a test tube, and sonicate for 1 min. Centrifuge for 10 min, and filter into a beaker. Evaporate to dryness with nitrogen and if necessary induce crystallization by etching the beaker.]

Mix approximately 2 mg of the residue with approximately 300 mg of potassium bromide, and record the IR spectrum.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

**Buffer:** 1.42 g/L of anhydrous dibasic sodium phosphate in water

**Diluent:** Methanol and *Buffer* (80:20)

**Mobile phase:** 0.77 mg/mL of dodecyltrimethylammonium bromide in *Diluent*

**Internal standard solution:** 0.25 mg/mL of USP

Citalopram Related Compound F RS in *Diluent*

**Standard stock solution:** 1.25 mg/mL of USP

Citalopram Hydrobromide RS in *Diluent*

**Standard solution:** 0.025 mg/mL of USP Citalopram Related Compound F RS and 0.125 mg/mL of USP

Citalopram Hydrobromide RS from the *Internal standard solution* and the *Standard stock solution*, respectively, in *Diluent*

**Sample solution:** Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of methanol, and sonicate for about 5 min. Allow to cool to room temperature, then dilute with *Diluent* to volume. Allow to stand until the residue settles before taking an aliquot for dilution. Transfer a volume of the clear supernatant to a 50-mL volumetric flask to obtain a final nominal concentration between 0.090 mg/mL and 0.10 mg/mL of citalopram. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume. Pass a portion through a filter (PTFE) having a 0.45-μm or finer pore size.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for citalopram related compound F and citalopram are about 1.36 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 1.5 between citalopram and citalopram related compound F

**Column efficiency:** NLT 2000 theoretical plates, calculated from the citalopram peak

**Relative standard deviation:** NMT 1.5% for the citalopram peak

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the label claim of citalopram in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = ratio of the peak response of citalopram to the internal standard from the *Sample solution*

$R_S$  = ratio of the peak response of citalopram to the internal standard from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Buffer solution:** pH 1.5 buffer (prepared by transferring 118 mL of 1 N hydrochloric acid and 82 mL of 1 N sodium hydroxide to a 1000-mL volumetric flask, diluting with water to volume, and adjusting with 1 N sodium hydroxide to a pH of 1.5)

**Medium:** *Buffer solution*; 800 mL, deaerated

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Detector:** UV at about 239 nm

**Sample solution:** Sample per *Dissolution* <711>. Pass through a PVDF filter having a 0.45-μm pore size, and dilute with *Medium* as needed.

**Standard solution:** 12 μg/mL of USP Citalopram Hydrobromide RS in *Medium*

**Analysis:** Calculate the percentage of citalopram dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (M_{r1}/M_{r2}) \times (100/L)$$

$A_U$  = absorbance from the *Sample solution*

$A_S$  = absorbance from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium* (800 mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

$L$  = Tablet label claim of citalopram (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{20}H_{21}FN_2O$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

## IMPURITIES

### Organic Impurities

### • PROCEDURE

**Buffer:** 3.15 g/L of potassium dihydrogen phosphate and 3.60 g/L of disodium hydrogen phosphate ( $Na_2HPO_4 \cdot 12H_2O$ ) in water

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (38:7:55). Adjust with phosphoric acid to a pH of 6.5.

**Standard stock solution:** 0.25 mg/mL of USP

Citalopram Hydrobromide RS in *Mobile phase*

**System suitability solution:** 1 μg/mL each of USP Citalopram Related Compound A RS, USP Citalopram Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E RS, in *Standard stock solution*

**Standard solution:** 0.625 μg/mL of citalopram hydrobromide from *Standard stock solution* in *Mobile phase*

**Sensitivity solution:** 0.05 μg/mL of citalopram hydrobromide from *Standard solution* in *Mobile phase*

**Sample solution:** Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of a mixture of methanol and water (1:1), mix, and sonicate for about 5 min. Allow to cool, dilute with a mixture of methanol and water (1:1) to volume, and mix thoroughly. Allow the excipients to settle. Dilute with *Mobile phase* as necessary to obtain a final concentration of 0.5 mg/mL of citalopram. Pass a portion of this solution through a polytetrafluoroethylene (PTFE) membrane filter having a 0.45-μm or finer pore size, and use the filtrate.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 239 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 45°**Flow rate:** 0.8 mL/min**Injection size:** 20 μL**System suitability****Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*[NOTE—The relative retention times are given in *Impurity Table 1*.]**Suitability requirements****Resolution:** NLT 3 between citalopram related compound C and citalopram, *System suitability solution***Tailing factor:** NMT 1.5, *Standard solution***Relative standard deviation:** NMT 5%, *Standard solution***Signal-to-noise ratio:** NLT 3, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

- $r_U$  = peak response for each citalopram related compound from the *Sample solution*  
 $r_S$  = peak response of the corresponding peak in the *Standard solution*  
 $C_S$  = concentration of citalopram hydrobromide in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of citalopram hydrobromide in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of citalopram, 324.39  
 $M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30  
 $F$  = relative response factor for each impurity relative to citalopram (free base)

**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 0.8%**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A	0.43	0.77	0.2
Citalopram related compound B	0.60	0.98	0.25
Citalopram related compound C	0.83	0.69	0.25
Citalopram	1.0	—	—
Citalopram related compound E	1.32	0.91	0.1
Any other individual unidentified impurity	—	1.0	0.2

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS** <11>
  - USP Citalopram Hydrobromide RS
  - USP Citalopram Related Compound A RS
  - 1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
  - $C_{20}H_{23}FN_2O_2$  342.22

USP Citalopram Related Compound B RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.

 $C_{20}H_{21}FN_2O_2$  340.22

USP Citalopram Related Compound C RS

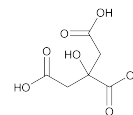
3-[3-(Dimethylamino)-1-propyl](4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone. $C_{20}H_{19}FN_2O_2$  338.22

USP Citalopram Related Compound E RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydrobenzofuran-5-carbonitrile-*N*-oxide. $C_{20}H_{21}FN_2O_2$  340.22

USP Citalopram Related Compound F RS

Dimethyl-(1-methyl-3,3-diphenylallyl)amine hydrochloride.

 $C_{18}H_{21}NHCl$  286.64**Anhydrous Citric Acid**Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact. $C_6H_8O_7$ 

1,2,3-Propanetricarboxylic acid, 2-hydroxy-; Citric acid [77-92-9].

192.1

**DEFINITION**Anhydrous Citric Acid contains NLT 99.5% and NMT 100.5% of  $C_6H_8O_7$ , calculated on the anhydrous basis.**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197K>: Dry the substance to be examined at 105° for 2 h.

**ASSAY****PROCEDURE****Sample:** 0.550 g of Anhydrous Citric Acid; record weight accurately.**Analysis:** Dissolve the *Sample* in 50 mL of water. Add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of  $C_6H_8O_7$ .**Acceptance criteria:** 99.5%–100.5% on the anhydrous basis**IMPURITIES**

- RESIDUE ON IGNITION** <281>: NMT 0.1%, determined on 1.0 g
- HEAVY METALS** <231>: NMT 10 μg/g♦
- SULFATE**

**Standard sulfate solution A:** 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 μg/mL of sulfate.**Standard sulfate solution B:** 1.81 mg/mL of potassium sulfate in water. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 μg/mL of sulfate.**Sample stock solution:** 66.7 mg/mL of Anhydrous Citric Acid**Sample solution:** To 4.5 mL of *Standard sulfate solution A*, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the

resulting suspension, add 15 mL of the *Sample stock solution* and 0.5 mL of 5 N acetic acid, and mix.

**Standard solution:** Prepare as directed for the *Sample solution*, except use 15 mL of *Standard sulfate solution B* instead of the *Sample stock solution*.

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any turbidity produced in the *Sample solution* after 5 min standing is not greater than that produced in the *Standard solution* (0.015%).

- **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)

**Standard aluminum solution:** To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, dilute with water to volume, and mix. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.

**pH 6.0 acetate buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.

**Sample solution:** Dissolve 20.0 g of Anhydrous Citric Acid in 100 mL of water, and add 10 mL of pH 6.0 acetate buffer. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.

**Standard solution:** Prepare a mixture of 2.0 mL of *Standard aluminum solution*, 10 mL of pH 6.0 acetate buffer, and 98 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

**Blank solution:** Prepare a mixture of 10 mL of pH 6.0 acetate buffer and 100 mL of water. Extract this mixture as described for *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

#### Fluorometric conditions

**Excitation wavelength:** 392 nm

**Emission wavelength:** 518 nm

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Determine the fluorescence intensities of the *Samples* in a fluorometer set as directed under *Fluorometric conditions*, using the *Blank solution* to set the instrument to zero.

**Acceptance criteria:** The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).

- **LIMIT OF OXALIC ACID**

**Sample stock solution:** 0.80 g of Anhydrous Citric Acid in 4 mL of water

**Sample solution:** To the *Sample stock solution* add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1 min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake, and allow to stand for 30 min.

**Standard solution:** Prepare as directed for the *Sample solution*, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the *Sample stock solution*. [NOTE—Prepare concomitantly with the *Sample solution*.]

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** The level of bacterial endotoxins is such that the requirement in the relevant

dosage form monograph(s) in which Anhydrous Citric Acid is used can be met. Where the label states that Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used can be met.♦

#### • CLARITY OF SOLUTION

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine sulfate solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

**Opalescence standard:** Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]

**Standard suspension A:** Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.

**Standard suspension B:** Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.

**Sample solution:** 200 mg/mL of Anhydrous Citric Acid in water

#### Analysis

**Samples:** *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]

**Acceptance criteria:** The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

#### • COLOR OF SOLUTION

**Standard stock solution A:** Ferric chloride CS, cobaltous chloride CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 7.0)

**Standard stock solution B:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)

**Standard stock solution C:** Ferric chloride CS, cobaltous chloride CS, and cupric sulfate CS (9.6: 0.2: 0.2) [NOTE—Prepare the *Standard solutions* immediately before use.]

**Standard solution A:** Dilute 2.5 mL of *Standard stock solution A* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution B:** Dilute 2.5 mL of *Standard stock solution B* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution C:** Dilute 0.75 mL of *Standard stock solution C* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Sample solution:** Prepare as directed in the test for *Clarity of Solution*.

**Analysis 1****Samples:** Water and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

**Acceptance criteria 1:** The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.

**Analysis 2****Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*, and *Standard solution C* in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

**Acceptance criteria 2:** The *Sample solution* is not more intensely colored than *Standard solutions A*, *B*, and *C*.

**• READILY CARBONIZABLE SUBSTANCES****Sample:** 1.0 g of powdered Anhydrous Citric Acid

**Analysis:** Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at 90 ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.

**Acceptance criteria:** The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity* (631)) in a matching tube, the tubes being observed vertically against a white background.

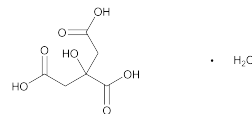
- **STERILITY TESTS** (71): Where the label states that Anhydrous Citric Acid is sterile, it meets the requirements for *Sterility Tests* (71) in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used.♦

**• WATER DETERMINATION, Method I** (921)**Sample:** 2.0 g of Anhydrous Citric Acid**Acceptance criteria:** NMT 1.0%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Anhydrous Citric Acid is sterile, it is so labeled.
- **USP REFERENCE STANDARDS** (11)  
USP Citric Acid RS  
USP Endotoxin RS.♦

**Citric Acid Monohydrate**

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



$C_6H_8O_7 \cdot H_2O$  210.14  
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, monohydrate  
[5949-29-1].

**DEFINITION**

Citric Acid Monohydrate contains one molecule of water of hydration. It contains NLT 99.5% and NMT 100.5% of  $C_6H_8O_7$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K): Dry the substance to be examined at 105° for 2 h.

**ASSAY****• PROCEDURE**

**Sample:** 0.550 g of Citric Acid Monohydrate. Record the weight accurately.

**Analysis:** Dissolve the *Sample* in 50 mL of water, and add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of  $C_6H_8O_7$ .

**Acceptance criteria:** 99.5%–100.5% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%, determined on 1.0 g

- **HEAVY METALS** (231): NMT 10 µg/g.♦

**• SULFATE**

**Standard sulfate solution A:** 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 µg/mL of sulfate.

**Standard sulfate solution B:** 1.81 mg/mL of potassium sulfate. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg/mL of sulfate.

**Sample stock solution:** 66.7 mg/mL of Citric Acid Monohydrate

**Sample solution:** To 4.5 mL of *Standard sulfate solution A*, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension add 15 mL of the *Sample stock solution* and 0.5 mL of 5 N acetic acid, and mix.

**Standard solution:** Prepare as directed in the *Sample solution*, except use 15 mL of *Standard sulfate solution B* instead of the *Sample stock solution*.

**Analysis****Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any turbidity produced in the *Sample solution* after 5 min standing is not greater than that produced in the *Standard solution* (0.015%).

- **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)

**Standard aluminum solution:** To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, and dilute with water to volume. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.

**pH 6.0 acetate buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.

**Sample solution:** Dissolve 20.0 g of Citric Acid Monohydrate in 100 mL of water, and add 10 mL of pH 6.0 acetate buffer. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloro-

form extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.

**Standard solution:** Prepare a mixture of 2.0 mL of *Standard aluminum solution*, 10 mL of pH 6.0 acetate buffer, and 98 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

**Blank solution:** Prepare a mixture of 10 mL of pH 6.0 acetate buffer and 100 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

#### Fluorometric conditions

**Excitation wavelength:** 392 nm

**Emission wavelength:** 518 nm

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Determine the fluorescence intensities of the *Samples* in a fluorometer set as directed under *Fluorometric conditions*, using the *Blank solution* to set the instrument to zero.

**Acceptance criteria:** The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).

#### • LIMIT OF OXALIC ACID

**Sample stock solution:** 0.80 g of Citric Acid Monohydrate in 4 mL of water

**Sample solution:** To the *Sample stock solution* add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1 min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake and allow to stand for 30 min.

**Standard solution:** Prepare as directed for the *Sample solution*, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the *Sample stock solution*. [NOTE—Prepare concomitantly with the *Sample solution*.]

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met. Where the label states that Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met.♦

#### • CLARITY OF SOLUTION

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine sulfate solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

**Opalescence standard:** Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]

**Standard suspension A:** Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.

**Standard suspension B:** Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.

**Sample solution:** 200 mg/mL of Citric Acid Monohydrate in water

#### Analysis

**Samples:** *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]

**Acceptance criteria:** The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

#### • COLOR OF SOLUTION

**Standard stock solution A:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 0: 7.0)

**Standard stock solution B:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)

**Standard stock solution C:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (9.6: 0.2: 0.2: 0)

[NOTE—Prepare the *Standard solutions* immediately before use.]

**Standard solution A:** Transfer 2.5 mL of *Standard stock solution A*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution B:** Transfer 2.5 mL of *Standard stock solution B*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution C:** Transfer 0.75 mL of *Standard stock solution C*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Sample solution:** Prepare as directed in the test for *Clarity of Solution*.

#### Analysis 1

**Samples:** Water and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

**Acceptance criteria 1:** The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.

#### Analysis 2

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*,

and *Standard solution C* in diffused daylight, viewing vertically against a white background (see *Spectrophotometry and Light-Scattering* <851>, *Visual Comparison*).

**Acceptance criteria 2:** The *Sample solution* is not more intensely colored than *Standard solutions A, B, and C*.

• **READILY CARBONIZABLE SUBSTANCES**

**Sample:** 1.0 g powdered Citric Acid Monohydrate

**Analysis:** Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at  $90 \pm 1^\circ$  for  $60 \pm 0.5$  min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.

**Acceptance criteria:** The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity* <631>) in a matching tube, the tubes being observed vertically against a white background.

- **STERILITY TESTS** <71>: Where the label states that Citric Acid Monohydrate is sterile, it meets the requirements for *Sterility Tests* <71> in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used.♦

• **WATER DETERMINATION, Method I** <921>

**Sample:** 0.5 g of Citric Acid Monohydrate

**Acceptance criteria:** 7.5%–9.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Citric Acid Monohydrate is sterile, it is so labeled.
- **USP REFERENCE STANDARDS** <11>  
USP Citric Acid RS  
USP Endotoxin RS.♦

## Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation

» Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation is a sterile solution of Citric Acid, Magnesium Oxide, and Sodium Carbonate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of citric acid ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ), magnesium oxide ( $\text{MgO}$ ), and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ).

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

**USP Reference standards** <11>—

USP Citric Acid RS  
USP Endotoxin RS

**Identification**—

**A:** It meets the requirements of the tests for *Sodium* <191> and for *Magnesium* <191>.

**B:** To 10 mL of the Irrigation add 1 mL of mercuric sulfate TS, heat to boiling, and add a few drops of potassium permanganate TS: a white precipitate is formed.

**Bacterial endotoxins** <85>—It contains not more than 2.80 Endotoxin Units per mL.

**pH** <791>: between 3.8 and 4.2.

**Other requirements**—It meets the requirements under *Injections* <1>, except that the container may be designed to empty rapidly, and may exceed 1000 mL in capacity.

**Assay for citric acid**—

*Mobile Phase, Standard Preparation 1, and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* <345>.

*Assay preparation*—Transfer an accurately measured volume of Irrigation, equivalent to about 130 mg of monohydrate citric acid into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under *Assay for Citric Acid/Citrate and Phosphate* <345>.

*Procedure*—Proceed as directed for *Procedure* under <345>, and calculate the quantity, in mg per mL, of monohydrate citric acid ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) in the Irrigation taken by the formula:

$$0.001(210.14 / 189.10)C_s(D/V)(r_u / r_s)$$

in which 210.14 is the molecular weight of citric acid monohydrate; 189.10 is the molecular weight of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ );  $C_s$  is the concentration, in  $\mu\text{g}$  per mL, of citrate in *Standard Preparation 1*;  $D$  is the dilution factor;  $V$  is the volume, in mL, of Irrigation used to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the citrate peak areas obtained from the *Assay preparation* and *Standard Preparation 1*, respectively.

**Assay for magnesium oxide**—Transfer an accurately measured volume of Irrigation, equivalent to about 40 mg of magnesium oxide, to a beaker containing 130 mL of water heated to  $75 \pm 5^\circ$ , and add 4 mL of ammonium chloride TS and then 5 mL of ammonium hydroxide. Mix, and add slowly, with stirring, 8 mL of 8-hydroxyquinoline TS. After allowing to stand for 30 minutes at  $75^\circ$ , filter through a sintered-glass crucible, previously dried and weighed, and wash the precipitate with 50 mL of a warm mixture of water and 6 N ammonium hydroxide (45:5), followed by 50 mL of cool water. Dry the crucible and contents at  $105^\circ$  for 3 hours, cool, and weigh. Determine the equivalent of  $\text{MgO}$  in the portion of Irrigation taken by multiplying the weight of  $\text{C}_{18}\text{H}_{12}\text{MgN}_2\text{O}_2 \cdot 2\text{H}_2\text{O}$  so obtained by 0.1156.

**Assay for sodium carbonate**—

*Sodium chloride stock solution*—Transfer 475 mg of sodium chloride, previously dried at  $105^\circ$  for 2 hours and accurately weighed, to a 100-mL volumetric flask. Dissolve in water, dilute with water to volume, and mix.

*Internal standard solution*—Transfer 636 mg of lithium chloride to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Standard preparation*—Quantitatively prepare a mixture of *Internal standard solution* and *Sodium chloride stock solution* (99:1).

*Assay preparation*—Dilute an accurately measured volume of Irrigation quantitatively with water to obtain a stock solution containing about 4.4 mg of sodium carbonate per mL. Quantitatively prepare a mixture of *Internal standard solution* and this stock solution (99:1).

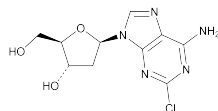
*Procedure*—Concomitantly determine the emittances of the *Standard preparation* and the *Assay preparation* at 591 nm and 671 nm with a suitable flame photometer, adjusting the instrument to zero emittance with *Internal standard solution*. Calculate the quantity, in mg, of  $\text{Na}_2\text{CO}_3$  in each mL of the Irrigation taken by the formula:

$$(105.99/116.88)(C)(L/D)(R_{u,591} / R_{u,671})(R_{s,671} / R_{s,591})$$

in which 105.99 is the molecular weight of sodium carbonate; 116.88 is two times the molecular weight of sodium chloride;  $C$  is the concentration, in mg per mL, of sodium chloride in the *Sodium chloride stock solution*;  $L$  is the labeled quantity, in mg per mL, of sodium carbonate in the Irriga-

tion;  $D$  is the concentration, in mg per mL, of sodium carbonate in the stock solution used to prepare the *Assay preparation*, on the basis of the labeled quantity in each mL, and the extent of dilution;  $R_{U,591}$  and  $R_{U,671}$  are the emittance readings obtained from the *Assay preparation* at the wavelengths indicated by the subscripts; and  $R_{S,671}$  and  $R_{S,591}$  are the emittance readings obtained from the *Standard preparation* at the wavelengths indicated by the subscripts.

## Cladribine



$C_{10}H_{12}ClN_5O_3$  285.69  
Adenosine, 2-chloro-2'-deoxy-  
2-Chloro-2'-deoxyadenosine [4291-63-8].

» Cladribine contains not less than 98.0 percent and not more than 102.0 percent of  $C_{10}H_{12}ClN_5O_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers, and protect from light. Store between 2° and 8°.

**USP Reference standards** (11)—

USP Cladribine RS

USP Cladribine Related Compound A RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between  $-17.0^\circ$  and  $-21.0^\circ$ .

*Test solution:* 10 mg per mL, in dimethylformamide.

**Water**, *Method I* (921): not more than 4.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Related compounds**—

*Buffer, Diluent, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cladribine and cladribine related compound A is not less than 1.5; and the tailing factor is not more than 2.0.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram,

and measure the peak responses. Calculate the percentage of each impurity in the portion of Cladribine taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response of each individual impurity, and  $r_s$  is the sum of the responses of all the peaks. Refer to *Table 1* of the impurity limits.

**Assay**—

*Buffer*—Dissolve 9.96 g of triethylamine phosphate, accurately weighed, in 500 mL of water, and add another 500 mL of water. Adjust with potassium hydroxide to a pH of 6.1.

*Diluent*—Prepare a mixture of water and methanol (90:10).

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer* and methanol (78:22). Make adjustments if necessary (see *System suitability* under *Chromatography* (621)).

*System suitability solution*—Prepare a solution of USP Cladribine RS and USP Cladribine Related Compound A RS in *Diluent* to obtain known concentrations of about 0.02 mg per mL each.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cladribine RS in *Diluent* to obtain a concentration of 0.5 mg per mL.

*Assay preparation*—Transfer about 25 mg of Cladribine, accurately weighed, to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cladribine and cladribine related compound A is not less than 1.5; the tailing factor for the cladribine peak in the *System suitability solution* is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{10}H_{12}ClN_5O_3$  in the portion of Cladribine taken by the formula:

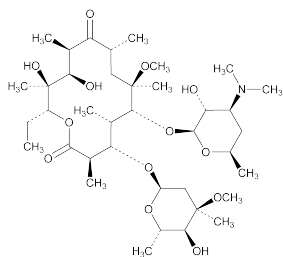
$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cladribine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Table 1**

Name	Relative Retention	
	Time	Limit (%)
2,6-Diaminopurine-2'-deoxyriboside	about 0.41	0.20
2'-Deoxyadenosine	about 0.47	0.20
2-Chloroadenine	about 0.60	0.20
2-Methoxy-2'-deoxyadenosine (cladribine related compound A)	about 0.91	0.20
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

## Clarithromycin



$C_{38}H_{69}NO_{13}$  747.95  
Erythromycin, 6-O-methyl-;  
6-O-Methylerythromycin [81103-11-9].

### DEFINITION

Clarithromycin contains NLT 96.0% and NMT 102.0% of clarithromycin ( $C_{38}H_{69}NO_{13}$ ), calculated on the anhydrous basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION <197K>

### ASSAY

#### Change to read:

#### PROCEDURE

**Solution A, Solution B, Mobile phase, Diluent, Standard solution 1, Standard solution 2, Standard solution 4, Sample solution, and Chromatographic system:** Proceed as directed in *Organic Impurities*.

#### System suitability

**Samples:** *Standard solution 2* and *Standard solution 4*  
[NOTE—See the relative retention times in *Table 2*. The typical retention time for clarithromycin is about 11 min.]

#### Suitability requirements

**Tailing factor:** NMT 1.7, *Standard solution 2*  
**Peak-to-valley ratio:** NLT 3.0, *Standard solution 4*  
The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

$H_P$  = height, above the baseline, of the  
▲clarithromycin▲*USP36* impurity D peak,  
*Standard solution 4*

$H_V$  = height, above the baseline, of the lowest point  
of the curve separating the  
▲clarithromycin▲*USP36* impurity D peak from  
the clarithromycin peak, *Standard solution 4*

**Relative standard deviation:** NMT 1.5%, *Standard solution 2*

#### Analysis

**Samples:** *Standard solution 1* and *Sample solution*  
Calculate the percentage of clarithromycin ( $C_{38}H_{69}NO_{13}$ )  
in the portion of Clarithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area response from the *Sample solution*

$r_S$  = peak area response from *Standard solution 1*

$C_S$  = concentration of USP Clarithromycin RS in  
*Standard solution 1* (mg/mL)

$C_U$  = concentration of Clarithromycin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 96.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Change to read:

#### RESIDUE ON IGNITION <281>

**Sample:** 0.5 g

▲*USP36*

**Acceptance criteria:** NMT 0.2%

#### Change to read:

#### HEAVY METALS

**Diluent:** 85% v/v dioxane in water

**Lead nitrate stock solution:** ▲Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid. Dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.▲*USP36*

**Sample solution:** 50 mg/mL in *Diluent*. Transfer 12 mL of this solution to a color-comparison tube.

**Standard solution:** 1 ppm of Pb, prepared by diluting *Lead nitrate stock solution* with *Diluent*. Add 10 mL of this solution and 2 mL of the *Sample solution* to a color-comparison tube.

**Blank:** Add 10 mL of *Diluent* and 2 mL of the *Sample solution* to a color-comparison tube.

#### Analysis

**Samples:** *Sample solution*, *Standard solution*, and *Blank*  
To each of the three tubes add 2 mL of pH 3.5 acetate buffer, mix, then add 1.2 mL of thioacetamide–glycerin base TS, and mix.

**Acceptance criteria:** Compared to the *Blank*, the *Standard solution* shows a slight brown color. After 2 min, any brown color in the *Sample solution* is not more intense than that in the *Standard solution* (NMT 20 µg/g).

#### Change to read:

#### ORGANIC IMPURITIES

**Solution A:** 4.76 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid (1 in 10) or potassium hydroxide (45% w/v) to a pH of 4.4. Pass this solution through a C18 filtration kit.

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

**Diluent:** Acetonitrile and water (1:1)

**Standard solution 1:** 1.5 mg/mL of USP Clarithromycin RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

**Standard solution 2:** 75 µg/mL of USP Clarithromycin RS from *Standard solution 1* in *Diluent*

**Standard solution 3:** 7.5 µg/mL of USP Clarithromycin RS from *Standard solution 2* in *Diluent*

**Standard solution 4:** 1.5 mg/mL of USP Clarithromycin Identity RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

**Sample solution:** 1.5 mg/mL of Clarithromycin in acetonitrile and water (1:1). Dissolve first in acetonitrile, us-



ing 50% of the final volume, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 10-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1.1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *Standard solution 2* and *Standard solution 4*

[NOTE—See the relative retention times in *Table 2*. The typical retention time for clarithromycin is about 11 min.]

#### Suitability requirements

**Tailing factor:** NMT 1.7, *Standard solution 2*

**Peak-to-valley ratio:** NLT 3.0, *Standard solution 4*

The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_p/H_v$$

$H_p$  = height, above the baseline, of the  
▲clarithromycin▲<sup>USP36</sup> impurity D peak,  
*Standard solution 4*

$H_v$  = height, above the baseline, of the lowest point  
of the curve separating the clarithromycin  
impurity D peak from the  
▲clarithromycin▲<sup>USP36</sup> peak, *Standard solution 4*

#### Analysis

**Samples:** *Diluent*, *Standard solution 2*, *Standard solution 3*, *Standard solution 4*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Clarithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times \frac{1}{F} \times 100$$

$r_U$  = peak response of any individual impurity from  
the *Sample solution*

$r_S$  = peak response of clarithromycin from *Standard solution 3*

$C_S$  = concentration of USP Clarithromycin RS in  
*Standard solution 3* (mg/mL)

$C_U$  = concentration of Clarithromycin in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

▲**Acceptance criteria:** See *Table 2*.

**Any individual impurity:** NMT 1.0%. NMT four impurities exceed 0.4%.

**Total impurities:** NMT 3.5%

**Table 2**

Name	Relative Retention Time	Relative Response Factor
Clarithromycin impurity I <sup>a</sup>	0.38	1.0
Clarithromycin impurity A <sup>b</sup> (clarithromycin F)	0.42	1.0
Clarithromycin impurity J <sup>c</sup>	0.63	1.0
Clarithromycin impurity L <sup>d</sup>	0.74	1.0
Clarithromycin impurity B <sup>e</sup>	0.79	1.0
Clarithromycin impurity M <sup>f</sup>	0.81	1.0
Clarithromycin impurity C <sup>g</sup>	0.89	1.0
Clarithromycin impurity D <sup>h</sup>	0.96	1.0
Clarithromycin	1.0	—
Clarithromycin impurity N <sup>i</sup>	1.15	1.0
Clarithromycin related compound A <sup>j</sup>	1.27	1.0
Clarithromycin impurity F <sup>k</sup>	1.33	1.0
Clarithromycin impurity P <sup>l</sup>	1.35	1.0
Clarithromycin impurity O <sup>m</sup>	1.38	1.0
Clarithromycin impurity K <sup>n</sup>	1.59	1.0
Clarithromycin impurity G <sup>o</sup>	1.72	3.7
Clarithromycin impurity H <sup>p</sup>	1.82	6.7

<sup>a</sup> 3-O-Decadinosyl-6-O-methylerythromycin A.

<sup>b</sup> 2-Demethyl-2-(hydroxymethyl)-6-O-methylerythromycin A.

<sup>c</sup> Erythromycin A (E)-9-oxime.

<sup>d</sup> 6-O-Methylerythromycin (Z)-9-oxime.

<sup>e</sup> 6-O-Methyl-15-norerythromycin A.

<sup>f</sup> 3''-N-Demethyl-6-O-methylerythromycin A (E)-9-oxime.

<sup>g</sup> 6-O-Methylerythromycin A (E)-9-oxime.

<sup>h</sup> 3''-N-Demethyl-6-O-methylerythromycin A.

<sup>i</sup> (10E)-10,11-Didehydro-11-deoxy-6-O-methylerythromycin A.

<sup>j</sup> 6,11-Di-O-methylerythromycin A.

<sup>k</sup> 6,12-Di-O-methylerythromycin A.

<sup>l</sup> 4',6-Di-O-methylerythromycin A.

<sup>m</sup> 6-O-Methylerythromycin A (Z)-9-(O-methyloxime).

<sup>n</sup> (1S,2R,5R,6S,7S,8R,9R,11Z)-2-Ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexapyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-O-decadinosyl-8,9:10,11-dianhydro-6-O-methylerythromycin A-9,12-hemiketal).

<sup>o</sup> 6-O-Methylerythromycin A (E)-9-(O-methyloxime).

<sup>p</sup> 3''-N-Demethyl-3''-N-formyl-6-O-methylerythromycin A.

▲<sup>USP36</sup>

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 10 mg/mL in methylene chloride

**Acceptance criteria:** −94° to −102° (at 20°)

##### • CRYSTALLINITY <69S>: Meets the requirements

##### • PH <791>

**Sample:** 2 mg/mL suspension in methanol and water (1:19)

**Acceptance criteria:** 8.0–10.0

##### • WATER DETERMINATION, *Method I* <921>: NMT 2.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight containers.

#### Change to read:

##### • USP REFERENCE STANDARDS <11>

USP Clarithromycin RS

USP Clarithromycin Identity RS

▲This is a mixture of clarithromycin, clarithromycin impurity D (3''-N-demethyl-6-O-methylerythromycin A; C<sub>37</sub>H<sub>67</sub>NO<sub>13</sub> 733.9), and other impurities.▲<sup>USP36</sup>

## Clarithromycin for Oral Suspension

### DEFINITION

Clarithromycin for Oral Suspension is a dry mixture of Clarithromycin, dispersing agents, diluents, preservatives, and flavorings. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ), the labeled amount being 25 mg or 50 mg/mL when constituted as directed in the labeling.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### Change to read:

#### PROCEDURE

**Buffer:** 0.067 M monobasic potassium phosphate  
**Mobile phase:** Methanol and *Buffer* (60:40), adjusted with phosphoric acid to a pH of 3.5. Pass through a suitable filter.

**Standard stock solution:** Equivalent to 2.1 mg/mL of clarithromycin from USP Clarithromycin RS in methanol  
**Standard solution:** 0.415 mg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Constitute the Clarithromycin for Oral Suspension as directed in the labeling. Transfer an aliquot of the suspension, equivalent to 1–2 g of clarithromycin, with the aid of 330 mL of *Buffer*, to a 1000-mL volumetric flask containing 50 mL of *Buffer*. Shake by mechanical means for 30 min, and dilute with methanol to volume. Sonicate for about 30 min, and allow to cool. Dilute with methanol to volume, add a magnetic stirring bar, and stir for 60 min. Allow to settle, and use the clear supernatant.

**Sample solution:** Transfer an aliquot of the *Sample stock solution*, nominally equivalent to 20 mg of clarithromycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

#### Columns

**Guard (optional):** Packing L1

**Analytical:** 4.6-mm × 15-cm; packing L1

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements** ▲<sup>USP36</sup>

**Tailing factor:** 1.0–1.7

▲<sup>USP36</sup>

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the portion of the constituted Clarithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area response from the *Sample solution*  
 $r_S$  = peak area response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clarithromycin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS** (905) (for powder packaged in single-unit containers): Meets the requirements
- DELIVERABLE VOLUME** (698) (for powder packaged in multi-unit containers): Meets the requirements

### SPECIFIC TESTS

#### pH (791)

**Sample:** Use the suspension constituted as directed in the labeling.

**Acceptance criteria:** 4.0–5.4

#### LOSS ON DRYING (731)

**Sample:** 1 g

**Analysis:** Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

**Acceptance criteria:** NMT 2.0%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS** (11)  
 USP Clarithromycin RS

## Clarithromycin Tablets

### DEFINITION

Clarithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{38}H_{69}NO_{13}$ .

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol and 0.067 M monobasic potassium phosphate (13:7). Adjust with phosphoric acid to a pH of 4.0, and pass through a suitable filter.

**Standard stock solution:** 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in methanol.

[NOTE—Shake and sonicate to facilitate dissolution.]

**Standard solution:** 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*. Pass through a suitable filter.

**System suitability stock solution:** 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol.

**System suitability solution:** 125 µg/mL of USP Clarithromycin RS from *Standard stock solution* and 125 µg/mL of USP Clarithromycin Related Compound A RS from *System suitability stock solution* in *Mobile phase*

**Sample stock solution:** Equivalent to 4 mg/mL of clarithromycin from finely powdered Tablets in methanol. [NOTE—Shake by mechanical means for 30 min to disperse and allow any insoluble matter to settle.]

**Sample solution:** 120 µg/mL of clarithromycin from *Sample stock solution* in *Mobile phase*. Pass through a filter of 0.5-µm or finer pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; packing L1

[NOTE—A guard column containing packing L1 may be added.]

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection size:** 20–50 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*

**Column efficiency:** NLT 750 theoretical plates from the clarithromycin peak, *Standard solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate percentage of label claim of  $C_{38}H_{69}NO_{13}$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of clarithromycin in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Buffer solution:** Prepare a solution containing 13.61 mg/mL of sodium acetate trihydrate in water. Prepare another solution by diluting 5.7 mL of glacial acetic acid to 1 L with water. Combine portions of the two solutions to obtain a pH of 5.0.

**Medium:** *Buffer solution*, 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** Proceed as directed in the *Assay*.

**Sample solution:** Dilute with *Mobile phase* to yield a nominal concentration of 125  $\mu\text{g/mL}$  of clarithromycin.

**Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed for *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amount of  $C_{38}H_{69}NO_{13}$  dissolved in the *Medium*, as directed in the *Assay*.

Calculate the percentage of clarithromycin dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{38}H_{69}NO_{13}$  is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

#### SPECIFIC TESTS

##### • LOSS ON DRYING <731>: Dry a portion of powdered Tablets in vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h: it loses NMT 6.0% of its weight.

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight containers.

##### • USP REFERENCE STANDARDS <11>

USP Clarithromycin RS

USP Clarithromycin Related Compound A RS

6,11-Di-O-methylerythromycin A.

$C_{39}H_{71}NO_{13}$  762.00

## Clarithromycin Extended-Release Tablets

#### DEFINITION

Clarithromycin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### Change to read:

##### • PROCEDURE

**Buffer A:** 0.067 M monobasic potassium phosphate  
**Mobile phase:** Methanol and *Buffer A* (13:7). Adjust with phosphoric acid to a pH of 4.0. Pass through a suitable filter.

**Standard stock solution:** 625  $\mu\text{g/mL}$  of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to facilitate dissolution.

**Standard solution:** 125  $\mu\text{g/mL}$  of clarithromycin in *Mobile phase* from *Standard stock solution*. Pass through a suitable filter.

**System suitability stock solution:** 625  $\mu\text{g/mL}$  of USP Clarithromycin Related Compound A RS in methanol

**System suitability solution:** 125  $\mu\text{g/mL}$  of USP Clarithromycin Related Compound A RS from *System suitability stock solution* and 125  $\mu\text{g/mL}$  of clarithromycin from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Transfer nominally 2000 mg of clarithromycin from finely powdered Tablets to a 500-mL volumetric flask with the aid of methanol. Add about 350 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and sonicate for 30 min. Cool to room temperature, and allow to stand for at least 16 h. Mix, allow any insoluble matter to settle, and use the supernatant.

**Sample solution:** Transfer 3.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

#### Columns

**Guard (optional):** Packing L1

**Analytical:** 4.6-mm  $\times$  15-cm; packing L1

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 20–50  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*

▲<sup>USP36</sup>

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of clarithromycin in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of clarithromycin in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****Change to read:****• DISSOLUTION <711>****Test 1**

**Buffer B:** Dissolve 816.5 g of monobasic potassium phosphate and 48 g of sodium hydroxide in about 4 L of water, mix, and dilute with water to 20 L. Adjust with either concentrated phosphoric acid or 1 N sodium hydroxide to a pH of  $6.0 \pm 0.05$ .

**Medium:** *Buffer B*; 900 mL

**Apparatus 2:** 75 rpm

**Times:** 30, 45, 60, and 120 min

**Standard solutions:** Prepare five solutions of USP Clarithromycin RS dissolved in acetonitrile and diluted with *Medium*, with known concentrations over a range of about 60–600  $\mu\text{g/mL}$ .

**Sample solution:** Use portions of the solution under test passed through a polyethylene filter of 35- $\mu\text{m}$  pore size.

**Chromatographic system:** Proceed as directed in the *Assay*, except the *Injection volume* is 50  $\mu\text{L}$ .

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*  
Perform a linear regression analysis to generate a standard curve using the peak area of each *Standard solution* versus its concentration. Determine the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each specified time interval, using the peak area of each *Sample solution* and the linear regression statistics for the *Standard solutions*.

**Tolerances:** The percentages of the labeled amounts of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to *Table 1*.

**Table 1**

Level	Time (min)	Amount Dissolved, Individual Limits (%)	Amount Dissolved, Average Limits (%)
L1	30	NMT 65	—
	45	55–85	—
	60	NLT 75	—
	120	NLT 85	—
L2	30	NMT 75	NMT 65
	45	45–95	55–85
	60	NLT 65	NLT 75
	120	NLT 75	NLT 85

**Table 1 (Continued)**

Level	Time (min)	Amount Dissolved, Individual Limits (%)	Amount Dissolved, Average Limits (%)
L3	30	NMT 2 Tablets release more than 75%, and no individual Tablet releases more than 85%	NMT 65
	45	NMT 2 Tablets are outside the range of 45%–95%, and no individual Tablet is outside the range of 35%–105%	55–85
	60	NMT 2 Tablets release less than 65%, and no individual Tablet releases less than 55%	NLT 75
	120	NMT 2 Tablets release less than 75%, and no individual Tablet releases less than 65%	NLT 85

**Test 2**

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Buffer C:** 0.05 M phosphate buffer with a pH of 6.8, containing 0.5% of sodium lauryl sulfate

**Medium:** *Buffer C*; 900 mL, degassed by sonication and vacuum

**Apparatus 1:** 100 rpm

**Times:** 2, 12, and 24 h

**Buffer D:** 9.2 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.5 prior to final dilution

**Mobile phase:** Methanol and *Buffer D* (65:35)

**Standard solution:** 0.56 mg/mL of USP Clarithromycin RS in a solution of methanol and *Medium* (1 in 10). Dissolve first in methanol using 10% of the final volume, and dilute with *Medium* to volume.

**Sample solution:** Centrifuge the solution under test at 2500 rpm for 10 min.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L1


**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 5  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements** 

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis****Samples:** *Standard solution* and *Sample solution*▲ Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each time point ( $Q_T$ ):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_{24} = [Q_2 \times (V_S/V)] + [Q_{12} \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of clarithromycin in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 900 mL $V_S$  = volume of the sample withdrawn at each time point (mL) $L$  = label claim (mg/Tablet) ▲<sup>USP36</sup>**Tolerances:** The percentages of the labeled amounts of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.**Table 2**

Time (h)	Amount Dissolved (%)
2	NMT 20
12	45–70
24	NLT 80

**Test 3**If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.**Buffer E:** Dissolve 3.59 g of sodium acetate trihydrate and 11.0 mL of 2 N acetic acid in 1000 mL of water. Adjust with 2 N acetic acid to a pH of 4.75.**Medium:** *Buffer E*; 1000 mL**Apparatus 1:** 10 mesh; 50 rpm**Times:** 1, 2, 4, 8, and 12 h**Buffer F:** 9.12 g/L of monobasic potassium phosphate in water**Mobile phase:** Methanol and *Buffer F* (65:35). Adjust with phosphoric acid to a pH of 4.0.**Standard stock solution:** 625 µg/mL of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to dissolve.**Standard solution:** 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase***System suitability stock solution:** 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol**System suitability solution:** 125 µg/mL of clarithromycin related compound A from *System suitability stock solution* and 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase***Sample solution:** Withdraw 10 mL of the solution under test from each vessel and replace with 10 mL of *Medium*. Transfer 3 mL of the withdrawn solution to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter of 0.45-µm pore size.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 15-cm; 5-µm packing L1**Column temperature:** 50°**Flow rate:** 1 mL/min**Injection volume:** 50 µL**System suitability****Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*▲<sup>USP36</sup>**Tailing factor:** 0.9–2, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*▲ Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each time point ( $Q_T$ ):

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_2 = [Q_1 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_4 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_8 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

$$Q_{12} = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [Q_8 \times V_S/(V - 4V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 4V_S) \times 100]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of clarithromycin in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 1000 mL $V_S$  = volume of the sample withdrawn at each time point (mL) $L$  = label claim (mg/Tablet) ▲<sup>USP36</sup>**Tolerances:** The percentages of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.**Table 3**

Time (h)	Amount Dissolved (%)
1	NMT 15
2	10–30
4	35–55
8	NLT 80
12	NLT 90

**Test 4**If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.**Buffer G:** 6.8 g/L of potassium dihydrogen phosphate and 0.18 g/L of sodium hydroxide in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 6.0 ± 0.1.**Medium:** *Buffer G*; 900 mL**Apparatus 2:** 50 rpm**Times:** 2, 4, 8, and 12 h**Buffer H:** 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 4.5 ± 0.1.

**Mobile phase:** Methanol and *Buffer H* (64:36)

**Standard solution:** 0.4 mg/mL of USP Clarithromycin RS in methanol and *Medium* (96:4). Dissolve first in *Medium*, using 60% of the final volume. Sonicate about 10 min until dissolved. Add methanol, using 4% of the final volume. Dilute with *Medium* to volume.

**Sample solution:** Use the solution under test, passed through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 203 nm

**Column:** 4.0-mm  $\times$  12.5-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the concentration, in mg/mL, of clarithromycin (C<sub>38</sub>H<sub>69</sub>NO<sub>13</sub>) in the *Sample solution* at each time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)

▲ Calculate the percentage of the labeled amount of clarithromycin (C<sub>38</sub>H<sub>69</sub>NO<sub>13</sub>) dissolved at each time point (Q<sub>7</sub>):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_8 = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [Q_8 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of clarithromycin in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $V_S$  = volume of the sample withdrawn at each time point (mL)  
 $L$  = label claim (mg/Tablet) ▲ USP36

**Tolerances:** The percentages of the labeled amount of clarithromycin (C<sub>38</sub>H<sub>69</sub>NO<sub>13</sub>) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Table 4**

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	45–75
12	NLT 80

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

## SPECIFIC TESTS

### Delete the following:

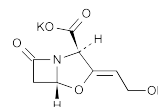
- ▲ **LOSS ON DRYING** <731>: Dry a portion of powdered Tablets in a vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h: it loses NMT 5.0% of its weight.

▲ USP36

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** <11>
  - USP Clarithromycin RS
  - USP Clarithromycin Related Compound A RS
  - 6,11-Di-O-methylethylerythromycin A.
  - C<sub>39</sub>H<sub>71</sub>NO<sub>13</sub> 762.00

## Clavulanate Potassium



C<sub>8</sub>H<sub>8</sub>KNO<sub>5</sub> 237.25  
 4-Oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3-(2-hydroxyethylidene)-7-oxo-, monopotassium salt, 2R-(2 $\alpha$ ,3Z,5 $\alpha$ )-;  
 Potassium (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [61177-45-5].

## DEFINITION

Clavulanate Potassium contains the equivalent of NLT 75.5% and NMT 92.0% of clavulanic acid (C<sub>8</sub>H<sub>9</sub>NO<sub>5</sub>), calculated on the anhydrous basis.

## IDENTIFICATION

- **A.** The retention time of the major peak for clavulanic acid in the *Sample solution* corresponds to that in the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Potassium** <191>: Meets the requirements

## ASSAY

### PROCEDURE

**Solution A:** 7.8 mg/mL of monobasic sodium phosphate in water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 4.4  $\pm$  0.1 before final dilution.

**Mobile phase:** Methanol and *Solution A* (1:19)

**Standard solution:** 0.25 mg/mL of USP Clavulanate Lithium RS in water

**System suitability solution:** 0.5 mg/mL of amoxicillin dissolved in *Standard solution*

**Sample solution:** 0.25 mg/mL of Clavulanate Potassium in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks, *System suitability solution*

**Column efficiency:** NLT 550 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_8H_9NO_5$  in each mg of Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)

$P$  = designated potency of USP Clavulanate Lithium RS, in  $\mu\text{g}/\text{mg}$  of clavulanic acid

$F$  = unit conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 75.5%–92.0% on the anhydrous basis

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE 1

**Solution A:** 0.05 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of  $4.0 \pm 0.1$ .

**Solution B:** Methanol and *Solution A* (1:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
15	50	50
18	50	50
24	100	0

**Standard solution:** 0.1 mg/mL of USP Clavulanate Lithium RS in *Solution A*

**Sample solution:** 10.0 mg/mL of Clavulanate Potassium in *Solution A*

**System suitability solution:** 0.1 mg/mL each of USP Clavulanate Lithium RS and amoxicillin in *Solution A*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu\text{m}$  packing L1

**Temperature:** 40°

**Flow rate:** 1 mL/min

[NOTE—The system is equilibrated for 15 min with 100% *Solution A*.]

**Injection size:** 20  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 1.0 and 2.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 13 between the clavulanic acid peak and the amoxicillin peak, *System suitability solution*

**Column efficiency:** NLT 2000 theoretical plates from the clavulanic acid peak, *System suitability solution*

**Tailing factor:** NMT 2.0 for the clavulanic acid peak, *System suitability solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage, in terms of clavulanate potassium equivalent, of each impurity in the Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of an individual impurity peak from the *Sample solution*

$r_S$  = peak response of clavulanic acid from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Clavulanate Potassium from the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of clavulanate potassium, 237.3

$M_{r2}$  = molecular weight of clavulanate lithium, 205.1

#### Acceptance criteria

**Total impurities:** NMT 2%

##### • PROCEDURE 2: LIMIT OF CLAVAM-2-CARBOXYLATE POTASSIUM

**Mobile phase:** 0.1 M monobasic sodium phosphate.

Adjust with phosphoric acid to a pH of  $4.0 \pm 0.1$ .

**Standard solution:** 5  $\mu\text{g}/\text{mL}$  of USP Clavam-2-Carboxylate Potassium RS in water

**Sample solution:** 10 mg/mL of Clavulanate Potassium in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu\text{m}$  packing L1

**Flow rate:** 0.5 mL/min

**Injection size:** 20  $\mu\text{L}$

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for clavam-2-carboxylic acid and clavulanic acid are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Column efficiency:** NLT 4000 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clavam-2-carboxylate potassium in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\mu\text{g}/\text{mL}$ )

$C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)

$F$  = unit conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** NMT 0.01%

##### • PROCEDURE 3: LIMIT OF ALIPHATIC AMINES

**Internal standard solution:** 50  $\mu\text{L}$  of 3-methyl-2-pentanone in water to 100 mL

**Standard solution:** Dissolve 80.0 mg of each of the following amines in 2 N hydrochloric acid: 1,1-dimethylethylamine, diethylamine, tetramethylethylenediamine, 1,1,3,3-tetramethylbutylamine, and *N,N'*-diisopropylethylenediamine. Dilute with 2 N hydrochloric acid to 200.0 mL. Transfer 5.0 mL of this solution to a centrifuge tube. Add 5.0 mL of *Internal standard solution*, 10.0 mL of 2 N sodium hydroxide, 5.0 mL of isopropyl alcohol, and 5 g of sodium chloride. Shake for 1

min, and centrifuge to separate the layers. Use the upper layer.

**Sample solution:** Transfer 1.0 g of Clavulanate Potassium to a centrifuge tube, add 5.0 mL of *Internal standard solution*, 5.0 mL of 2 N sodium hydroxide, 10.0 mL of water, 5.0 mL of isopropyl alcohol, and 5 g of sodium chloride. Shake for 1 min, and centrifuge to separate the layers. Use the upper layer.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 50-m capillary fused silica column that contains a 5-μm film coating of stationary phase G41

**Temperature**

**Injector:** 200°

**Detector:** 250°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	7
35	30	150	15

**Carrier gas:** Helium

**Flow rate:** 8 mL/min

**Split ratio:** 1:10

**Injection size:** 1 μL

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE— See the table below for relative retention times.]

Name	Relative Retention Time
1,1-Dimethylethylamine	0.55
Diethylamine	0.76
3-Methyl-2-pentanone (internal standard)	1.0
Tetramethylethylenediamine	1.07
1,1,3,3-Tetramethylbutylamine	1.13
N,N'-Diisopropylethylenediamine	1.33
Bis(2-methylamino)ethyl ether <sup>a</sup>	1.57

<sup>a</sup> The relative retention time for this compound is provided for information only; bis(2-methylamino)ethyl ether is not a component of the *Standard solution*.

Calculate the percentage of each impurity in the Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for an individual impurity from the *Sample solution*

$r_S$  = peak response for the relevant analyte from the *Standard solution*

$C_S$  = concentration of the relevant analyte in the *Standard solution*

$C_U$  = nominal concentration of Clavulanate Potassium in the *Standard solution*

Calculate the percentage of any individual impurity for which no relevant reference compound is provided in the *Standard solution* by the same formula, except for  $r_S$  use the peak response corresponding to the 1,1-dimethylethylamine peak.

#### Acceptance criteria

**Total of all aliphatic amines:** NMT 0.2%

#### PROCEDURE 4: LIMIT OF 2-ETHYLHEXANOIC ACID

**Internal standard solution:** 1 mg/mL of 3-cyclohexylpropionic acid in cyclohexane

**Standard solution:** 1.5 mg/mL of 2-ethylhexanoic acid in *Internal standard solution*. Transfer 1.0 mL of this solution to a centrifuge tube, and add 4.0 mL of 4 N hydrochloric acid. Shake for 1 min, and allow the phases to separate, centrifuging if necessary. Withdraw the lower phase, and reserve the upper phase. To the lower phase add 1.0 mL of *Internal standard solution*, and shake for 1 min. Allow the phases to separate, centrifuging if necessary. Withdraw the upper phase, and combine with the reserved upper layer.

**Sample solution:** Transfer 300 mg of Clavulanate Potassium to a centrifuge tube. Add 4.0 mL of 4 N hydrochloric acid, and shake with two successive 1.0-mL portions of the *Internal standard solution*. Allow the phases to separate, centrifuging if necessary. Use the combined upper phases.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 25-m capillary fused silica; 1-μm film coating of stationary phase G35

**Temperature**

**Injector temperature:** 200°

**Detector temperature:** 300°

**Column temperature:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	2
40	30	200	3

**Carrier gas:** Hydrogen

**Flow rate:** 100 cm/s

**Injection size:** 1 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between the 2-ethylhexanoic acid peak and the 3-cyclohexylpropionic acid peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2-ethylhexanoic acid in the Clavulanate Potassium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Sample solution*

$R_S$  = peak area response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Standard solution*

$C_S$  = concentration of 2-ethylhexanoic acid in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.8%

#### SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Clavulanate Potassium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.03 USP Endotoxin Unit/mg.

• **STERILITY TESTS (71):** Where the label states that Clavulanate Potassium is sterile, it meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.



- **pH** <791>: 5.5–8.0, in a 10 mg/mL solution
- **WATER DETERMINATION**, *Method I* <921>: NMT 1.5%

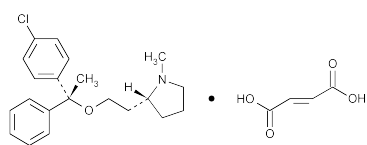
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** <11>
  - USP Clavam-2-Carboxylate Potassium RS
  - USP Clavulanate Lithium RS
  - USP Endotoxin RS

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**Clemastine Fumarate**


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$C_{21}H_{26}ClNO \cdot C_4H_4O_4$  459.96  
 Pyrrolidine, 2-[2-[1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methyl-, [R-(R\*,R\*)]-, (E)-2-butenedioate (1:1).  
 (+)-(2R)-2-[2-[[[(R)-p-Chloro- $\alpha$ -methyl- $\alpha$ -phenylbenzyl]oxy]ethyl]-1-methylpyrrolidine fumarate (1:1) [14976-57-9].

» Clemastine Fumarate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 25°.

**USP Reference standards** <11>—

USP Clemastine Fumarate RS

**Clarity and color of solution**—Dissolve 100 mg of Clemastine Fumarate in 10.0 mL of methanol, and mix to obtain the *Test solution*. Prepare a *Comparison solution* by mixing 2.5 mL of 0.0002 M sodium chloride, 2.5 mL of water, 5.0 mL of 2.5 N nitric acid, and 1.0 mL of 0.1 N silver nitrate, and use this solution within 5 minutes. Prepare a *Color matching fluid* by mixing 1 volume of *Matching Fluid C* (see *Color and Achromicity* <631>) with 3 volumes of water. Transfer the *Test solution*, the *Comparison solution*, and 10 mL of *Color matching fluid* to separate test tubes having the same nominal diameter (about 12 mm). View the *Test solution* and the *Comparison solution* horizontally against a dull black background: the *Test solution* is clear or not more opalescent than the *Comparison solution*. View the *Test solution* and *Color matching fluid* horizontally against a dull white background: the *Test solution* is colorless or not more intensely colored than *Color matching fluid*.

**Identification**—

**A:** *Infrared Absorption* <197M>.

**B:** Prepare a *Test preparation* by dissolving 40 mg of Clemastine Fumarate in 2.0 mL of dilute alcohol (8 in 10) with slight warming. Similarly prepare a *Standard preparation* by dissolving 50 mg of fumaric acid in 10.0 mL of dilute alcohol (8 in 10). Separately apply 5- $\mu$ L portions of the *Test preparation* and the *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture, and dry the spots with the aid of a current of air.

Develop the chromatogram in a solvent system consisting of a mixture of diisopropyl ether, formic acid, and water (70:25:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, dry at 100° for 30 minutes, cool, and spray the plate with 0.1 M potassium permanganate. Dry briefly with the aid of a current of warm air, and examine the chromatogram: the principal spot obtained from the *Test preparation* corresponds in  $R_f$  value, color, and intensity to that obtained from the *Standard preparation*.

**Specific rotation** <781S>: between +15.0° and +18.0° ( $t = 20^\circ$ ).

*Test solution:* 10 mg per mL, in methanol.

**pH** <791>: between 3.2 and 4.2, in a suspension (1 in 10).

**Loss on drying** <731>—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

**Heavy metals**, *Method II* <231>: 0.002%.

**Chromatographic purity**—

**Spray reagent**—Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water, and mix (*Solution A*). Dissolve 8 g of potassium iodide in 20 mL of water (*Solution B*). Mix 5.0 of *Solution A*, 5.0 mL of *Solution B*, and 20 mL of glacial acetic acid in a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Dissolve a suitable quantity of USP Clemastine Fumarate RS in a mixture of chloroform and methanol (1:1) to obtain a solution having a known concentration of 20 mg per mL. Dilute portions of this solution quantitatively with the mixture of chloroform and methanol (1:1) to prepare 5 *Comparison solutions* having known concentrations of 0.10, 0.08, 0.06, 0.04, and 0.02 mg per mL, respectively (0.5%, 0.4%, 0.3%, 0.2%, and 0.1% of the *Standard preparation*, respectively).

**Test preparation**—Dissolve 100 mg of Clemastine Fumarate in 5.0 mL of a mixture of chloroform and methanol (1:1), and mix.

**Procedure**—Separately apply 5- $\mu$ L portions of the *Standard preparation*, each of the 5 *Comparison solutions*, and the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (90:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at room temperature with the aid of a current of air. Locate the spots on the plate by spraying first with *Spray reagent*, then with 3% hydrogen peroxide: the principal spot obtained from the *Test preparation* corresponds in  $R_f$  value, color, and intensity to that obtained from the *Standard preparation*; the sum of the intensities of any secondary spots, if present in the chromatogram from the *Test preparation*, corresponds to not more than 1.0%; and the intensities of any secondary spots do not exceed 0.5% of that of the principal spot in the chromatogram from the *Standard preparation* on the basis of comparison with spots obtained from the *Comparison solutions*.

**Assay**—Transfer about 350 mg of Clemastine Fumarate, accurately weighed, to a small conical flask, and dissolve in 60 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 46.00 mg of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ .

## Clemastine Fumarate Tablets

» Clemastine Fumarate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Clemastine Fumarate RS

**Identification**—

*Spray reagent*—Prepare as directed in the test for *Chromatographic purity* under *Clemastine Fumarate*.

*Standard preparation*—Prepare a solution in a mixture of chloroform and methanol (1:1) having a concentration of about 2.5 mg of USP Clemastine Fumarate RS per mL.

*Test preparation*—Place a portion of powdered Tablets, equivalent to about 2.5 mg of clemastine fumarate, in a glass-stoppered flask. Add 10 mL of a mixture of chloroform and methanol (1:1), and shake for 20 minutes. Filter, wash the residue with two 5-mL portions of the mixture of chloroform and methanol (1:1), and evaporate the combined filtrate and washings to dryness under vacuum. Dissolve the residue so obtained in 1 mL of the mixture of chloroform and methanol (1:1), and mix.

*Procedure*—Proceed as directed for *Procedure* in the test for *Chromatographic purity* under *Clemastine Fumarate*, applying 5- $\mu$ L portions of the *Standard preparation* and the *Test preparation* on the thin-layer chromatographic plate: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation*.

**Dissolution** (711)—

*pH 4.0 citrate buffer*—Dissolve 20.0 g of monohydrate citric acid in about 1000 mL of water, add 22.0 mL of sodium hydroxide solution (3 in 10) and 8.8 mL of hydrochloric acid, and dilute with water to 2000 mL. Adjust, if necessary, with sodium hydroxide solution (1 in 2) to a pH of 4.0.

*Medium*: pH 4.0 citrate buffer; 500 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

*Procedure*—Centrifuge 60 mL of the solution under test for 20 minutes at 4000 rpm, and transfer 50.0 mL of the supernatant to a 125-mL separator. To a second 125-mL separator transfer 50.0 mL of a *Standard preparation* that is prepared by dissolving an accurately weighed quantity of USP Clemastine Fumarate RS in *Dissolution Medium* and diluting quantitatively and stepwise with *Dissolution Medium* to yield a solution having a known concentration comparable with that of the solution under test. To a third 125-mL separator transfer 50.0 mL of *Dissolution Medium* to provide a blank. Treat each of the solutions in the three separators as follows. Add 10 mL of methyl orange solution (2 in 10,000), mix, add 20.0 mL of chloroform, shake simultaneously by mechanical means for 10 minutes, remove the chloroform layer, and centrifuge the chloroform layer for 10 minutes at 4000 rpm. Determine the amount of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$  dissolved from absorbances at the wavelength of maximum absorbance at about 420 nm of the chloroform solutions obtained from the solution under test and the *Standard preparation*, using the chloroform solution obtained from the blank to set the instrument.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

*Dye solution*—Dissolve 100 mg of bromocresol purple in 1000 mL of 0.33 N acetic acid, and mix.

*Acetous methanol*—Dilute 100 mL of methanol with sufficient 0.33 N acetic acid to prepare 1000 mL of solution, and mix.

*Standard preparation*—Transfer about 27 mg of USP Clemastine Fumarate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with 0.33 N acetic acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Acetous methanol* to volume, and mix.

*Test preparation*—Mix 1 finely powdered Tablet with an accurately measured volume of *Acetous methanol*, sufficient to obtain a solution having a concentration of about 27  $\mu$ g of clemastine fumarate per mL. Shake for 30 minutes, and filter, discarding the first few mL of the filtrate.

*Procedure*—Transfer 15.0 mL each of the *Standard preparation*, the *Test preparation*, and *Acetous methanol* to provide the blank to individual 125-mL separators. Add 25 mL of *Dye solution* and 50.0 mL of chloroform to each, and shake by mechanical means for 15 minutes. Allow the layers to separate, and filter the chloroform layers. Concomitantly determine the absorbances of the filtered solutions obtained from the *Test preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 406 nm, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of clemastine fumarate in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Clemastine Fumarate RS in the *Standard preparation*;  $D$  is the concentration, in  $\mu$ g per mL, of clemastine fumarate in the *Test preparation*, based on the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay**—

*pH 7 phosphate buffer*—Transfer 9.47 g of anhydrous dibasic sodium phosphate to a 1000-mL volumetric flask, dilute with water to volume, and mix (*flask A*). Transfer 9.08 g of monobasic potassium phosphate to a 1000-mL volumetric flask, dilute with water to volume, and mix (*flask B*). Mix 612 mL of *A* with 388 mL of *B*.

*Dilute phosphate buffer*—Prepare a mixture of 1 volume of pH 7 phosphate buffer and 3 volumes of water.

*Mobile phase*—Prepare a suitable and degassed solution of methanol and *Dilute phosphate buffer* (83:17).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clemastine Fumarate RS in a mixture of methanol and water (1:1) to obtain a solution having a known concentration of about 0.14 mg per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 14 mg of clemastine fumarate, to a 200-mL conical flask. Pipet 100 mL of a mixture of methanol and water (1:1) into the flask, shake for 30 minutes, centrifuge, and filter the supernatant.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 4 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%.

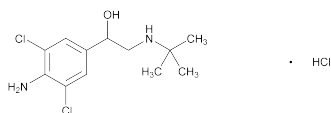
*Procedure*—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate

the quantity, in mg, of  $C_{21}H_{26}ClNO \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clemastine Fumarate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of clemastine fumarate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clenbuterol Hydrochloride



$C_{12}H_{18}Cl_2N_2O \cdot HCl$  313.65  
Ethanol, 1-(4-amino-3,5-dichlorophenyl)-2-(*tert*-butylamino), hydrochloride;  
4-Amino- $\alpha$ -[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol, hydrochloride [21898-19-1].

### DEFINITION

Clenbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$ , calculated on the anhydrous basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

[NOTE—Alternatively, *Infrared Absorption* (197A) may be used.]

#### B. IDENTIFICATION TESTS—GENERAL, Chloride (191):

Meets the requirements

### ASSAY

#### PROCEDURE

**Sample solution:** Dissolve 0.25 g in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.

**Analysis:** Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Read the volume added between the two points of inflection. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium hydroxide is equivalent to 31.37 mg of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$ .

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%, from 1–2 g
- **HEAVY METALS, Method II** (231): NMT 10 ppm

#### Organic Impurities

##### PROCEDURE

**Buffer:** Dissolve 3.0 g of sodium 1-decanesulfonate and 5.0 g of monobasic potassium phosphate in 900 mL of water, adjust with dilute phosphoric acid (1 in 10) to a pH of 3.0, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (2:2:6)

**System suitability solution:** 0.2 mg/mL each of USP Clenbuterol Related Compound B RS and Clenbuterol Hydrochloride in *Mobile phase*

**Sample solution 1:** 2.0 mg/mL of Clenbuterol Hydrochloride in *Mobile phase*

**Sample solution 2:** 2.0  $\mu$ g/mL of Clenbuterol Hydrochloride in *Mobile phase*, from *Sample solution 1*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.0-mm  $\times$  12.5-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 0.5 mL/min

**Injection size:** 5  $\mu$ L

### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 4.0 between clenbuterol related compound B and clenbuterol

**Relative standard deviation:** NMT 2.0% for the clenbuterol peak

### Analysis

**Samples:** *Sample solution 1* and *Sample solution 2*

Calculate the percentage of impurities in the portion of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$  taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from *Sample solution 1*

$r_S$  = peak response of clenbuterol from *Sample solution 2*

$C_S$  = concentration of Clenbuterol Hydrochloride in *Sample solution 2* (mg/mL)

$C_U$  = concentration of Clenbuterol Hydrochloride in *Sample solution 1* (mg/mL)

### Acceptance criteria

**Individual impurities:** 0.1%

**Total impurities:** NMT 0.2%

[NOTE— The reporting level for impurities is 0.05%.]

### SPECIFIC TESTS

#### OPTICAL ROTATION, Specific Rotation (781S)

**Sample:** 30 mg/mL in water, filter as necessary

**Acceptance criteria:**  $-10^\circ$  to  $+10^\circ$  at 20°

#### PH (791):

5.0–7.0

**Sample:** 50 mg/mL in carbon dioxide-free water

#### WATER DETERMINATION, Method I (921):

NMT 1.0%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

• **LABELING:** Label it to indicate that it is for veterinary use only.

#### USP REFERENCE STANDARDS (11)

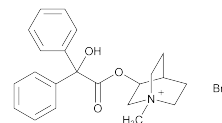
USP Clenbuterol Hydrochloride RS

USP Clenbuterol Related Compound B RS

1-(4-Amino-3,5-dichlorophenyl)-2-*tert*-butyl-aminoethanone hydrochloride.

$C_{12}H_{16}Cl_2N_2O \cdot HCl$  311.64

## Clidinium Bromide



$C_{22}H_{26}BrNO_3$

432.35

1-Azoniabicyclo[2.2.2]octane, 3-[(hydroxydiphenylacetyl)oxy]-1-methyl-, bromide, ( $\pm$ );

( $\pm$ )-3-Hydroxy-1-methylquinuclidinium bromide benzilate [3485-62-9].

### DEFINITION

Clidinium Bromide contains NLT 99.0% and NMT 100.5% of  $C_{22}H_{26}BrNO_3$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.
- **C. BROMIDE**  
*Sample solution:* 50 mg/mL  
*Analysis:* To 2 mL of the *Sample solution* add a few drops of 2 N nitric acid and 1 mL of silver nitrate TS.  
*Acceptance criteria:* A yellowish white precipitate is formed.

**ASSAY**

- **PROCEDURE**  
*Sample:* 1.2 g  
*Analysis:* Dissolve the *Sample* in 80 mL of glacial acetic acid, warming if necessary to effect solution. Cool, and add 15 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid in dioxane VS, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 43.24 mg of  $C_{22}H_{26}BrNO_3$ .  
*Acceptance criteria:* 99.0%–100.5% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS** (231)  
*Sample solution:* 1 g in 25 mL of water  
*Acceptance criteria:* NMT 20 ppm
- **ORGANIC IMPURITIES**  
*Standard solution:* 100 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid  
*Sample solution:* 100 mg/mL of Clidinium Bromide in 0.1 N methanolic hydrochloric acid  
*Reference solution:* Dissolve 100 mg of USP Clidinium Bromide RS in 1.0 mL of 0.1 N methanolic hydrochloric acid, and add 20  $\mu$ L of a solution of 25.0 mg of USP Clidinium Bromide Related Compound A RS in 1.0 mL of 0.1 N methanolic hydrochloric acid.  
**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*).  
*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture  
*Application volume:* 20  $\mu$ L  
*Developing solvent system:* Acetone, methanol, hydrochloric acid, and water (70:20:5:5)  
*Spray reagent:* Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute with dilute sulfuric acid (1 in 10) to 500 mL. Add 7.5 g  $\pm$  2.5 g of iodine, and mix until the solution is complete.  
**Chromatographic plates:** Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with the *Developing solvent system*, and allow the *Developing solvent system* to move about 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.

**Analysis 1 (3-quinuclidinyl benzilate):** Apply the *Standard solution* and the *Sample solution* to a *Chromatographic plate*. Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and allow the solvent front to move 10 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with potassium iodoplatinate TS.

**Acceptance criteria 1:** The *Sample solution* shows no spot at an  $R_f$  value (about 0.8) corresponding to that of 3-quinuclidinyl benzilate.

**Analysis 2 (limit of clidinium bromide related compound A):** Apply the *Sample solution* and *Reference solution* to a second *Chromatographic plate*. Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and

allow the solvent front to move 15 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with the *Spray reagent*.

**Acceptance criteria 2:** Any spot from the *Sample solution* at an  $R_f$  value of about 0.4 is not greater in size or intensity than the minor spot of the *Reference solution*: NMT 0.5% of clidinium bromide related compound A is found.

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

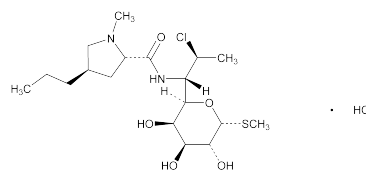
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Clidinium Bromide RS  
USP Clidinium Bromide Related Compound A RS  
3-Hydroxy-1-methylquinuclidinium bromide.  
 $C_8H_{16}BrNO$  222.13

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**Clindamycin Hydrochloride**


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- $C_{18}H_{33}ClN_2O_5S \cdot HCl$  461.44  
 $C_{18}H_{33}ClN_2O_5S \cdot HCl \cdot H_2O$  479.47  
L-threo- $\alpha$ -D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-, (2S-trans)-, monohydrochloride; Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo- $\alpha$ -D-galacto-octopyranoside monohydrochloride [21462-39-5]. Monohydrate [58207-19-5].

**DEFINITION**

Clindamycin Hydrochloride is the hydrated hydrochloride salt of clindamycin, a substance produced by the chlorination of lincomycin. It has a potency equivalent to NLT 800  $\mu$ g/mg of  $C_{18}H_{33}ClN_2O_5S$ .

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**  
*Buffer:* 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.  
*Mobile phase:* Acetonitrile and *Buffer* (9:11)  
*Standard solution:* 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*  
*Sample solution:* 1 mg/mL of Clindamycin Hydrochloride in *Mobile phase*  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*).  
*Mode:* LC  
*Detector:* UV 210 nm  
*Column:* 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

[NOTE—USP Clindamycin Hydrochloride RS contains clindamycin B and 7-epiclidamycin as minor components.]

**Resolution:** NLT 2.4 between clindamycin B and 7-epiclidamycin and NLT 3.0 between 7-epiclidamycin and clindamycin

**Tailing factor:** NMT 1.2 for the clindamycin peak

**Relative standard deviation:** NMT 1.0% for the clindamycin peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms for a period of time that is twice the retention time of the clindamycin peak.

Calculate the potency of  $C_{18}H_{33}ClN_2O_5S$ , in µg/mg, in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)

**Acceptance criteria:** NLT 800 µg/mg

**IMPURITIES**

**Organic Impurities**

• **PROCEDURE**

**Buffer and Mobile phase:** Prepare as directed in the Assay.

**Standard stock solution:** 0.5 mg/mL of USP Lincomycin Hydrochloride RS and 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*

**Standard solution:** 50 µg/mL of USP Lincomycin Hydrochloride RS and 100 µg/mL of USP Clindamycin Hydrochloride RS from *Standard stock solution* in *Mobile phase*

**Sample solution:** 5 mg/mL of Clindamycin Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms for a period of time that is six times the retention time of clindamycin.

Calculate the percentage of lincomycin in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of lincomycin from the *Sample solution*

$r_S$  = peak response of lincomycin from the *Standard solution*

$C_S$  = concentration of USP Lincomycin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)

$P$  = potency of USP Lincomycin Hydrochloride RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

Calculate the percentage of all other related compounds in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each individual related compound, other than lincomycin, from the *Sample solution*

$r_S$  = peak response of clindamycin from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$P$  = potency of USP Clindamycin Hydrochloride RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lincomycin <sup>a</sup>	0.4	—
Clindamycin B	0.65	2.0
7-Epiclidamycin	0.8	4.0
Clindamycin	1.0	—
Any other individual related compound	—	1.0
Total related compounds <sup>b</sup>	—	6.0

<sup>a</sup> Lincomycin is controlled in the total of all related compounds. There is no individual acceptance criterion for this compound.

<sup>b</sup> Total of all related compounds including lincomycin.

**SPECIFIC TESTS**

• **CRYSTALLINITY** <695>: Meets the requirements

• **pH** <791>: 3.0–5.5, in a 100-mg/mL solution

• **WATER DETERMINATION, Method 1** <921>: 3.0%–6.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** <11>

USP Clindamycin Hydrochloride RS

L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, (2S-trans)-, monohydrochloride.

$C_{18}H_{33}ClN_2O_5S \cdot HCl$  461.45

USP Lincomycin Hydrochloride RS

D-erythro-α-D-galacto-Octopyranoside, methyl 6,8-dideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2S-trans)-.

$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$  461.02

**Clindamycin Hydrochloride Capsules**

**DEFINITION**

Clindamycin Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

**IDENTIFICATION**

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, relative to the internal standard, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Mobile phase:** Add 2 g of *dl*-10-camphorsulfonic acid, 1 g of ammonium acetate, and 1 mL of glacial acetic acid to 200 mL of water in a 500-mL volumetric flask, and mix to dissolve. Dilute with methanol to volume, and mix. Adjust, if necessary, with hydrochloric acid or a sodium hydroxide solution (1 in 2) to a pH of  $6.0 \pm 0.1$ .

**Internal standard solution:** Add 0.5 mL of phenylethyl alcohol to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Standard solution:** 18 mg/mL of USP Clindamycin Hydrochloride RS in *Internal standard solution*

**Sample solution:** Equivalent to 15 mg/mL of clindamycin, from the contents of NLT 20 Capsules, in *Internal standard solution*; shake for 30 min; centrifuge or filter, if necessary, to obtain a clear solution.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 4-mm  $\times$  30-cm stainless steel; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for the internal standard and clindamycin are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 5.0 between the analyte and internal standard

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times 100$$

$R_U$  = peak response ratio of clindamycin to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clindamycin to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Medium:** pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Mobile phase:** Dissolve 16 g of *dl*-10-camphorsulfonic acid, 8 g of ammonium acetate, and 8 mL of glacial acetic acid in 1600 mL of water. Add 2400 mL of methanol, and adjust with hydrochloric acid or 5 N sodium hydroxide to a pH of  $6.0 \pm 0.05$ .

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Standard solution:** Prepare a solution of USP Clindamycin Hydrochloride RS in *Medium* having a known concentration similar to that expected in the *Sample solution*.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 50  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I <921>:** NMT 7.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS <11>**

USP Clindamycin Hydrochloride RS

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## Clindamycin Hydrochloride Oral Solution

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**DEFINITION**

Clindamycin Hydrochloride Oral Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

**IDENTIFICATION**

- **A.** The retention time of the clindamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.

**Mobile phase:** Acetonitrile and *Buffer* (450:550). Increasing the proportion of acetonitrile in the *Mobile phase* decreases the retention time, and decreasing it increases the resolution between 7-epiclindamycin and clindamycin.

**Standard solution:** 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*

**Sample solution:** Equivalent to 0.85 mg/mL of clindamycin from Oral Solution in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.4 between the clindamycin B and 7-epiclindamycin peaks, and NLT 3.0 between the 7-epiclindamycin and clindamycin peaks

**Column efficiency:** NLT 4000 theoretical plates from the clindamycin peak

**Tailing factor:** NMT 1.2 for the clindamycin peak  
**Relative standard deviation:** NMT 1.0% for the clindamycin peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in each mL of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak area response from the *Sample solution*  
 $r_S$  = peak area response from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for solution packaged in single-unit containers
- **DELIVERABLE VOLUME** (698): Meets the requirements

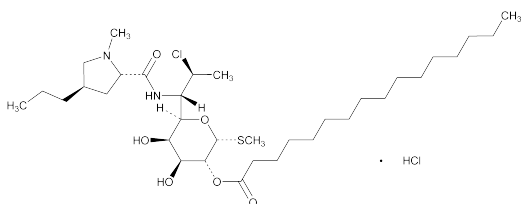
**SPECIFIC TESTS**

- **pH** (791): 2.5–6.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label Oral Solution to indicate that it is intended for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
 USP Clindamycin Hydrochloride RS

## Clindamycin Palmitate Hydrochloride



$C_{34}H_{63}ClN_2O_6S \cdot HCl$  699.85  
 L-threo- $\alpha$ -D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-2-hexadecanoate, monohydrochloride, (2S-trans)-;  
 Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo- $\alpha$ -D-galacto-octopyranoside 2-palmitate monohydrochloride [25507-04-4].

**DEFINITION**

Clindamycin Palmitate Hydrochloride has a potency equivalent to NLT 540  $\mu\text{g}$  of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ )/mg.

**IDENTIFICATION**

- **INFRARED ABSORPTION** (197M)

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 2 g of docusate sodium and 1.54 g of ammonium acetate in a mixture of 2 mL of glacial acetic acid and 75 mL of water. Dilute with methanol to 1 L. Pass through a suitable filter, and degas.

**Standard solution:** 14 mg/mL of USP Clindamycin Palmitate Hydrochloride RS in *Mobile phase*

**Sample solution:** 14 mg/mL of Clindamycin Palmitate Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu\text{m}$  packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 20  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the potency in  $\mu\text{g}/\text{mg}$  of  $C_{18}H_{33}ClN_2O_5S$  in the portion of Clindamycin Palmitate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response of clindamycin palmitate from the *Sample solution*  
 $r_S$  = peak response of clindamycin palmitate from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of Clindamycin Palmitate Hydrochloride in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )

**Acceptance criteria:** NLT 540  $\mu\text{g}$

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.5%

**SPECIFIC TESTS**

- **pH** (791): 2.8–3.8, in a 10 mg/mL solution
- **WATER DETERMINATION, Method I** (921): NMT 3.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Clindamycin Palmitate Hydrochloride RS

## Clindamycin Palmitate Hydrochloride for Oral Solution

**DEFINITION**

Clindamycin Palmitate Hydrochloride for Oral Solution is a dry mixture of Clindamycin Palmitate Hydrochloride and one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ), the labeled amount being 15 mg/mL when constituted as directed in the labeling.

**ASSAY**• **PROCEDURE**

**Solution A:** 300 mg/mL of sodium carbonate

**Internal standard solution:** 5 mg/mL of cholesteryl benzoate in chloroform

**Standard solution:** Transfer 150 mg of USP Clindamycin Palmitate Hydrochloride RS to a glass-stoppered, 15-mL conical centrifuge tube. Add 5 mL of water, 5.0 mL of *Internal standard solution*, and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and cen-

trifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

**Sample solution:** Constitute the Clindamycin Palmitate Hydrochloride for Oral Solution as directed in the labeling, and transfer 5.0 mL of the constituted solution to a glass-stoppered, 15-mL conical centrifuge tube. Add 5.0 mL of *Internal standard solution* and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and centrifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.6-m × 3-mm glass; packing 1% phase G36 on support S1AB

**Temperature**

**Column:** 290°

**Detector:** 320°

**Carrier gas:** Dry helium

**Flow rate:** 60 mL/min

**Injection size:** 1.0 µL

#### System suitability

**Sample:** *Standard solution*

The elution order is: cholesteryl benzoate, clindamycin palmitate.

**Suitability requirements:** In a suitable chromatogram, the peaks are completely resolved.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S) in each mL of the solution constituted from Clindamycin Palmitate Hydrochloride for Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

$R_U$  = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Sample solution*

$R_S$  = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin palmitate hydrochloride in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS (µg/mg)

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

For solids packaged in single-unit containers: Meets the requirements

- **DELIVERABLE VOLUME** <698>: Meets the requirements

#### SPECIFIC TESTS

- **pH** <791>: 2.5–5.0, in the solution constituted as directed in the labeling
- **WATER DETERMINATION**, *Method I* <921>: NMT 3.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
USP Clindamycin Palmitate Hydrochloride RS

### Clindamycin Phosphate

C<sub>18</sub>H<sub>34</sub>ClN<sub>2</sub>O<sub>8</sub>PS 504.96

*L-threo-α-D-galacto-Octopyranoside*, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, 2-(dihydrogen phosphate), (2*S-trans*)-.

Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo-α-D-galacto*-octopyranoside 2-(dihydrogen phosphate) [24729-96-2].

» Clindamycin Phosphate has a potency equivalent to not less than 758 µg of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### USP Reference standards <11>—

USP Clindamycin Phosphate RS

USP Endotoxin RS

#### Identification, Infrared Absorption <197M>—

*Test specimen:* undried.

**Crystallinity** <695>: meets the requirements.

**pH** <791>: between 3.5 and 4.5, in a solution containing 10 mg per mL.

**Water**, *Method I* <921>: not more than 6.0%.

**Other requirements**—Where the label states that Clindamycin Phosphate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Clindamycin for Injection*. Where the label states that *Clindamycin Phosphate* must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Clindamycin for Injection*.

#### Assay—

**Buffer solution**—Add 14 mL of phosphoric acid to 4000 mL of HPLC grade water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of 3.90 ± 0.05.

**Organic solution**—Prepare a mixture of acetonitrile and methanol (900:100).

**Diluent**—Prepare a degassed mixture of *Buffer solution* and *Organic solution* (80:20).

**Solution A**—Prepare a degassed mixture of *Buffer solution* and *Organic solution* (920:80).

**Solution B**—Prepare a degassed mixture of *Buffer solution* and *Organic solution* (520:480).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).



**Standard preparation**—Accurately weigh about 22 mg of USP Clindamycin Phosphate RS. Add 10.0 mL of *Diluent*, shake briefly, and sonicate for about 5 minutes to dissolve. Allow to cool to ambient temperature.

**Assay preparation**—Accurately weigh about 22 mg of Clindamycin Phosphate. Add 10.0 mL of *Diluent*, shake briefly, and sonicate for about 5 minutes to dissolve. Allow to cool to ambient temperature.

**Chromatographic system**—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.2 mL per minute. Maintain the column at a constant temperature of about 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95.0	5.0	equilibrium
0–40	95.0→5.0	5.0→95.0	linear gradient
40–41	5.0→95.0	95.0→5.0	linear gradient
41–46	95.0	5.0	isocratic

Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the resolution between clindamycin phosphate and its nearest related compound is not less than 1.0, determined as follows. Between the peaks, draw a line perpendicular to the base line at the valley that separates the two peaks. The height of the valley from the base line is not more than 40 percent of the height of the related compound peak. Calculate the resolution, *R*, using peak widths at half height. The relative standard deviation determined from the clindamycin phosphate peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the clindamycin phosphate peaks. Calculate the quantity, in µg, of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S) in the portion of Clindamycin Phosphate taken by the formula:

$$(PW_S / W_U)(r_U / r_S)$$

in which *P* is the potency, in µg of clindamycin per mg, of USP Clindamycin Phosphate RS; *W<sub>S</sub>* is the weight, in mg, of USP Clindamycin Phosphate RS taken to prepare the *Standard preparation*; *W<sub>U</sub>* is the weight, in mg, of Clindamycin Phosphate taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas for clindamycin phosphate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clindamycin Phosphate Vaginal Cream

### DEFINITION

Clindamycin Phosphate Vaginal Cream contains an amount of clindamycin phosphate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S).

### IDENTIFICATION

- A.** The relative retention time of the major peak for clindamycin phosphate of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phos-

phoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Cream, prepared as follows. Transfer a suitable portion of Cream to a stoppered conical flask, and add *Mobile phase*. Add about 10 glass beads (about 10 mm in diameter). Insert the stopper securely in the flask, and shake by mechanical means at 50° for 1 h. Cool in an ice bath for 20 min, and centrifuge. Pass a portion of the cloudy lower layer through a filter of 2-µm or finer pore size, and use the filtrate.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]

### Suitability requirements

**Resolution:** NLT 6.0 between clindamycin phosphate and clindamycin, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

*P* = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)

*F* = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

- pH** <791>: 3.0–6.0, determined on the undiluted Cream

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- USP REFERENCE STANDARDS** <11>  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

## Clindamycin Phosphate Gel

### DEFINITION

Clindamycin Phosphate Gel contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin (C<sub>18</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>5</sub>S).

**IDENTIFICATION**

- **A.** The retention time of the clindamycin phosphate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Gel. Shake by mechanical means for 30 min. Centrifuge a portion of the solution, and if necessary, filter a portion of the supernatant. Use the clear filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are 1.0 and 1.5, respectively.]

**Suitability requirements**

**Resolution:** NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*  
Calculate as follows:

$$\text{Result} = (t_r/W_{h/2})^2 \times 5.545$$

$t_r$  = retention time

$W_{h/2}$  = peak width at half height

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** <755>: Meets the requirements

**SPECIFIC TESTS**

- **pH** <791>: 4.5–6.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** <11>  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

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**Clindamycin Injection**

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**DEFINITION**

Clindamycin Injection contains an amount of Clindamycin Phosphate in Water for Injection equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ). It may be frozen.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.

**System suitability stock solution:** 0.1 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

**System suitability solution:** 25 µg/mL of USP Benzyl Alcohol RS from *System suitability stock solution* and 0.25 mg/mL of USP Clindamycin Phosphate RS, in *Mobile phase*

**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**Sample stock solution:** Nominally 3 mg/mL of clindamycin from Injection in *Mobile phase*

**Sample solution:** 0.21 mg/mL of clindamycin from *Sample stock solution* in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and benzyl alcohol are 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clindamycin phosphate and benzyl alcohol, *System suitability solution*

**Relative standard deviation:** NMT 2.5, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response of clindamycin phosphate from the *Sample solution*  
 $r_S$  = peak response of clindamycin phosphate from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** 90.0%–120.0%

**SPECIFIC TESTS**

- **PH** (791): 5.5–7.0
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, or in suitable plastic containers.
- **LABELING:** Meets the requirements in *Injections* (1), *Labeling*. Where it is maintained in the frozen state, the label states that it is to be thawed just before use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)  
 USP Benzyl Alcohol RS  
 USP Clindamycin Phosphate RS  
 USP Endotoxin RS

## Clindamycin for Injection

**DEFINITION**

Clindamycin for Injection contains an amount of Clindamycin Phosphate equivalent to NLT 758  $\mu\text{g}/\text{mg}$  of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)  
 Sample: Undried specimen  
 Acceptance criteria: Meets the requirements

**ASSAY****PROCEDURE**

**Buffer:** Add 14 mL of phosphoric acid to 4000 mL of HPLC grade water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of  $3.90 \pm 0.05$ .

**Solution A:** Acetonitrile and methanol (9:1)

**Diluent:** *Solution A* and *Buffer* (1:4)

**Solution B:** *Solution A* and *Buffer* (2:23)

**Solution C:** *Solution A* and *Buffer* (12:13)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	95	5
40	5	95
41	95	5
46	95	5

**Standard solution:** 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*, prepared as follows. Shake

briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.

**Sample solution:** Nominally 2.2 mg/mL of clindamycin phosphate in *Diluent* from Clindamycin for Injection, prepared as follows. Shake briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.0 between clindamycin phosphate and its nearest related compound. Calculate the resolution using peak widths at half height.

**Peak-to-valley ratio:** NLT 2.5

Calculate the peak-to-valley ratio:

$$\text{Result} = H_p/H_v$$

$H_p$  = height above the baseline of the nearest related compound peak

$H_v$  = height above the baseline of the valley between clindamycin phosphate and its nearest related compound

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ), in  $\mu\text{g}/\text{mg}$ , in the portion of Clindamycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin phosphate in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in the USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

**Acceptance criteria:** NLT 758  $\mu\text{g}/\text{mg}$  on the anhydrous basis

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): Contains NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **STERILITY TESTS** (71)  
 Sample solution: 6 g of Clindamycin for Injection aseptically dissolved in 200 mL of *Fluid A*  
 Acceptance criteria: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PH** (791)  
 Sample solution: Nominally 10 mg/mL of clindamycin in water from Clindamycin for Injection  
 Acceptance criteria: 3.5–4.5
- **WATER DETERMINATION, Method I** (921): NMT 6.0%
- **CRYSTALLINITY** (695): Meets the requirements
- **OTHER REQUIREMENTS:** It responds to the *Identification* test in *Clindamycin Phosphate*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve as described in *Injections* (1), *Containers for Sterile Solids*.

- **USP REFERENCE STANDARDS** <11>  
USP Clindamycin Phosphate RS  
USP Endotoxin RS

## Clindamycin Phosphate Topical Solution

### DEFINITION

Clindamycin Phosphate Topical Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.

**System suitability stock solution 1:** 4 mg/mL of 4'-hydroxyacetophenone in acetonitrile

**System suitability stock solution 2:** 0.04 mg/mL of 4'-hydroxyacetophenone from *System suitability stock solution 1* in *Mobile phase*

**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**System suitability solution:** Mix 1 part of *System suitability stock solution 2* with 3 parts of *Standard solution*.

**Sample solution:** Equivalent to 0.2 mg/mL of clindamycin from Topical Solution in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clindamycin phosphate and 4'-hydroxyacetophenone are about 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clindamycin phosphate and 4'-hydroxyacetophenone, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of the Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)  
 $F$  = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **pH** <791>: 4.0–7.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
USP Clindamycin Phosphate RS

## Clindamycin Phosphate Topical Suspension

### DEFINITION

Clindamycin Phosphate Topical Suspension contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS, in the *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in the *Mobile phase*

**Sample solution:** Equivalent to 0.2 mg/mL of clindamycin from Topical Suspension in *Mobile phase*. Prepare as follows. Using a suitable hypodermic needle and syringe, transfer a suitable aliquot of Topical Suspension to a suitable volumetric flask, dilute with *Mobile phase* to volume, and mix.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*, calculated from the peak width at half height

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of the Topical Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*

- $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

- **PH** (791): 4.5–6.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Clindamycin Hydrochloride RS  
 USP Clindamycin Phosphate RS

## Clindamycin Phosphate Vaginal Inserts

**DEFINITION**

Clindamycin Phosphate Vaginal Inserts contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)

**Sample:** Transfer a Vaginal Insert into a suitable container, add 120 mL of methylene chloride, insert a stopper, and shake until the Vaginal Insert is completely dissolved. Using a vacuum, pass through a methylene chloride-compatible filter having a 0.45- $\mu\text{m}$  pore size. Rinse the filter with several portions of methylene chloride, and allow the filter to air-dry. Use the white residue to prepare the mineral oil dispersion for the test.

**Acceptance criteria:** The IR absorption of the *Sample* exhibits maxima at the same wavelengths as that of a similar preparation of USP Clindamycin Phosphate RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

**Buffer:** 10.54 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS and 6  $\mu\text{g}/\text{mL}$  of USP Clindamycin Hydrochloride RS, in *Buffer*

**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Buffer*

**Sample solution:** Transfer 1 Vaginal Insert to a suitable 100-mL container. Add 40 mL of isooctane, and seal the container tightly with a teflon-lined septum and crimp cap. Shake vigorously for about 15 min until all of the Vaginal Insert is dissolved. Add 40.0 mL of *Buffer*. Recap the container tightly, and shake vigorously for NLT 30 min, taking care to avoid leakage. Allow the layers to separate, and remove a volume of the lower aqueous layer sufficient to perform the following steps. Pass the aqueous solution through a filter having a 5- $\mu\text{m}$  or finer pore size, discarding the first 2 mL of the filtrate. Collect the remaining filtrate, and prepare a solution equivalent to 0.2 mg/mL of clindamycin with *Buffer*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 35  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between clindamycin phosphate and clindamycin hydrochloride, *System suitability solution*

Calculate as follows.

$$\text{Result} = [(t_2 - t_1)/(w_{h1} + w_{h2})] \times 1.177$$

$t_2$  = retention time of the second peak

$t_1$  = retention time of the first peak

$w_{h1}$  = height at half width of the first peak

$w_{h2}$  = height at half width of the second peak

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ) equivalent in the Vaginal Insert taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in the USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 90.0%–110.0%. Use as the *Assay* value the average of the determinations obtained in the test for *Uniformity of Dosage Units* (905), *Content Uniformity*.

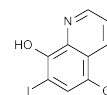
**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, at controlled room temperature, or in a cool place.
- **USP REFERENCE STANDARDS** (11)  
 USP Clindamycin Hydrochloride RS  
 USP Clindamycin Phosphate RS

## Clioquinol



$\text{C}_9\text{H}_5\text{ClINO}$  305.50

8-Quinololinol, 5-chloro-7-iodo-

5-Chloro-7-iodo-8-quinolinol [130-26-7].

» Clioquinol, dried over phosphorus pentoxide for 5 hours, contains not less than 93.0 percent

and not more than 100.5 percent of  $C_9H_5ClINO$  (the 5-chloro-7-iodo-8-quinolinol isomer).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clioquinol RS

**Identification**—

**A:** Prepare a Standard solution as directed for *Standard preparation* in the *Assay*, except to use 1.0 mL of pyridine instead of the *Internal standard solution*, and chromatograph as directed in the *Assay*: the chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a peak for clioquinol, the retention time of which corresponds with that exhibited by the Standard solution.

**B: Ultraviolet Absorption** (197U)—

*Solution:* 5 µg per mL.

*Medium:* 3 N hydrochloric acid.

Absorptivities at 267 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Heat 100 mg with 5 mL of sulfuric acid: copious violet vapors of iodine are evolved.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 5 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Free iodine and iodide**—Shake 1.0 g with 20 mL of water for 30 seconds, allow to stand for 5 minutes, and filter. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid, then add 2 mL of chloroform, and shake: no violet color appears in the chloroform (*free iodine*). To the mixture add 5 mL of 2 N sulfuric acid and 1 mL of potassium dichromate TS, and shake for 15 seconds: the color in the chloroform layer is no deeper than that produced in a control test made in the following manner: Dilute 2.0 mL of potassium iodide solution (1 in 6000) with water to 10 mL, add 6 mL of 2 N sulfuric acid, 1 mL of potassium dichromate TS, and 2 mL of chloroform, and shake for 15 seconds (0.05% of iodide).

**Assay**—

*Internal standard solution*—Prepare a solution of pyrene in pyridine containing 2 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1) to obtain a Standard solution having a known concentration of about 3 mg per mL. Transfer 1.0 mL of the Standard solution to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 minutes, and then cool to ambient temperature.

*Assay preparation*—Transfer about 75 mg of Clioquinol, previously dried and accurately weighed, to a 25-mL volumetric flask, dissolve in a mixture of pyridine and *n*-hexane (4:1), dilute with the same solvent to volume, and mix. Transfer 1.0 mL of this solution to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 minutes, then cool to ambient temperature.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector, and contains a 1.83-m × 2-mm glass column packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB. The injection port and detector temperatures are maintained at 170° and 250°, respectively, and the initial column temperature is 200° for a conditioning period of not less than 16 hours (not connected to the detector) and is then reduced to 165°. Helium is used as the carrier gas at a flow rate of about 30 mL per minute, and hydrogen and air are introduced into the detector at rates of 25 mL and 500 mL per minute, respectively. Chromatograph the *Standard preparation*, and record the peak responses as directed under

*Procedure*: the resolution,  $R$ , between the clioquinol and the internal standard peaks is not less than 3.

*Procedure*—Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times for clioquinol and pyrene are about 0.6 and 1.0, respectively. Calculate the quantity, in mg, of  $C_9H_5ClINO$  in the Clioquinol taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clioquinol RS in the Standard solution used to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the clioquinol peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clioquinol Cream

» Clioquinol Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_5ClINO$  in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clioquinol RS

**Identification**—

**A:** Prepare a Standard solution as directed for *Standard preparation* in the *Assay*, except to use 1.0 mL of pyridine instead of the *Internal standard solution*, and chromatograph as directed in the *Assay*: the chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a peak for clioquinol, the retention time of which corresponds with that exhibited by the Standard solution.

**B:** Place a quantity of Cream, equivalent to about 25 mg of clioquinol, in a 100-mL volumetric flask, add about 75 mL of dilute hydrochloric acid (1 in 4), and heat on a steam bath to melt the cream, shaking vigorously to extract the clioquinol. Cool under running water, and add dilute hydrochloric acid (1 in 4) to volume. Filter through paper, and dilute 3 mL of the filtrate with dilute hydrochloric acid (1 in 4) to 100 mL: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clioquinol RS, concomitantly measured.

**Assay**—

*Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under Clioquinol.

*Assay preparation*—Transfer an accurately weighed portion of Cream, equivalent to about 150 mg of clioquinol, to a 60-mL separator. Place the separator on its side in a vacuum oven at a pressure of about 10 mm of mercury at 45° for 4 hours. Remove the separator from the oven, allow to cool, add 15 mL of a mixture of pyridine and *n*-hexane (4:1), insert a polytef stopper, and mix. Transfer the mixture to a 50-mL volumetric flask, and rinse the separator with two 15-mL portions of the same solvent, shaking each time for 30 seconds. Transfer both rinsings to the volumetric flask, dilute with the same solvent to volume, and mix. Transfer 1.0 mL to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 minutes, and then cool to ambient temperature.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Clioquinol*. Calculate the quantity, in mg, of  $C_9H_5ClINO$  in the portion of Cream taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clioquinol RS in the Standard solution used to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the clioquinol peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clioquinol Ointment

» Clioquinol Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_5ClINO$  in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or tight, light-resistant containers.

**USP Reference standards** {11}—

USP Clioquinol RS

**Identification**—It responds to the *Identification* tests under *Clioquinol Cream*.

**Assay**—

*Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Clioquinol*.

*Assay preparation*—Transfer an accurately weighed portion of Ointment, equivalent to about 150 mg of clioquinol, to a 125-mL separator. Add 75 mL of *n*-hexane, and mix. Add 15 mL of dimethylformamide, and mix for 1 minute. Allow the layers to separate, and transfer the lower layer to a 50-mL volumetric flask. Repeat the extraction with separate 15-mL and 10-mL portions of dimethylformamide, and transfer the lower layers to the 50-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a screw-capped glass vial fitted with a septum, and evaporate at about 60° under a stream of nitrogen to dryness. Add 1.0 mL of a mixture of pyridine and *n*-hexane (4:1) to the residue, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 minutes, then cool to ambient temperature.

*Procedure*—Proceed as directed for *Procedure* under *Clioquinol*. Calculate the quantity, in mg, of  $C_9H_5ClINO$  in the portion of Ointment taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clioquinol RS in the Standard solution used to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the clioquinol peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Compound Clioquinol Topical Powder

» Compound Clioquinol Topical Powder contains not less than 22.5 percent and not more than 27.5 percent of  $C_9H_5ClINO$ .

Clioquinol	250 g
Lactic Acid	25 g
Zinc Stearate	200 g
Lactose	525 g
To make	1000 g

Mix the Lactic Acid with the Lactose, then add the Clioquinol and the Zinc Stearate, and mix.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** {11}—

USP Clioquinol RS

**Identification**—Place a quantity of Topical Powder, equivalent to about 30 mg of clioquinol, in a glass-stoppered, 50-mL conical flask, add 20 mL of 1 N sulfuric acid, and shake for 5 minutes. Filter, transfer 5 mL of the filtrate to a glass-stoppered test tube, add 5 drops of potassium dichromate TS and 2 mL of chloroform, and shake well: a red-violet color develops in the chloroform layer.

**Assay**—Transfer an accurately weighed quantity of Topical Powder, equivalent to about 50 mg of clioquinol, to a 200-mL volumetric flask, add 100 mL of 3 N hydrochloric acid, and shake by mechanical means for 15 minutes. Dilute with 3 N hydrochloric acid to volume, and mix. Filter a portion of the solution, dilute 4.0 mL of the filtrate with 3 N hydrochloric acid to 200.0 mL, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Clioquinol RS in the same medium having a known concentration of about 5 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 267 nm, with a suitable spectrophotometer, using 3 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_9H_5ClINO$  in the portion of Topical Powder taken by the formula:

$$10C(A_U / A_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Clioquinol RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Topical Powder and the Standard solution, respectively.

## Clioquinol and Hydrocortisone Cream

» Clioquinol and Hydrocortisone Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of clioquinol ( $C_9H_5ClINO$ ) and of hydrocortisone ( $C_{21}H_{30}O_5$ ) in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

**USP Reference standards** {11}—

USP Clioquinol RS

USP Hydrocortisone RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for clioquinol* exhibits a peak for clioquinol, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone* exhibits a peak for hydrocortisone, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**Minimum fill** (755): meets the requirements.

#### Assay for clioquinol—

*Internal standard solution*—Prepare a solution of pyrene in pyridine containing about 2 mg per mL.

*Standard solution*—Transfer about 75 mg of USP Clioquinol RS to a 25-mL volumetric flask, add a mixture of pyridine and hexane (4:1) to volume, and mix to obtain a *Standard solution* having a known concentration of about 3 mg of USP Clioquinol RS per mL.

*Standard preparation*—Transfer 1.0 mL of *Standard solution*, 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution* to a suitable screw-capped glass vial, fitted with a polytef-lined septum, and mix. Heat on a water bath at 50° for 15 minutes, and cool to room temperature.

*Assay preparation*—Transfer an accurately weighed quantity of Cream, equivalent to about 150 mg of clioquinol, to a 60-mL separator. Place the separator on its side in a vacuum oven at about 45° for 4 hours. Remove the separator, cool to room temperature, and add 15.0 mL of a mixture of pyridine and hexane (4:1). Insert the stopper in the separator, and mix until the specimen is completely dispersed. Quantitatively transfer the contents of the separator to a 50-mL volumetric flask, rinse the separator with two 15-mL portions of a mixture of pyridine and hexane (4:1), collecting the rinsings in the volumetric flask, dilute with the same solvent mixture to volume, and mix. Immediately transfer 1 mL of this solution to a dry, screw-capped glass vial, and evaporate with the aid of gentle heat and a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), add 1 mL each of *N,O*-bis(trimethylsilyl)acetamide and *Internal standard solution* to the screw-capped glass vial, fitted with a polytef-lined septum, and mix. Heat on a water bath at 50° for 15 minutes, and cool to room temperature.

*Chromatographic system* (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m column packed with 3% liquid phase G3 on 80- to 100-mesh support SIAB. The column, injection port, and detector block temperatures are maintained at 165°, 170°, and 250°, respectively. Dry helium is used as the carrier gas at a flow rate of about 30 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for clioquinol and 1.0 for pyrene; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms so as to obtain not less than 40% of maximum recorder response, and measure the peak response of each component. Calculate the quantity, in mg, of C<sub>9</sub>H<sub>5</sub>ClINO in the portion of Cream taken by the formula:

$$150C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Clioquinol RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for hydrocortisone—

*Mobile phase*—Prepare a filtered and degassed solution of water, acetonitrile, and methanol (2.75:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Hydrocortisone RS in alcohol to obtain a solution having a known concentration of about 1 mg per mL (*Solution A*). Pipet 1 mL of this solution into a 10-mL

volumetric flask, dilute with alcohol to volume, and mix to obtain a solution having a known concentration of about 100 µg of USP Hydrocortisone RS per mL.

*Resolution solution*—Dissolve an accurately weighed quantity of methylparaben in alcohol to obtain a solution having a known concentration of about 0.5 mg of methylparaben per mL. Pipet 2 mL of this solution and 20 mL of *Solution A* into a 200-mL volumetric flask, dilute with alcohol to volume, and mix.

*Assay preparation*—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of hydrocortisone, to a 50-mL centrifuge tube. Add 30 mL of alcohol and heat on a steam bath just to boiling. Shake for 15 minutes and centrifuge. Quantitatively transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

*Chromatographic system*—(see *Chromatography* <621>). The liquid chromatograph is equipped with a 254-nm detector, a 3.9-mm × 30-cm column that contains packing L1, and a guard column that contains packing L2. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for methylparaben and 1.0 for hydrocortisone; the resolution, *R*, between the hydrocortisone and methylparaben peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>21</sub>H<sub>30</sub>O<sub>5</sub> in the portion of Cream taken by the formula:

$$0.1C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Hydrocortisone RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clioquinol and Hydrocortisone Ointment

» Clioquinol and Hydrocortisone Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of clioquinol (C<sub>9</sub>H<sub>5</sub>ClINO) and of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Clioquinol RS

USP Hydrocortisone RS

#### Identification—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for clioquinol* exhibits a peak for clioquinol, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone* exhibits a peak for hydrocortisone, the retention time of which corresponds to that exhibited by the *Standard preparation*.



**Minimum fill** (755): meets the requirements.

#### Assay for clioquinol—

*Internal standard solution, Standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay for clioquinol under Clioquinol and Hydrocortisone Cream.

*Assay preparation*—Transfer an accurately weighed quantity of Ointment, equivalent to about 150 mg of clioquinol, to a 125-mL separator. Add 75 mL of *n*-hexane, insert the stopper in the separator, and mix until the specimen is completely dispersed. Extract with 25 mL of dimethylformamide, collecting the extract in a 50-mL volumetric flask. Repeat the extraction with two 10-mL portions of dimethylformamide, collecting the extracts in the 50-mL volumetric flask, dilute with dimethylformamide to volume, and mix. Transfer 1.0 mL of this solution to a suitable size screw-capped vial, and evaporate the solution with the aid of nitrogen at about 60° to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), and pipet 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution* into the glass vial, fitted with a polytetrafluoroethylene-lined septum, securely close, and mix. Heat the vial on a water bath at 50° for 15 minutes, and cool to room temperature.

*Procedure*—Proceed as directed for *Procedure* in the Assay for clioquinol under Clioquinol and Hydrocortisone Cream. Calculate the quantity, in mg, of  $C_9H_5ClINO$  in the portion of Ointment taken by the formula:

$$150C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Clioquinol RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for hydrocortisone—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the Assay for hydrocortisone under Clioquinol and Hydrocortisone Cream.

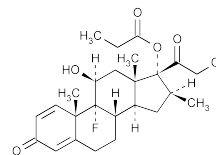
*Assay preparation*—Transfer an accurately weighed quantity of Ointment, equivalent to about 10 mg of hydrocortisone, to a 50-mL centrifuge tube. Add 30 mL of alcohol and heat on a steam bath just to boiling. Shake for 15 minutes, and centrifuge. Quantitatively transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

*Procedure*—Proceed as directed for *Procedure* in the Assay for hydrocortisone under Clioquinol and Hydrocortisone Cream. Calculate the quantity, in mg, of  $C_{21}H_{30}O_5$  in the portion of Ointment taken by the formula:

$$0.1C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Hydrocortisone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clobetasol Propionate



$C_{25}H_{32}ClFO_5$  466.97  
Pregna-1,4-diene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (11β,16β)-; 21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [25122-46-7; 25122-41-2].

#### DEFINITION

Clobetasol Propionate contains NLT 97.0% and NMT 102.0% of  $C_{25}H_{32}ClFO_5$ , calculated on the dried basis.

#### IDENTIFICATION

- **INFRARED ABSORPTION** (197M)

#### ASSAY

##### • PROCEDURE

**Solution A:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (19:4:17)

**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**Standard solution:** Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate.

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Sample solution:** Transfer 4 mg of Clobetasol Propionate to a 100-mL volumetric flask, add 40.0 mL of *Internal standard solution*, and dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

**Column efficiency:** NLT 5000 theoretical plates for the clobetasol peak

**Tailing factor:** NMT 2.0 for the clobetasol peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of  $C_{25}H_{32}ClFO_5$  in the portion of Clobetasol Propionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
- $R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*
- $C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)
- Acceptance criteria:** 97.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%, using a platinum crucible
- **HEAVY METALS, Method II** (231): NMT 20 ppm

**Organic Impurities****• PROCEDURE**

**Solution A, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sample solution:** 0.1 mg/mL of Clobetasol Propionate in *Mobile phase*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Clobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area for each impurity

$r_T$  = sum of the areas of all of the peaks

**Acceptance criteria**

**Any individual impurity:** NMT 1.0%

**Total impurities:** NMT 2.5%

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): Approximately 196°
- **OPTICAL ROTATION, Specific Rotation** (781S): +98° to +104° at 20°  
**Sample solution:** 10 mg/mL in dioxane
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 2.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Clobetasol Propionate RS  
USP Clobetasol Propionate Related Compound A RS  
9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].  
 $C_{25}H_{30}ClFO_4$  448.96

**Clobetasol Propionate Cream****DEFINITION**

Clobetasol Propionate Cream is Clobetasol Propionate in a suitable cream base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.6 mg/mL of USP Clobetasol Propionate RS in chloroform

**Test solution:** Transfer a portion of Cream equivalent to 0.75 mg of clobetasol propionate to a 25-mL, plastic-

stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 60° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Cool to room temperature, add 3.5 mL of water, and mix. Centrifuge at 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a 100-mL separator, add 1 g of sodium chloride and 10 mL of water, and mix. Extract with 5 mL of chloroform by shaking for 1 min, collect the lower layer, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of chloroform.

**Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

**ASSAY****• PROCEDURE**

**Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 50% sodium hydroxide solution to a pH of 5.5.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (95:20:85)

**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Standard solution:** 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.

**Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. In a suitable flask, dissolve a portion of Cream equivalent to 1.0 mg of clobetasol propionate in 10.0 mL of the *Internal standard solution* and 15.0 mL of methanol, and shake vigorously to disperse the Cream. Centrifuge at about 3500 rpm for 10 min, and pass a portion of the supernatant through a filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak

**Tailing factor:** NMT 2.0 for the clobetasol propionate peak

**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
- $R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*
- $C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)
- Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** <755>: Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **PH** <791>: 4.5–7.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** <11>
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - $C_{25}H_{30}ClFO_4$  448.96

## Clobetasol Propionate Ointment

**DEFINITION**

Clobetasol Propionate Ointment is Clobetasol Propionate in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>
  - Standard solution:** 0.5 mg/mL of USP Clobetasol Propionate RS in chloroform
  - Test solution:** Nominally 0.5 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 70° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Freeze the mixture in an ice bath for 5 min, and centrifuge at about 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a suitable vial. Evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of chloroform.
  - Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)
  - Acceptance criteria:** The  $R_F$  value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

**ASSAY**

- **PROCEDURE**
  - Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.
  - Mobile phase:** Acetonitrile, methanol, and *Buffer* (95:20:85)

**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Standard solution:** Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate

**Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate into a 125-mL separatory funnel. Add 30 mL of hexane, 10.0 mL of the *Internal standard solution*, and shake. Collect the lower layer in a 25-mL volumetric flask. Extract the hexane remaining in the separatory funnel with two 5-mL portions of *Mobile phase*, and combine all of the extracts in the 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and pass a portion through a filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak

**Tailing factor:** NMT 2.0 for the clobetasol propionate peak

**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

$R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

$C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

- **MINIMUM FILL** <755>: Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** <11>
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - C<sub>25</sub>H<sub>30</sub>ClFO<sub>4</sub> 448.96

### Clobetasol Propionate Topical Solution

#### DEFINITION

Clobetasol Propionate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of clobetasol propionate (C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>).

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>
  - Standard solution:** 0.75 mg/mL of USP Clobetasol Propionate RS in chloroform
  - Test solution:** Nominally 0.75 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.5 mg of clobetasol to a 50-mL separatory funnel. Add 5 mL of water, and extract with 5 mL of chloroform. Collect the lower layer through a cotton wool plug, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 2 mL of chloroform.
  - Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)
  - Acceptance criteria:** The *R<sub>f</sub>* value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

#### ASSAY

- **PROCEDURE**
  - Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.
  - Mobile phase:** Acetonitrile, methanol, and Buffer (95:20:85)
  - Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol
  - System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*
  - Standard solution:** 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.
  - Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.0 mg of clobetasol propionate to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.
- Chromatographic system**
  - (See *Chromatography* <621>, *System Suitability*.)
  - Mode:** LC
  - Detector:** UV 240 nm
  - Column:** 4.6-mm  $\times$  15-cm; packing L1
  - Flow rate:** 1 mL/min
  - Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*  
 [NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A  
**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak  
**Tailing factor:** NMT 2.0 for the clobetasol propionate peak  
**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 [NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]  
 Calculate the percentage of clobetasol propionate (C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>) in the portion of Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- R<sub>U</sub>* = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*  
*R<sub>S</sub>* = ratio of the clobetasol propionate peak area to the Internal standard peak area from the *Standard solution*  
*C<sub>S</sub>* = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)  
*C<sub>U</sub>* = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** <755>: Meets the requirements

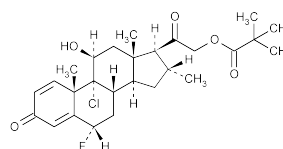
#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed 10<sup>2</sup> cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **pH** <791>: 4.5–6.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** <11>
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - C<sub>25</sub>H<sub>30</sub>ClFO<sub>4</sub> 448.96

### Clocortolone Pivalate



C<sub>27</sub>H<sub>36</sub>ClFO<sub>5</sub> 495.02  
 Pregna-1,4-diene-3,20-dione, 9-chloro-21-(2,2-dimethyl-1-oxopropoxy)-6-fluoro-11-hydroxy-16-methyl-, (6 $\alpha$ ,11 $\beta$ ,16 $\alpha$ )-.

9-Chloro-6 $\alpha$ -fluoro-11 $\beta$ ,21-dihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 21-pivalate [34097-16-0].

» Clotocortolone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{36}ClFO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clotocortolone Pivalate RS

**Color and clarity of solution**—A 1 in 100 solution in chloroform is clear and practically colorless.

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 15  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +125° and +135°.

*Test solution:* 40 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%, a 100-mg test specimen being used.

**Chromatographic purity**—

*Test solution*—Accurately weigh about 100 mg of Clotocortolone Pivalate, and transfer to a 25-mL volumetric flask. Dissolve in a mixture of chloroform and methanol (1:1), and dilute with the same solvent to volume.

*Standard solution*—Using an accurately weighed quantity of USP Clotocortolone Pivalate RS, prepare a solution in a mixture of chloroform and methanol (1:1) having a known concentration of about 4 mg per mL.

*Procedure*—Score a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture into three equal sections to be used for the *Test solution*, the blank, and the *Standard solution*, respectively. Activate the plate at 105° for 30 minutes before use. Apply 100  $\mu$ L each of the *Test solution* and the *Standard solution* as streaks 2.5 cm from the bottom of the appropriate section of the plate, and dry the streaks with a gentle current of air. Using a solvent system consisting of a mixture of cyclohexane and ethyl acetate (2:1), develop the chromatogram in a suitable chromatographic chamber lined with absorbent paper and previously equilibrated, until the solvent front has moved 15 cm above the line of application. Air-dry the plate, and develop the chromatogram a second time using the same chromatographic system. Air-dry the plate, and locate the principal band occupied by the *Standard solution* by viewing under UV light. Mark this band as well as corresponding bands in the blank and *Test solution* sections of the plate. Quantitatively remove the silica gel from each band, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of methanol to each tube, shake for not less than 20 minutes, and centrifuge. Concomitantly determine the absorbances of the supernatants from the *Test solution* and the *Standard solution* against the blank at the wavelength of maximum absorbance at about 238 nm, with a suitable spectrophotometer. Calculate the percentage of chromatographic impurities in the *Test solution* taken by the formula:

$$100 - [100(C_s / C_u)(A_u / A_s)]$$

in which  $C_s$  is the concentration, in mg per mL, of USP Clotocortolone Pivalate RS in the *Standard solution*;  $C_u$  is the concentration, in mg per mL, of the *Test solution*; and  $A_u$  and  $A_s$  are the absorbances of the solutions from the *Test*

*solution* and the *Standard solution*, respectively: not more than 3.0% of total impurities is found.

**Assay**—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clotocortolone Pivalate RS in chloroform to obtain a solution having a known concentration of about 0.75 mg per mL. Dilute an accurately measured volume of this solution with methanol, and mix to obtain a *Standard preparation* having a known concentration of about 30  $\mu$ g per mL.

*Assay preparation*—Accurately weigh about 75 mg of Clotocortolone Pivalate, and transfer to a 100-mL volumetric flask. Dissolve in chloroform, dilute with chloroform to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

*Procedure*—Transfer 10.0-mL portions of the *Standard preparation* and the *Assay preparation* to separate glass-stoppered, 50-mL conical flasks, and evaporate on a steam bath to dryness. To each flask, and to a third flask to provide the blank, add 15.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of methanol. Swirl the contents of the flasks to dissolve the residues. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours. Cool to room temperature. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells against the blank at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $C_{27}H_{36}ClFO_5$  in the portion of Clotocortolone Pivalate taken by the formula:

$$2.5C(A_u / A_s)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Clotocortolone Pivalate RS in the *Standard preparation*, and  $A_u$  and  $A_s$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Clotocortolone Pivalate Cream

» Clotocortolone Pivalate Cream contains not less than 90.0 percent and not more than 110.0 percent of  $C_{27}H_{36}ClFO_5$  in a suitable cream base. It may contain suitable preservatives.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clotocortolone Pivalate RS

**Identification**—Place a portion of Cream, equivalent to about 1 mg of clotocortolone pivalate, in a suitable separator. Add 5 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform layer to dryness, and dissolve the residue in 2 mL of methanol. Apply 20  $\mu$ L of this test solution and 20  $\mu$ L of a *Standard solution* of USP Clotocortolone Pivalate RS in chloroform containing about 0.5 mg per mL about 1.5 cm from the bottom of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of cyclohexane and ethyl acetate (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 5.0 and 7.0, in a 1 in 10 aqueous dispersion.

**Particle size determination**—Place a small portion of Cream on a microscope slide, apply a cover slide, press slightly, and examine under 40× objective magnification using a suitable microscope equipped with polarized light. Scan the complete slide preparation, and record the size of the largest crystal found in reference to a calibrated grid: no particle in the Cream is greater than 50 microns when measured in the longitudinal axis.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clocortolone Pivalate RS in methanol to obtain a solution having a known concentration of about 0.06 mg per mL.

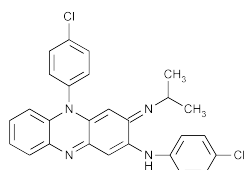
**Assay preparation**—Using a plastic syringe equipped with a suitable cannula, transfer an accurately weighed quantity of Cream, equivalent to about 3 mg of clocortolone pivalate, to a 50-mL volumetric flask. Add about 25 mL of methanol, and warm the flask in a 60° water bath for about 10 minutes, with occasional swirling, to disperse the Cream. Cool to room temperature, dilute with methanol to volume, and mix. Allow any insoluble material to settle.

**Procedure**—Transfer 10.0 mL of the *Standard preparation* to a 25-mL volumetric flask. Transfer 10.0-mL portions of the *Assay preparation* into two separate 25-mL volumetric flasks labeled *Assay preparation* and *Assay blank*, respectively. Evaporate the contents of the three flasks with the aid of a stream of air or nitrogen to dryness. Transfer 20.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of methanol to the flasks containing the *Standard preparation*, the *Assay preparation*, and a fourth 25-mL volumetric flask labeled *Reagent blank*. Pipet 20 mL of acidified methanol solution (0.3 mL of hydrochloric acid diluted with methanol to 500 mL) into the flask labeled *Assay blank*. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours, occasionally swirling the contents of each flask. Cool the flasks to room temperature, dilute with the acidified methanol solution to volume, and mix. Centrifuge the *Assay preparation* at high speed for 10 minutes. Concomitantly determine the absorbances of the *Standard preparation*, the *Assay preparation*, the *Assay blank*, and the *Reagent blank* against acidified methanol solution as the solvent blank in 1-cm cells at the wavelength of maximum absorbance at about 390 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $C_{27}H_{36}ClFO_5$  in the portion of Cream taken by formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Clocortolone Pivalate RS in the *Standard preparation*;  $A_U$  is the absorbance of the *Assay preparation*, corrected for the *Assay blank* and the *Reagent blank*; and  $A_S$  is the absorbance of the *Standard preparation* corrected for the *Reagent blank*.

## Clofazimine



$C_{27}H_{22}Cl_2N_4$  473.40  
2-Phenazinamine, *N*,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-

3-(*p*-Chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine [2030-63-9].

» Clofazimine contains not less than 98.5 percent and not more than 101.5 percent of  $C_{27}H_{22}Cl_2N_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, at room temperature.

**USP Reference standards** (11)—

USP Clofazimine RS

**Identification**—

**A: Infrared Absorption** (197S): 5% solution in methylene chloride.

**B:** The  $R_f$  value of the principal spot observed in the chromatogram of the *Test preparation* corresponds to that of *Standard preparation A* as obtained in the test for *Chromatographic purity*.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281)—not more than 0.1%.

**Chromatographic purity**—

**Standard preparations**—Dissolve an accurately weighed quantity of USP Clofazimine RS in methylene chloride, and mix to obtain *Standard preparation A* having a known concentration of about 0.5 mg per mL. Dilute portions of *Standard preparation A* quantitatively with methylene chloride to obtain *Standard preparations B* and *C* having known concentrations of 0.25 and 0.1 mg per mL, respectively.

**Test preparation**—Dissolve an accurately weighed quantity of Clofazimine in methylene chloride to obtain a solution having a known concentration of about 50 mg per mL.

**Ammonia solution**—Pipet 1 mL of ammonium hydroxide into a 100-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Prepare this solution fresh daily.]

**Chromatographic plate**—Use a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Immediately before use, expose the plate to ammonia vapors for 30 minutes by suspending the plate in a tank containing a shallow layer of approximately 25 mL of *Ammonia solution*. [NOTE—Prevent the plate from coming into contact with the liquid.]

**Procedure**—Separately apply 5  $\mu$ L of the *Test preparation* and 5  $\mu$ L of each *Standard preparation* to the *Chromatographic plate*. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride and *n*-propyl alcohol (10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: no secondary spot from the chromatogram of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation A* (1.0%), and the sum of the intensities of the secondary spots obtained from the *Test preparation* corresponds to not more than 2.0%.

**Assay**—Dissolve about 300 mg of Clofazimine, accurately weighed, in 5 mL of chloroform, with the aid of heat if necessary. Add 20 mL of acetone and 5 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS in glacial acetic acid, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode with a saturated solution of potassium chloride as the bridge fluid and agar gel as the bridge. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.34 mg of  $C_{27}H_{22}Cl_2N_4$ .

## Clofazimine Capsules

» Clofazimine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{27}H_{22}Cl_2N_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Clofazimine RS

**Identification**—

**A:** The  $R_f$  value of the principal spot observed in the chromatogram of the *Test preparation* corresponds to that of the *Standard preparation* in the test for *Chromatographic purity*.

**B:** The UV absorption spectrum of the *Assay preparation*, prepared as directed in the *Assay*, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clofazimine RS, concomitantly measured.

**Dissolution** (711)—

*Medium:* water; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Chromatographic purity**—

*Ammonia solution, Chromatographic plate, and Procedure*—Proceed as directed in the test for *Chromatographic purity* under *Clofazimine*.

**Standard preparations**—Dissolve an accurately weighed quantity of USP Clofazimine RS in methylene chloride, and mix to obtain *Standard preparation A* having a known concentration of about 0.5 mg per mL. Dilute portions of *Standard preparation A* quantitatively with methylene chloride to obtain *Standard preparations B* and *C* having known concentrations of 0.1 and 0.04 mg per mL, respectively.

**Test preparation**—To a portion of Capsule contents, equivalent to about 500 mg of clofazimine, add 25 mL of methylene chloride and 25 mL of 0.1 N sodium hydroxide, and sonicate for 30 minutes. Withdraw the methylene chloride layer, and filter through anhydrous sodium sulfate.

**Assay**—

**0.1 N Methanolic hydrochloric acid**—Pipet 10 mL of hydrochloric acid into a 1000-mL volumetric flask containing about 500 mL of methanol, mix, and dilute with methanol to volume.

**Reference solution**—Pipet 5 mL of methylene chloride into a 50-mL volumetric flask, dilute with 0.1 N *Methanolic hydrochloric acid* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clofazimine RS in methylene chloride, and dilute quantitatively, and stepwise if necessary, with methylene chloride to obtain a solution having a known concentration of about 0.075 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.1 N *Methanolic hydrochloric acid* to volume, and mix.

**Assay preparation**—Remove, as completely as possible, the contents of not less than 20 Capsules with the aid of

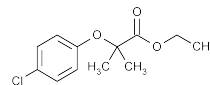
methylene chloride. Dissolve in methylene chloride, filter the solution through a pledget of cotton, and dilute quantitatively, and stepwise if necessary, with methylene chloride to obtain a solution having a concentration of about 0.075 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.1 N *Methanolic hydrochloric acid* to volume, and mix.

**Procedure**—Concomitantly determine the UV absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 491 nm, using the *Reference solution* as the blank. Calculate the quantity, in mg, of  $C_{27}H_{22}Cl_2N_4$  in the Capsules taken by the formula:

$$(L / D)C(A_U / A_S)$$

in which *L* is the labeled quantity, in mg, of clofazimine in each Capsule; *D* is the concentration, in mg per mL, of clofazimine in the *Assay preparation*, based on the labeled quantity and extent of dilution; *C* is the concentration, in mg per mL, of USP Clofazimine RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Clofibrate



$C_{12}H_{15}ClO_3$  242.70

Propanoic acid, 2-(4-chlorophenoxy)-2-methyl-, ethyl ester.  
Ethyl 2-(*p*-chlorophenoxy)-2-methylpropanoate [637-07-0].

» Clofibrate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{12}H_{15}ClO_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clofibrate RS

**Identification**—

**A:** *Infrared Absorption* (197F).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 20 µg per mL.

*Medium:* methanol.

**Refractive index** (831): between 1.500 and 1.505, at 20°.

**Acidity**—Mix 10.0 g with 100 mL of neutralized alcohol, add 3 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 0.90 mL is required for neutralization.

**Water, Method I** (921): not more than 0.2%.

**Chromatographic purity**—

**Standard preparation**—Prepare a solution in chloroform having known concentrations of about 0.1 mg of USP Clofibrate RS and 0.03 mg of *p*-chlorophenol per mL. To 10.0 mL of this solution add 5.0 µL of tributyrin, and mix.

**Test preparation**—To 10.0 mL of Clofibrate add 5.0 µL of tributyrin, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a split injector with a 20:1 split ratio, a flame-ionization detector, and a 0.53-mm × 15-m column to the internal walls of which is bonded a 1.5-µm film of phase G1. The chromatograph is programmed to maintain the column temperature at 120° for

1 minute, then to increase the temperature at a rate of 5° per minute for 12 minutes, and finally to maintain a temperature of 180° for 9 minutes. Maintain the injection port at 210° and the detector block at 220°. Helium is used as the carrier gas flowing at a rate of about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.2 for *p*-chlorophenol, 0.55 for clofibrate, and 1.0 for tributyrin.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity, other than *p*-chlorophenol, in the Clofibrate taken by the formula:

$$0.1C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Clofibrate RS in the *Standard preparation*,  $R_U$  is the ratio of the response of each individual impurity peak (other than the *p*-chlorophenol peak) to that of the tributyrin peak obtained from the *Test preparation*, and  $R_S$  is the ratio of the response of the clofibrate peak to that of the tributyrin peak obtained from the *Standard preparation*: the percentage of any impurity, other than *p*-chlorophenol, does not exceed 0.01%, and the total percentage of all such impurities does not exceed 0.12%.

**Limit of *p*-chlorophenol**—Use the chromatograms obtained in the test for *Chromatographic purity*. Calculate the percentage of *p*-chlorophenol in the portion of Clofibrate taken by the formula:

$$0.1C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of *p*-chlorophenol in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of the responses of the *p*-chlorophenol and tributyrin peaks obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 0.003% of *p*-chlorophenol is found.

#### Assay—

**Ion-exchange resin**—To a beaker containing 75 mL of 1 N sodium hydroxide add about 3 g of a 50- to 100-mesh strongly basic styrene-divinylbenzene anion-exchange resin, and allow the mixture to stand for about 15 minutes, with occasional stirring. Wash the resin with water until the last washing is neutral to litmus paper, then wash with three 50-mL portions of methanol.

**Ion-exchange column**—Place a plug of glass wool in the base of a 1- × 15-cm ion-exchange tube, and transfer to the tube a sufficient amount of *ion-exchange resin*, slurried in methanol, to produce a column bed height of from 6 cm to 8 cm.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clofibrate RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 20 µg per mL.

**Assay preparation**—Transfer about 200 mg of Clofibrate, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsing in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 226 nm, with a suitable spectrophotometer, using methanol

as the blank. Calculate the quantity, in mg, of  $C_{12}H_{15}ClO_3$  in the portion of Clofibrate taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Clofibrate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Clofibrate Capsules

» Clofibrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{12}H_{15}ClO_3$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Clofibrate RS

**Identification**—Capsules respond to *Identification test A* under *Clofibrate*.

#### Dissolution (711)—

**Medium:** sodium lauryl sulfate solution (5 in 100); 1000 mL.

**Apparatus 2:** 100 rpm.

**Time:** 180 minutes.

Determine the amount of  $C_{12}H_{15}ClO_3$  dissolved by employing the following method.

**Mobile phase**—Prepare a degassed and filtered mixture of methanol and water (80:20).

**Standard solution**—Transfer about 20 mg of USP Clofibrate RS, accurately weighed, to a 50-mL volumetric flask. Add 20 mL of methanol, mix to dissolve the clofibrate, dilute with water to volume, and mix. Dilute an aliquot quantitatively with methanol to obtain a final solution having a known concentration of about 80 µg per mL.

**Test solution**—Dilute a 5.0-mL portion of the solution under test to 25.0 mL, using methanol. Allow to stand for 5 minutes, and filter.

**Chromatographic system**—The liquid chromatograph is equipped with a 226-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal portions (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of  $C_{12}H_{15}ClO_3$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{12}H_{15}ClO_3$  is dissolved in 180 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Assay**—Proceed with Capsules as directed in the *Assay under Clofibrate*, using the following as the *Assay preparation*: Weigh accurately not less than 20 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any liquid from the emptied capsules by washing with several small portions of ether. Discard the washings, and allow the capsules to dry in a jet of dry air until the odor of ether no longer is perceptible. Weigh the empty capsules in the tared weighing bottle, and calculate the average net weight per capsule. Transfer an accurately weighed amount of capsule contents,

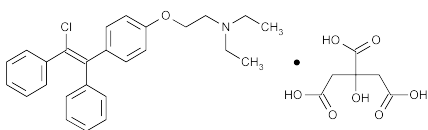


equivalent to about 200 mg of clofibrate, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *Ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsings in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Calculate the quantity, in mg, of  $C_{12}H_{15}ClO_3$  in the portion of Capsules taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Clomiphene RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Clomiphene Citrate



$C_{26}H_{28}ClNO \cdot C_6H_8O_7$  598.08

Ethanamine, 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1). 2-[p-(2-Chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate (1:1) [50-41-9].

» Clomiphene Citrate contains not less than 98.0 percent and not more than 102.0 percent of a mixture of the (E)- and (Z)-geometric isomers of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ , calculated on the anhydrous basis. It contains not less than 30.0 percent and not more than 50.0 percent of the Z-isomer, [(Z)-2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1).

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Clomiphene Citrate RS

USP Clomiphene Related Compound A RS

(E,Z)-2-[4-(1,2-Diphenylethenyl)phenoxy]-N,N-diethylethanamine hydrochloride.

$C_{26}H_{29}NO \cdot HCl$  407.98

### Identification—

**A:** It meets the requirements under *Identification—Organic Nitrogenous Bases* (181).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 20  $\mu\text{g}$  per mL.

*Medium:* 0.1 N hydrochloric acid.

**C:** It responds to the tests for *Citrate* (191).

**Water, Method I** (921): not more than 1.0%.

**Heavy metals, Method II** (231): 0.002%.

### Content of (Z)-isomer—

*Mobile phase, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Test solution*—Use the Assay preparation prepared as directed in the Assay.

*Procedure*—Inject a volume (about 50  $\mu\text{L}$ ) of the *Test solution* into the chromatograph, record the chromatograms,

and measure the responses for the major peaks. Calculate the percentage of (Z)-isomer in the portion of Clomiphene Citrate taken by the formula:

$$100(r_Z / r_i)$$

in which  $r_Z$  is the peak response of the (Z)-isomer in the *Test solution*, and  $r_i$  is the sum of all of the peak responses obtained from the *Test solution*.

### Related compounds—

*Mobile phase and System suitability solution*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Clomiphene Citrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0  $\mu\text{g}$  per mL.

*Test solution*—Use the Assay preparation.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 290-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L26. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for clomiphene related compound A, 1.0 for the (Z)-isomer, and 1.2 for the (E)-isomer; the resolution,  $R$ , between clomiphene related compound A and the (Z)-isomer is not less than 1.0; and the resolution,  $R$ , between the (Z)-isomer and the (E)-isomer is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates for the (E)-isomer; the tailing factor is not more than 3.0 for the (E)-isomer; and the relative standard deviation for replicate injections is not more than 2.0% determined from both the (E)- and (Z)-isomers.

*Procedure*—Inject a volume (about 50  $\mu\text{L}$ ) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Clomiphene Citrate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 2.0% of clomiphene related compound A is found; not more than 0.5% of any other individual impurity is found; and not more than 2.0% of total impurities is found.

### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol, water, and triethylamine (55:45:0.3). Adjust with phosphoric acid to a pH of 2.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve suitable quantities of USP Clomiphene Related Compound A RS and USP Clomiphene Citrate RS in *Mobile phase* to obtain a solution containing about 0.002 and 0.05 mg per mL, respectively.

*Standard preparation*—[NOTE—Use actinic glassware for all solutions.] Dissolve an accurately weighed quantity of USP Clomiphene Citrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

*Assay preparation*—Transfer about 50 mg of the Clomiphene Citrate, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 233-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L26. The flow rate is about 1 mL per minute. Chromatograph the

*System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for clomiphene related compound A, 1.0 for (Z)-isomer, and 1.2 for (E)-isomer; and the resolution, *R*, between clomiphene related compound A and (Z)-isomer is not less than 1.0 and between (Z)-isomer and (E)-isomer is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates for the (E)-isomer; the tailing factor is not more than 3.0 for the (E)-isomer; and the relative standard deviation for replicate injections is not more than 2.0% for both (E)- and (Z)-isomers.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$  in the portion of Clomiphene Citrate taken by the formula:

$$1000C(r_{UE} + r_{UZ} / r_{SE} + r_{SZ})$$

in which *C* is the concentration, in mg per mL, of USP Clomiphene Citrate RS in the *Standard preparation*; *r<sub>UE</sub>* and *r<sub>UZ</sub>* are the peak responses obtained from the *Assay preparation* for the (E)- and (Z)-isomers, respectively; and *r<sub>SE</sub>* and *r<sub>SZ</sub>* are the peak responses obtained from the *Standard preparation* for the (E)- and (Z)-isomers, respectively.

## Clomiphene Citrate Tablets

» Clomiphene Citrate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ .

**Packaging and storage**—Preserve in well-closed containers, protected from light.

### USP Reference standards (11)—

USP Clomiphene Citrate RS

USP Clomiphene Related Compound A RS

(E,Z)-2-[4-(1,2-Diphenylethenyl)phenoxy]-N,N-diethylethanamine hydrochloride.

$C_{26}H_{29}NO \cdot HCl$  407.98

**Identification**—Place a portion of finely powdered Tablets, equivalent to about 30 mg of clomiphene citrate, in a centrifuge tube containing about 30 mL of a 1 in 2 solution of methanol in 0.1 N hydrochloric acid. Insert the stopper, and place the tube in a water bath at about 37° for 15 minutes. Shake occasionally. Centrifuge, and place the clear supernatant in a separator. Extract with one 40-mL and two 25-mL portions of hexanes, and discard the extract. Render the aqueous solution alkaline with 1 N sodium hydroxide, and extract the precipitated base with one 50-mL and two 25-mL portions of hexanes. Wash the combined extracts with two portions of water. Dry the extract with anhydrous sodium sulfate, and remove the hexanes by evaporation under reduced pressure. Add about 1.0 mL of carbon disulfide to the residue, and dissolve. Determine the absorption spectra of the test solution and of a Standard solution of USP Clomiphene Citrate RS, similarly prepared, as directed under *Identification*—*Organic Nitrogenous Bases* (181).

### Dissolution (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the amount of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$  dissolved from UV absorbances at the wavelength

of maximum absorbance at about 232 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Clomiphene Citrate RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Mobile phase*, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Clomiphene Citrate*.

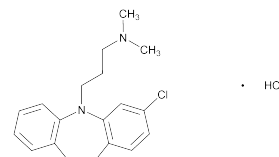
*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of clomiphene citrate, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and stir using a magnetic bar for about 30 minutes. Remove the magnetic bar from the flask, dilute with *Mobile phase* to volume, mix, and filter. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter, discarding the first 10 mL. [NOTE—This solution is stable for at least 24 hours.]

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{26}H_{28}ClNO \cdot C_6H_8O_7$  in the portion of Tablets taken by the formula:

$$1000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clomiphene Citrate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clomipramine Hydrochloride



$C_{19}H_{23}ClN_2 \cdot HCl$  351.31

5*H*-Dibenz[*b,f*]azepine-5-propanamine, 3-chloro-10,11-dihydro-*N,N*-dimethyl-, monohydrochloride.

3-Chloro-5-[3-(dimethylamino)propyl]-10,11-dihydro-5*H*-dibenz[*b,f*]azepine monohydrochloride [17321-77-6].

» Clomipramine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{23}ClN_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Clomipramine Hydrochloride RS

USP Desipramine Hydrochloride RS

USP Imipramine Hydrochloride RS

**Identification—****A: Infrared Absorption** (197K).**B: Ultraviolet Absorption** (197U)—*Solution:* 100 µg per mL.*Medium:* 0.1 N hydrochloric acid.

Absorptivities at the wavelength of maximum absorbance, calculated on the dried basis, do not differ by more than 1.0%.

**pH** (791): between 3.5 and 5.0, in a solution having a concentration of about 100 mg per mL.**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.**Residue on ignition** (281): not more than 0.1%.**Heavy metals, Method II** (231): 0.01%.**Chromatographic purity—****TEST 1—***Sodium 1-heptanesulfonate solution, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.*Mobile phase*—Transfer 20.0 mL of *Sodium 1-heptanesulfonate solution*, 2.0 mL of triethylamine, and 500 mL of water to a suitable container, mix, adjust with phosphoric acid to a pH of  $3.2 \pm 0.1$ , and dilute with water to 625 mL. Transfer to a 1-liter volumetric flask, dilute with acetonitrile to volume, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).*Test solution*—Transfer about 100 mg of Clomipramine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.*Procedure*—Inject a volume (about 5 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks.**TEST 2—***Sodium 1-heptanesulfonate solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.*Test solution*—Prepare as directed for *Test 1*.*Procedure*—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks. Not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found, the results for *Test 1* and *Test 2* being considered.**Assay—***Sodium 1-heptanesulfonate solution*—Transfer about 5.5 g of sodium 1-heptanesulfonate, accurately weighed, to a 100-mL volumetric flask, dissolve in 50.0 mL of water, and dilute with glacial acetic acid to volume.*Mobile phase*—Transfer 20.0 mL of *Sodium 1-heptanesulfonate solution* and 2.0 mL of triethylamine to a 500-mL volumetric flask, and dilute with water to volume. Transfer this solution to a 1-L volumetric flask, adjust with phosphoric acid to a pH of  $3.2 \pm 0.1$ , dilute with acetonitrile to volume, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).*System suitability solution*—Transfer about 7.0 mg of USP Desipramine Hydrochloride RS and 10.0 mg of USP Imipramine Hydrochloride RS, both accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.*Standard preparation*—Dissolve an accurately weighed quantity of USP Clomipramine Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.8 mg per mL. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, and mix.*Assay preparation*—Transfer about 80 mg of Clomipramine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, and mix.*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*; the relative retention times are about 0.85 for desipramine and 1.0 for imipramine; the resolution,  $R$ , between desipramine and imipramine is not less than 0.5; and the relative standard deviation for replicate injections is not more than 2.0%.*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{19}H_{23}ClN_2 \cdot HCl$  in the portion of Clomipramine Hydrochloride taken by the formula:

$$250C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Clomipramine Hydrochloride RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.**Clomipramine Hydrochloride Capsules**» Clomipramine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ).**Packaging and storage**—Preserve in well-closed containers.**USP Reference standards** (11)—  
USP Clomipramine Hydrochloride RS**Identification, Infrared Absorption** (197K)—*Test specimen*—Transfer the contents of a number of Capsules, equivalent to about 125 mg of clomipramine hydrochloride, to a suitable container, add 25 mL of chloroform, stir for 5 minutes, and filter. Evaporate on a steam bath to a volume of about 5 mL, chill in an ice bath, add ethyl ether, and stir until crystals form. Filter, and dry at 100° for 1 hour.**Dissolution** (711)—*Medium:* 0.1 N hydrochloric acid; 500 mL.*Apparatus 2:* 50 rpm.*Time:* 30 minutes.*Procedure*—Determine the amount of  $C_{19}H_{23}ClN_2 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 252 nm on filtered portions of the solution under test, suitably diluted with *Dissolution*

*Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Clomipramine Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{19}H_{23}ClN_2 \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Standard solution**—Prepare a solution of USP Clomipramine Hydrochloride RS in methanol having a known concentration of about 30 µg per mL.

**Test solution**—Quantitatively transfer the contents of 1 Capsule to a 100-mL volumetric flask with the aid of methanol, add about 75 mL of methanol, shake by mechanical means for 1 hour, and dilute with methanol to volume. Further dilute an aliquot of this solution quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 30 µg per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells, at the wavelength of maximum absorbance at about 252 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) in the Capsule taken by the formula:

$$(TC/D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of clomipramine hydrochloride in the Capsule; *C* is the concentration, in µg per mL, of USP Clomipramine Hydrochloride RS in the *Standard solution*; *D* is the concentration, in µg per mL, of clomipramine hydrochloride in the *Test solution*, based on the labeled quantity per Capsule and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Test solution* and the *Standard solution*, respectively.

#### Assay—

*Sodium 1-heptanesulfonate solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Clomipramine Hydrochloride.

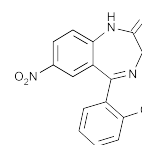
**Assay preparation**—Remove as completely as possible the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the contents, equivalent to about 160 mg of clomipramine hydrochloride, to a 200-mL volumetric flask, add 130 mL of methanol, shake by mechanical means for 1 hour, and dilute with methanol to volume. Transfer 10 mL of this solution to a 25-mL volumetric flask, dilute with methanol to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) in the portion of Capsules taken by the formula:

$$500C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clomipramine Hydrochloride RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clonazepam



$C_{15}H_{10}ClN_3O_3$  315.71  
2H-1,4-Benzodiazepin-2-one, 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-  
5-(o-Chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one [1622-61-3].

» Clonazepam contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{10}ClN_3O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, at room temperature.

**USP Reference standards** (11)—

USP Clonazepam RS

USP Clonazepam Related Compound A RS

3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

$C_{15}H_{10}ClN_3O_3$  315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$  276.68

USP Clonazepam Related Compound C RS

2-Bromo-2'-(2-chlorobenzoyl)-4'-nitroacetanilide.

**Identification, Infrared Absorption** (197K).

**Melting range** (741): between 237° and 240°.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Limit of clonazepam related compound C—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution**—Dissolve an accurately weighed quantity of Clonazepam in acetone to obtain a solution having a concentration of 25 mg per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Clonazepam Related Compound C RS in acetone to obtain a solution having a known concentration of 50 µg per mL.

**Application volume:** 20 µL.

**Developing solvent system:** a mixture of acetone and *n*-heptane (3:2).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After air-drying the plate, heavily spray the plate with 2 M sulfuric acid, and dry at 105° for 15 minutes. Successively spray the plate with 0.01 M sodium nitrite, 9 mM ammonium sulfamate, and *N*-(1-naphthyl)ethylenediamine dihydrochloride TS, and dry the plate with a current of air. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Standard solution*: no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from the *Standard solution* (0.2%).

**Related compounds—**

*Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay.

*Test preparation*—Use the *Assay preparation*.

*Procedure*—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Clonazepam taken by the formula:

$$100P_i / (r_c + \Sigma P_i)$$

in which *P* is the relative response factor, which is 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test preparation*; and *r<sub>c</sub>* is the peak response for clonazepam in the *Test preparation*: not more than 0.1% of clonazepam related compound A or of clonazepam related compound B is found, not more than 0.2% of any other impurity is found, and the sum of all other impurities is not more than 0.3%.

#### Assay—

*Buffer solution*—Transfer about 6.6 g of anhydrous dibasic ammonium phosphate to a 1-L volumetric flask, dissolve in 950 mL of water, adjust with 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 8.0, dilute with water to volume, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution*, methanol, and tetrahydrofuran (60:52:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of water, methanol, and tetrahydrofuran (60:52:13).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL.

*System suitability solution*—Dissolve suitable quantities of USP Clonazepam Related Compound A RS, USP Clonazepam Related Compound B RS, and USP Clonazepam RS in *Diluent* to obtain a solution containing about 0.04 mg per mL of each Reference Standard.

*Assay preparation*—Transfer about 10 mg of Clonazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 2.2 for clonazepam related compound A, 2.5 for clonazepam related compound B, and 1.0 for clonazepam; and the resolution, *R<sub>s</sub>*, between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>15</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>3</sub> in the portion of Clonazepam taken by the formula:

$$100C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clonazepam Oral Suspension

### DEFINITION

Clonazepam Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clonazepam (C<sub>15</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>3</sub>).

Prepare Clonazepam Oral Suspension 0.1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Clonazepam	10 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

If using tablets, comminute the tablets into a fine powder in a suitable mortar, or add *Clonazepam* powder to the mortar. Add approximately 10 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### PROCEDURE

*Mobile phase*: Methanol, acetonitrile, and water (30:30:40). Filter and degas.

*Standard solution*: 25 µg/mL of USP Clonazepam RS in acetonitrile

*Sample solution*: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 2.5 mL of the *Sample solution* into a 10-mL volumetric flask, and dilute with acetonitrile to volume to obtain a solution having a nominal concentration of 25 µg/mL of clonazepam.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

*Mode*: LC

*Detector*: UV 254 nm

*Column*: 4.6-mm × 10-cm; 5-µm packing L1

*Flow rate*: 1 mL/min

*Injection volume*: 20 µL

#### System suitability

*Sample*: *Standard solution*

[NOTE—The retention time for clonazepam is about 7 min.]

#### Suitability requirements

*Relative standard deviation*: NMT 1.8% for replicate injections

#### Analysis

*Samples*: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonazepam (C<sub>15</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>3</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

*r<sub>u</sub>* = peak response from the *Sample solution*

*r<sub>s</sub>* = peak response from the *Standard solution*

*C<sub>s</sub>* = concentration of clonazepam in the *Standard solution* (µg/mL)

*C<sub>u</sub>* = nominal concentration of clonazepam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **PH** (791): 3.6–4.6

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a cold place.
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded
- **USP REFERENCE STANDARDS** (11)  
USP Clonazepam RS

### Clonazepam Tablets

» Clonazepam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, at room temperature.

#### USP Reference standards (11)—

- USP Clonazepam RS
- USP Clonazepam Related Compound A RS  
3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.  
 $C_{15}H_{10}ClN_3O_3$  315.72
- USP Clonazepam Related Compound B RS  
2-Amino-2'-chloro-5-nitrobenzophenone.  
 $C_{13}H_9ClN_2O_3$  276.68

#### Identification—

**A:** Place an amount of finely powdered Tablets, equivalent to about 10 mg of clonazepam, in a 125-mL separator. Add 25 mL of water, shake for 2 minutes, and extract with two 40-mL portions of chloroform. Pass the extracts through anhydrous sodium sulfate, combine them, and evaporate at room temperature with the aid of a stream of nitrogen to dryness. Wash the residue with three 10-mL portions of solvent hexane: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Clonazepam RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

*Medium:* degassed water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

Determine the amount of Clonazepam dissolved, using the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and acetonitrile (40:30:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Prepare a solution of USP Clonazepam RS in methanol having a known concentration of about 0.05 mg per mL. Quantitatively dilute a portion of this solution with *Dissolution Medium* to obtain a *Standard solution* having a known concentration similar to the expected concentration in the solution under test.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector

and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%; and the tailing factor is not more than 2.0.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of  $C_{15}H_{10}ClN_3O_3$  dissolved by comparison of the peak responses obtained from the *Standard solution* and the test solution.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{15}H_{10}ClN_3O_3$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Related compounds—

*Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam*.

**Test preparation**—Use the *Assay preparation*.

**Procedure**—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100Pr_i / (r_c + \Sigma Pr_i)$$

in which *P* is the relative response factor, which is 2.45 for the peak with a relative retention time of 0.7 (if present), 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test preparation*; and *r<sub>c</sub>* is the peak response for clonazepam in the *Test preparation*: not more than 0.8% for the peak at relative retention time 0.7, not more than 0.4% of clonazepam related compound A, and not more than 1.0% of clonazepam related compound B are found; not more than 0.2% of any other impurity is found; and the sum of all other impurities is not more than 0.5%.

#### Assay—

*Buffer solution, Mobile phase, Diluent, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam*.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed amount of powder, equivalent to about 10 mg of clonazepam, to a 100-mL volumetric flask; dissolve, with sonication, in 75 mL of *Diluent*; cool to room temperature, dilute with *Diluent* to volume, mix, and filter, discarding the first few mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ) in the portion of Tablets taken by the formula:

$$100C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clonazepam Orally Disintegrating Tablets

» Clonazepam Orally Disintegrating Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clonazepam  $C_{15}H_{10}ClN_3O_3$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Clonazepam RS

USP Clonazepam Related Compound A RS

3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

$C_{15}H_{10}ClN_3O_3$  315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$  276.68

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Disintegration** (701): not more than 60 seconds.

### Dissolution (711)—

*Medium:* water; 900 mL, degassed.

*Apparatus 2:* 50 rpm.

*Time:* 60 minutes.

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Quantitatively dilute with *Medium* the *Standard preparation* as directed in the *Assay* according to the Tablet strength. The final concentration of the *Standard solution* corresponding to each Tablet strength is given in Table 1.

Table 1

Tablet Strength mg per Tablet	Final Standard solution µg/mL of Clonazepam
0.125	0.125
0.25	0.25
0.5	0.50
1.0	1.0
2.0	2.0

*Test solution*—Pass a portion of the solution under test through a 0.45-µm nylon membrane filter, discarding the first few mL.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the clonazepam peak. Calculate the percentage of clonazepam dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration of USP Clonazepam RS, in mg per mL, in the *Standard solution*; 900 is the volume of the *Medium*; and  $L$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 75% (Q) of the labeled amount of clonazepam is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Related compounds—

*Mobile phase and Standard preparation*—Prepare as directed in the *Assay*.

*System suitability solution*—Dissolve weighed quantities of USP Clonazepam Related Compound A RS and USP Clonazepam Related Compound B RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 µg per mL each of USP Clonazepam Related Compound A RS and USP Clonazepam Related Compound B RS.

*Standard solution*—Quantitatively dilute with *Mobile phase* the *Standard preparation* to obtain a solution having a known concentration of about 0.2 µg per mL of clonazepam.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Grind the Tablets into a fine powder and transfer an accurately weighed portion of the powder, equivalent to about 2 mg of clonazepam, to a 50-mL volumetric flask, pipet 20.0 mL of *Mobile phase* into the flask, and sonicate for about 2 minutes with intermittent shaking. DO NOT dilute to volume. Shake the flask for 30 minutes on a mechanical shaker. Pass a portion of this solution through a nylon membrane filter having a 0.45-µm or finer porosity, and use the filtrate after discarding the first 4 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*. Identify the peaks due to clonazepam related compound A and clonazepam related compound B using the relative retention times given in Table 2; the resolution,  $R$ , between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for six replicate injections is not more than 6.0%.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least four times the retention time of clonazepam, and measure the responses for all the peaks. [NOTE—Disregard any peaks with a retention time less than 3.5 minutes.] Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(C_S / C_T)(r_T / r_S)(1 / F)100$$

in which  $C_S$  is the concentration, in µg per mL, of USP Clonazepam RS in the *Standard solution*;  $C_T$  is the nominal concentration, in µg per mL, of clonazepam in the *Test solution*;  $r_T$  is the peak response of each impurity obtained from the *Test solution*;  $r_S$  is the peak response for clonazepam obtained from the *Standard solution*; and  $F$  is the relative response factor for the impurity given in Table 2. The limits of each impurity along with relative retention times and relative response factors are given in Table 2.

Table 2

Peak ID	Relative Retention Time	Relative Response Factor	Limit % (w/w)
Clonazepam	1.0	1.0	—
Clonazepam related compound A <sup>1</sup>	1.71	0.67	0.4
Clonazepam related compound B <sup>2</sup>	2.25	0.79	1.0

<sup>1</sup>3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

<sup>2</sup>2-Amino-2'-chloro-5-nitrobenzophenone.

Table 2 (Continued)

Peak ID	Relative Retention Time	Relative Response Factor	Limit % (w/w)
Any other unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

<sup>1</sup>3-Amino-4-(2-chlorophenyl)-6-nitrocarbostryl.<sup>2</sup>2-Amino-2'-chloro-5-nitrobenzophenone.**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (2:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Mobile phase*, and dilute, if necessary, to obtain a solution having a known concentration of about 0.01 mg per mL of USP Clonazepam RS.

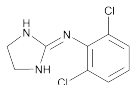
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of clonazepam, to a 200-mL volumetric flask, add 120 mL of *Mobile phase*, and sonicate for about 15 minutes with intermittent shaking. Shake the flask on a mechanical shaker for about 30 minutes. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a nylon membrane filter having a 0.45-μm or finer porosity, and use the filtrate after discarding the first 4 mL of the filtrate. [NOTE—The solution is stable for 48 hours at room temperature.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L7. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not fewer than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 60 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the clonazepam peak. Calculate the quantity, in percentage, of label claim of clonazepam (C<sub>15</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>3</sub>) in the portion of Tablets taken by the formula:

$$(C_s / C_u)(r_u / r_s)100$$

in which  $C_s$  is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard solution*;  $C_u$  is the nominal concentration based on label claim, in mg per mL, of clonazepam in the *Assay preparation*;  $r_u$  is the peak response for clonazepam obtained from the *Assay preparation*; and  $r_s$  is the peak response for clonazepam obtained from the *Standard preparation*.

**Clonidine**

C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub> 230.09  
Benzenamine, 2,6-dichloro-N-2-imidazolidinylidene-;  
2-[(2,6-Dichlorophenyl)imino]imidazolidine [4205-90-7].

**DEFINITION**

Clonidine contains NLT 99.0% and NMT 101.0% of clonidine (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>), calculated on the dried basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** <197K>• **B. ULTRAVIOLET ABSORPTION** <197U>

**Medium:** 0.01 N hydrochloric acid

**Sample solution:** 0.3 mg/mL of Clonidine in *Medium*

**Acceptance criteria:** Absorptivities are about 2.1 and 1.8 for the maxima at 271 and 279, respectively, calculated on the dried basis.

**ASSAY**• **PROCEDURE**

**Sample solution:** 190 mg of Clonidine in 100 mL of glacial acetic acid

**Analysis:** Titrate with 0.1 N perchloric acid VS, using a silver-silver chloride glass combination electrode with liquid junction. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each mL of 0.1 N perchloric acid is equivalent to 23.01 mg of clonidine (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>).

**Acceptance criteria:** 99.0%–101.0% on the dried basis

**IMPURITIES**• **RESIDUE ON IGNITION** <281>

**Analysis:** Ignite at 500 ± 25° to constant weight.

**Acceptance criteria:** NMT 0.1%

• **HEAVY METALS, Method II** <231>: NMT 10 μg/g• **ORGANIC IMPURITIES**

**1 M phosphoric acid:** 115 g/L of phosphoric acid in water

**Buffer:** 4 g/L of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 4.0.

**Solution A:** Acetonitrile and *Buffer* (75:25)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	90	10
15	30	70
15.1	90	10
20	90	10

**Acetylclonidine solution:** 0.05 mg/mL of USP Clonidine Related Compound A RS, prepared as follows. Initially dissolve USP Clonidine Related Compound A RS in acetonitrile (1 mg/mL), and dilute with *Buffer* to volume.

**System suitability solution:** 0.86 mg/mL of USP Clonidine RS and 0.86 μg/mL of clonidine related compound A from *Acetylclonidine solution* in *Buffer*

**Standard solution:** 8.6 μg/mL of USP Clonidine RS in *Buffer*

**Sample solution:** 0.86 mg/mL of Clonidine in *Buffer*

**Blank:** *Buffer*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.0-mm × 15-cm; 5-μm packing L56

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 5 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 5 between clonidine and acetylclonidine, *System suitability solution*

**Tailing factor:** NMT 2.5 for clonidine, *System suitability solution*



Relative standard deviation: NMT 5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Calculate the percentage of each impurity in the portion of Clonidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of clonidine from the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

#### Acceptance criteria

**Individual impurity:** NMT 0.1%

**Total impurities:** NMT 0.2%

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Analysis:** Dry a sample at 60° under vacuum to constant weight.

**Acceptance criteria:** NMT 0.5%

##### • APPEARANCE OF SOLUTION

#### Color of solution

**Standard solution:** 4.8  $\mu\text{g/mL}$  of potassium chromate in water

**Sample solution:** 100 mg/mL in methanol

**Acceptance criteria:** The color of 10 mL of *Sample solution* is not more intense than that of 10 mL of *Standard solution* when compared in matched color-comparison tubes (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

#### Turbidity

**Sample solution:** Prepare as directed in *Color of solution*.

**Acceptance criteria:** 10 mL of *Sample solution* has no more turbidity than 10 mL of methanol when compared in matched color-comparison tubes (see *Spectrophotometry and Light-Scattering* (851), *Visual Comparison*).

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Clonidine RS

USP Clonidine Related Compound A RS

1-Acetyl-2-(2,6-dichlorophenylamino)-2-(4,5-dihydroimidazol).

$\text{C}_{11}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}$  272.13

#### USP Reference standards (11)—

USP Clonidine Hydrochloride RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 330  $\mu\text{g}$  per mL.

*Medium:* 0.01 N hydrochloric acid.

Absorptivities at 272 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** It responds to the tests for *Chloride* (191).

**pH** (791): between 3.5 and 5.5, in a solution (1 in 20).

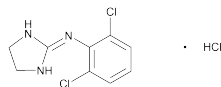
**Loss on drying** (731)—Dry it at 105° to constant weight; it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—Dissolve 200 mg in methanol, and dilute with methanol to 2.0 mL to obtain the test solution. Dissolve a suitable quantity of USP Clonidine Hydrochloride RS in methanol to obtain a *Standard solution* having a known concentration of 100 mg per mL. Dilute a portion of this solution quantitatively and stepwise with methanol to obtain a diluted *Standard solution* having a concentration of 100  $\mu\text{g}$  per mL. Apply separate 2- $\mu\text{L}$  portions of the test solution, the *Standard solution*, and the diluted *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a freshly prepared solvent system consisting of a mixture of toluene, dioxane, dehydrated alcohol, and ammonium hydroxide (10:8:2:1), until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and dry it at 100° for 1 hour. Dip the plate into a dipping chamber filled to three-fourths of its height with sodium hypochlorite solution, diluted to contain 0.5% available chlorine, dry in a fume hood with a current of air for 1 hour, and spray with starch-potassium iodide TS: the  $R_F$  value of the principal spot from the test solution corresponds to that of the *Standard solution*. Any other spot obtained from the test solution does not exceed, in size or intensity, the principal spot obtained from the diluted *Standard solution* (0.1%), and the total of any spots does not exceed 0.2%.

**Assay**—Dissolve about 200 mg of Clonidine Hydrochloride, accurately weighed, in about 80 mL of glacial acetic acid, add 15 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a sleeve-type calomel electrode containing 0.1 N lithium perchlorate in glacial acetic acid (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 26.66 mg of  $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3 \cdot \text{HCl}$ .

## Clonidine Hydrochloride



$\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3 \cdot \text{HCl}$  266.55

Benzenamine, 2,6-dichloro-N-2-imidazolidinylidene-, monohydrochloride.

2-[(2,6-Dichlorophenyl)imino]imidazolidine monohydrochloride [4205-91-8].

» Clonidine Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of  $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

## Clonidine Hydrochloride Tablets

#### DEFINITION

Clonidine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3 \cdot \text{HCl}$ ).

#### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

##### • B. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 10 mg/mL of USP Clonidine Hydrochloride RS in methanol

**Sample solution:** Transfer an equivalent to 1 mg of clonidine hydrochloride, from a quantity of finely powdered Tablets, to a separator containing 30 mL of water

and 5 mL of 1 N sodium hydroxide. Swirl gently to dissolve the sample specimen, and extract with 20 mL of chloroform. Allow the layers to separate, and filter the chloroform extract. Evaporate the filtrate to dryness, and dissolve the residue in 0.1 mL of methanol.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 2  $\mu$ L

**Developing solvent system:** Methanol and ammonium hydroxide (200:3)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Position the plate in a chromatographic chamber, and develop in *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Solution A:** 2.2 mg/mL of sodium 1-octanesulfonate in water

**Mobile phase:** Methanol, *Solution A*, and phosphoric acid (500:500:1). Adjust with 1 N sodium hydroxide to a pH of 3.0, and pass through a suitable filter of 0.45- $\mu$ m pore size.

**2,6-Dichloroaniline stock solution:** 12  $\mu$ g/mL of 2,6-dichloroaniline in *Mobile phase*

**Standard stock solution:** 100  $\mu$ g/mL of USP Clonidine Hydrochloride RS in *Mobile phase*

**Standard solution:** 1  $\mu$ g/mL of clonidine hydrochloride from the *Standard stock solution* in *Mobile phase*

**System suitability solution:** 2  $\mu$ g/mL of USP Clonidine Hydrochloride RS and 2.4  $\mu$ g/mL of 2,6-dichloroaniline in *Mobile phase*, from the *Standard stock solution* and *2,6-Dichloroaniline stock solution*, respectively

**Sample solution:** Weigh and finely powder NLT 20 Tablets. Transfer the equivalent to 0.1 mg of clonidine hydrochloride from powder to a 100-mL volumetric flask. Add about 60 mL of *Mobile phase*, shake by mechanical means for 15–30 min, dilute with *Mobile phase* to volume, and mix. Centrifuge a portion of this solution to obtain a clear solution.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L7, deactivated for basic compounds

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clonidine and 2,6-dichloroaniline are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Tailing factor:** NMT 1.5 for the clonidine peak, *System suitability solution*

**Column efficiency:** NLT 3500 theoretical plates for the clonidine peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the clonidine peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of clonidine hydrochloride in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** Proceed as directed in the *Assay*, except use *Medium* instead of *Mobile phase*.

**Analysis:** Proceed as directed in the *Assay*.

**Tolerances:** NLT 75% (Q) of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS <11>**  
USP Clonidine Hydrochloride RS

## Clonidine Hydrochloride and Chlorthalidone Tablets

#### DEFINITION

Clonidine Hydrochloride and Chlorthalidone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) and NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ).

#### IDENTIFICATION

##### • A.

**Sample solution:** Transfer an amount of powdered Tablets, equivalent to 3 mg of clonidine hydrochloride, to a beaker. Add 30 mL of water, stir for 5 min, and pass through a filter of medium pore size into a sintered-glass funnel. Transfer the filtrate to a separator, add 5 mL of 0.1 N sodium hydroxide, and extract with 20 mL of chloroform, collecting the chloroform extract in a separator. Extract the chloroform phase with 15 mL of 0.01 N hydrochloric acid, collecting the acid extract in a beaker. Remove any residual chloroform from the acid extract by heating on a steam bath.

**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clonidine Hydrochloride RS, concomitantly measured.

##### • B. INFRARED ABSORPTION

**Sample:** Transfer 10 powdered Tablets to a 50-mL beaker. Add 10 mL of methanol, boil on a steam bath for 5 min, and filter. Add 20 mL of water to the filtrate, and boil on a steam bath for 5 min under a current of air. Cool, with stirring, in ice until crystals form. Filter the crystals, and dry at 105° for 1 h.

**Acceptance criteria:** The IR absorption spectrum of a mineral oil dispersion of the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Chlorthalidone RS.

- **C.** The retention times of the chlorthalidone and clonidine hydrochloride peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 1 g/L of monobasic ammonium phosphate in water

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (100:100:800)

**Clonidine hydrochloride standard stock solution:** 1500/μg/mL of USP Clonidine Hydrochloride RS in *Buffer*; *J* is the ratio of the labeled amount, in mg, of clonidine hydrochloride to the labeled amount, in mg, of chlorthalidone per Tablet.

**Standard solution:** 150/μg/mL of USP Clonidine Hydrochloride RS and 150 μg/mL of USP Chlorthalidone RS prepared as follows. Transfer 15 mg of USP Chlorthalidone RS to a 100-mL volumetric flask, dissolve in 10 mL of methanol, and add 25 mL of *Buffer* and 10.0 mL of *Clonidine hydrochloride standard stock solution*. Dilute with *Buffer* to volume.

**Sample solution:** Transfer an amount equivalent to 15 mg of chlorthalidone from powdered Tablets (NLT 20). Add 10 mL of methanol, and sonicate for 5 min. Add 40 mL of *Buffer*, and sonicate until the solution is free from agglomerates. Allow to cool to ambient temperature, dilute with *Buffer* to volume, and centrifuge.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 10-cm; packing L7

**Flow rate:** 2 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clonidine hydrochloride and chlorthalidone are about 0.2 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between the clonidine hydrochloride and chlorthalidone peaks

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clonidine hydrochloride or chlorthalidone from the *Sample solution*

$r_S$  = peak response of clonidine hydrochloride or chlorthalidone from the *Standard solution*

$C_S$  = concentration of USP Clonidine Hydrochloride RS or USP Chlorthalidone RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of clonidine hydrochloride or chlorthalidone in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 100 rpm

**Time:** 60 min

**Sample solution:** Pipet 20 mL of a centrifuged portion of the solution under test into a 25-mL volumetric flask, and dilute with 0.5% monobasic ammonium phosphate

solution to volume. Use the resulting solution as the *Sample solution*.

**Analysis:** Proceed as directed in the *Assay*, making any necessary volumetric adjustments.

**Tolerances:** NLT 50% (Q) of the labeled amount of chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) and NLT 80% (Q) of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Content Uniformity* with respect to both clonidine hydrochloride and chlorthalidone

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** <11>

USP Chlorthalidone RS

USP Clonidine Hydrochloride RS

**Clonidine Transdermal System****DEFINITION**

Clonidine Transdermal System contains NLT 80.0% and NMT 120.0% of the labeled amount of clonidine ( $C_9H_9Cl_2N_3$ ). [NOTE—Throughout the following procedures, avoid the use of tetrahydrofuran stabilized with butylated hydroxytoluene (BHT). In the presence of peroxides, BHT may react with clonidine, producing impurity peaks.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>

**Buffer solution:** 242.28 g/L of tris-(hydroxymethyl)aminomethane in water. Adjust with dilute hydrochloric acid to a pH of 9.2.

**Sample:** Carefully peel the release liner from each Transdermal System, and place a number of Transdermal Systems equivalent to 25 mg of clonidine into a 50-mL screw-capped centrifuge tube. Add 5 mL of chloroform, and mix on a vortex mixer for 5 min. Allow to stand for 30 min, and mix intermittently on a vortex mixer.

Transfer the chloroform solution to another 50-mL centrifuge tube, and wash the residue with an additional 3 mL of chloroform, combining the extracts. Add 2 mL of 0.5 N hydrochloric acid to the extract, mix on a vortex mixer for 1 min, and centrifuge at about 1000 rpm for 4 min. Remove and discard the bottom chloroform layer. Extract the aqueous layer with 4 mL of chloroform. Centrifuge at 1000 rpm for an additional 5 min, and again discard the bottom chloroform layer. Add 5 mL of *Buffer solution* and 3 mL of methylene chloride. Mix on a vortex mixer for 1 min. Centrifuge at 1000 rpm for 4 min. Transfer the bottom methylene chloride layer into a 100-mL beaker, and dry the methylene chloride with anhydrous sodium sulfate (about 1/4 liquid height). Decant, and evaporate to dryness with a stream of nitrogen. Dry at 105° for 30 min, and allow to cool in a desiccator.

**Analysis:** Determine the IR spectrum of the *Sample solution* and USP Clonidine RS in the wavelength region of 3500–600 cm<sup>-1</sup>.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer solution:** 2.5 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer solution* (60:40).  
[NOTE—Stir the solution for 30 min.]

**Diluent:** Tetrahydrofuran and methanol (1:1)

**System suitability solution:** 250 µg/mL of USP Clonidine RS and 10 µg/mL of USP Clonidine Related Compound B RS in *Diluent*

**Standard stock solution:** 1 mg/mL of USP Clonidine RS in tetrahydrofuran

**Standard solutions:** Prepare a minimum of four *Standard solutions* from the *Standard stock solution* in *Diluent* that bracket the expected clonidine concentration in the sample. The standard concentrations should be within the range of 50–300 µg/mL. [NOTE—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

**Sample solution:** 357 µg/mL of clonidine prepared as follows. Remove each Transdermal System from its package, discard the release liner from each system, and transfer into a 50-mL centrifuge tube with a Teflon-lined screw cap. Add the appropriate volume of tetrahydrofuran as listed in *Table 1*.

**Table 1**

For systems containing about 2.5 mg of clonidine	7.0 mL
For systems containing about 5.0 mg of clonidine	14.0 mL
For systems containing about 7.5 mg of clonidine	21.0 mL

Mix vigorously on a vortex mixer until the systems are washed down and fully submerged in the tetrahydrofuran. Let the systems soak in tetrahydrofuran for about 5 min, and mix on a vortex mixer until the systems are completely delaminated. Allow the systems to remain submerged for an additional 60 min, mixing on a vortex mixer every 30 min. Add methanol in a volume equal to the volume of tetrahydrofuran, and mix vigorously on a vortex mixer. The solution turns milky. Centrifuge for 10 min at 2000 rpm. Use the supernatant as the *Sample solution*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 242 nm

[NOTE—The detector is programmed initially to 242 nm and switched to 210 nm after the elution of the clonidine peak but before the elution of the clonidine related compound B peak.]

**Column:** 4.6-mm × 15-cm; packing L10

**Flow rate:** 1 mL/min

**Injection size:** 25 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clonidine and clonidine related compound B are 1.0 and 1.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clonidine and clonidine related compound B

**Capacity factor (*k'*):** NLT 0.6 for clonidine

**Tailing factor:** NMT 3.0 for both clonidine and clonidine related compound B

**Relative standard deviation:** NMT 2.0% for the clonidine peak area

#### Analysis

**Samples:** At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995.

Calculate the percentage of the labeled amount of clonidine (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>) in the Transdermal System taken:

$$\text{Result} = (C_s/C_u) \times 100$$

C<sub>s</sub> = concentration of clonidine from the linear regression analysis (µg/mL)

C<sub>u</sub> = nominal concentration of clonidine in the *Sample solution* (µg/mL)

**Acceptance criteria:** 80.0%–120.0%

## PERFORMANCE TESTS

### • DRUG RELEASE <724>

#### Test 1

**Medium:** 0.001 M phosphoric acid; 80 mL for systems containing 5 mg or less of clonidine; 200 mL for systems containing more than 5 mg of clonidine

**Times:** 8, 24, 96, and 168 h

**Apparatus 7:** Proceed as directed in the chapter, using the transdermal system holder-angled disk (see *Figure 4a*). The appropriate size of the holder, 1.42 or 1.98 inches, should be chosen based on the size of the system to prevent overhang. Use 100-mL beakers for *Medium* volumes of 80 mL and 300-mL beakers for *Medium* volumes of 200 mL. Gently press the Transdermal System to a dry, smooth, square piece of cellulose membrane, or equivalent, with the adhesive side against the membrane. Attach the membrane/system to a suitable inert sample holder with a Viton O-ring, or equivalent, so that the backing of the system is adjacent to and centered on the bottom of the sample holder. Trim the excess cellulose membrane with scissors. Suspend each sample holder from the arm of a reciprocating shaker so that each system is continuously immersed in a beaker containing the specified volume of *Medium*. The filled beakers are weighed and pre-equilibrated to 32.0 ± 0.3° before immersing the test sample. Agitate the sample in an up-down motion at a frequency of 30 cycles/min with an amplitude of 2.0 ± 0.1 cm. The *Medium* must be added daily to the beakers during each interval to maintain sample immersion. At the end of each time interval, transfer the test sample to a fresh beaker containing the appropriate volume of *Medium*, weighed and pre-equilibrated to 32.0 ± 0.3°.

**Mobile phase:** 0.1% solution of triethylamine in a mixture of methanol and water (30:70). Adjust with phosphoric acid to a pH of 6.0 ± 0.2.

**System suitability solution:** 10 µg/mL of USP Clonidine RS in 0.001 M phosphoric acid

**Standard solutions:** Prepare a minimum of four *Standard solutions* of USP Clonidine RS in 0.001 M phosphoric acid having known concentrations of clonidine similar to those of the *Sample solutions*.

**Sample solutions:** At the end of each release interval, allow the beakers to cool to room temperature, and make up for evaporative *Medium* losses by adding *Medium* to obtain the original weight, then mix.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Capacity factor ( $k'$ ):** NLT 0.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*  
Construct a standard curve of concentration ( $\mu\text{g/mL}$ ) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.995.

Calculate the release rate of clonidine:

$$\text{Result} = CV/TA$$

$C$  = concentration of clonidine in the sample of the standard curve ( $\mu\text{g/mL}$ )

$V$  = volume of the *Medium* (mL)

$T$  = time (h)

$A$  = area of the Transdermal System ( $\text{cm}^2$ )

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Time for Sampling (h)	Release Rate ( $\mu\text{g/h/cm}^2$ )
0–8	8	7.5–16.0
8–24	24	1.5–4.6
24–96	96	1.5–4.6
96–168	168	1.5–3.3

The release rate of clonidine ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3$ ) from the Transdermal System, expressed as  $\mu\text{g/h/cm}^2$  at the times specified, conforms to *Acceptance Table 1* in <724>.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.

**Medium:** 0.01 N hydrochloric acid; 500 mL for systems labeled as 0.1 mg/day, 900 mL for systems labeled as 0.2 or 0.3 mg/day

**Apparatus 6:** 100 rpm. Apply double-sided tape around the lower-most circumference of the cylinder, overlapping the ends to prevent peeling of the tape end from the cylinder. Remove the outer layer of the tape. Attach the Transdermal System to the cylinder with the backing side against the double-sided tape and the longitudinal axis parallel to the bottom of the cylinder. Carefully smooth the system to remove any air bubbles, and remove the release liner from the system. For systems requiring 500 mL of *Medium*, apply the double-sided tape to the system such that the bottom edge of each is NMT 2 mm from the bottom of the cylinder to prevent evaporation during the test from exposure to air. After setting the cylinder in the vessel, cover the vessel to minimize evaporation.

**Times:** 6, 48, 96, and 168 h

**Buffer:** 0.3% triethylamine in 0.025 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of  $6.20 \pm 0.10$ .

**Mobile phase:** *Buffer* and tetrahydrofuran (94:6)

**Standard solutions:** Solutions containing 0.7, 3.0, 5.3, 7.5, and 9.8  $\mu\text{g/mL}$  of USP Clonidine RS in *Medium*. A small amount of methanol (not exceeding 10% of the final volume) can be used to solubilize clonidine.

**Sample solution:** 1.5 mL aliquots of the solution under test. After sampling the last time point, measure the volume of *Medium* remaining in the vessel.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

#### Columns

**Guard:** 3.0-mm  $\times$  4-mm; packing L1

**Analytical:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 50  $\mu\text{L}$

#### System suitability

**Sample:** 5.3  $\mu\text{g/mL}$  of the *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*  
Construct a standard curve of concentration ( $\mu\text{g/mL}$ ) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.997. Calculate the release rate of clonidine. Calculate the volume loss rate in mL/h ( $L$ ):

$$L = [V - F + (N \times 1.5)]/T$$

$V$  = initial volume of *Medium* (mL)

$F$  = final volume of *Medium* (mL)

$N$  = number of sampling time points

$T$  = total elapsed time between start of run and final volume measurement (h)

Calculate the volume (mL) at each sampling time adjusted for evaporation ( $V_{adj}$ ):

$$V_{adj} = V - (L \times t_c) - [(n - 1) \times 1.5]$$

$t_c$  = cumulative time for the sample withdrawal (6, 48, 96, or 168 h)

$n$  = sampling number (1, 2, 3, or 4 for the 6-, 48-, 96-, and 168-h sampling times, respectively)

Calculate the release rate of clonidine ( $\mu\text{g/h/cm}^2$ ):

$$\text{Result} = [(r_u - b) \times V_{adj}]/(m \times A \times t_i)$$

$r_u$  = peak response from the *Sample solution*

$b$  = y-intercept of the standard curve

$m$  = slope of the standard curve

$A$  = area of the system ( $\text{cm}^2$ )

$t_i$  = interval time (h)

**Tolerances:** See *Table 3*.

**Table 3**

Time (h)	Time for Sampling (h)	Interval Time (h)	Release Rate ( $\mu\text{g/h/cm}^2$ )
0–6	6	6	7.6–12.0
6–48	48	42	1.7–2.5
48–96	96	48	2.0–2.9
96–168	168	72	1.7–2.6

The release rate of clonidine ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3$ ) from the Transdermal System, expressed as  $\mu\text{g/h/cm}^2$  at the times specified, conforms to *Acceptance Table 1* in <724>.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.

**Medium:** 100 mM acetate buffer, pH 5.0, with 0.01% of cetyltrimethylammonium bromide (13.6 g/L of sodium acetate monohydrate in water, adjust with glacial acetic acid to a pH of 5.0, and add 0.1 g/L of cetyltrimethylammonium bromide); 900 mL

**Apparatus 5:** 100 rpm, with the 76-mm disk

**Times:** 8, 24, 96, and 168 h

**Solution A:** 2.4 g/L of octanesulfonic acid sodium salt and 2 mL/L of phosphoric acid in water

**Mobile phase:** Methanol and *Solution A* (45:55). Adjust with 10 N sodium hydroxide to a pH of 3.0.

**Standard stock solution:** 1 mg/mL of USP Clonidine RS in methanol

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration similar to the expected concentration in the *Sample solution*, considering complete drug release.

**Sample solution:** Apply double-sided adhesive tape to the stainless steel disk to cover enough of the disk area so that the entire patch is secured by the tape. Apply a Transdermal System with the release liner intact to the adhesive layer on the stainless steel disk. Press the backing film of the patch to the adhesive tape with the clear release liner film of the system facing up. Peel the release liner from the affixed system on the disk assembly, and place the disk assembly flat on the bottom of the vessel with the exposed transdermal adhesive side up and parallel to the bottom edge of the paddle blade. Lower the paddle, and start the equipment. At each sampling time withdraw an appropriate volume of the solution under test.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; packing L7

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 30 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 2.0%

#### Analysis:

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of clonidine ( $C_9H_9Cl_2N_3$ ) in the *Medium* (mg/mL) at each time point:

$$C_i = (r_U/r_S) \times C_S$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$i$  = interval, where  $i = 1$  at 8 h,  $i = 2$  at 24 h,  $i = 3$  at 96 h,  $i = 4$  at 168 h

Calculate the rate of clonidine ( $C_9H_9Cl_2N_3$ ) released in µg/h/cm<sup>2</sup> at each time point:

$$\text{Result} = [(C_i - C_{i-1}) \times V_i \times 1000] / [S \times (T_i - T_{i-1})]$$

$$V_i = V_0 - [(i - 1) \times V_A]$$

$V_i$  = volume of *Medium* at a given time point

$V_0$  = initial volume of *Medium*, 900 mL

$V_A$  = volume of *Medium* withdrawn at each time point

1000 = conversion factor from mg to µg

$S$  = system size in cm<sup>2</sup>

$T_i$  = current time point

$T_{i-1}$  = previous time point

**Tolerances:** See *Table 4*.

**Table 4**

Time (h)	Release Rate (µg/h/cm <sup>2</sup> )
8	6.5–11.0
24	2.5–5.5
96	2.5–5.0
168	2.0–3.8

The release rate of clonidine ( $C_9H_9Cl_2N_3$ ) from the Transdermal System, expressed as µg/h/cm<sup>2</sup> at the times specified, conforms to *Acceptance Table 1* in <724>.

- UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1 mg/mL of USP Clonidine Related Compound B RS in tetrahydrofuran

**Standard solutions:** Prepare a minimum of four *Standard solutions* in *Diluent* that bracket the expected clonidine related compound B concentration in the sample. The standard concentrations should be within the range of 0.2–10.0 µg/mL.

[NOTE—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

#### Analysis

**Samples:** At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Measure the responses for clonidine related compound B. Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995. Determine the concentration of clonidine related compound B.

Calculate the amount, in µg/cm<sup>2</sup>, of clonidine related compound B in the portion of the Transdermal System taken:

$$\text{Result} = CV/A$$

$C$  = concentration of clonidine related compound B from the linear regression analysis (µg/mL)

$V$  = volume of the *Sample solution* (mL)

$A$  = area of the sample system (cm<sup>2</sup>)

**Acceptance criteria:** NMT 10.0 µg/cm<sup>2</sup>

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in sealed, single-dose containers at a temperature not exceeding 30°.

- LABELING:** The label states the total amount of clonidine in the Transdermal System and the release rate, in mg/day, for the duration of the application of one system. When more than one *Drug Release* test is given, the labeling states the *Drug Release* test used only if *Test 1* is not used.

### • USP REFERENCE STANDARDS <11>

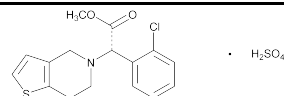
USP Clonidine RS

USP Clonidine Related Compound B RS

2-[(E)-2,6-Dichlorophenylimino]-1-(1-{2-[(E)-2,6-dichlorophenylimino]-imidazolidin-1-yl}-ethyl)imidazolidine.

$C_{20}H_{20}Cl_4N_6$  486.23

## Clopidogrel Bisulfate



$C_{16}H_{16}ClNO_2S \cdot H_2SO_4$  419.90

Thieno[3,2-c]pyridine-5(4H)-acetic acid, α-(2-chlorophenyl)-6,7-dihydro-, methyl ester, (S)-, sulfate (1:1).

Methyl (+)-(S)-α-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, sulfate (1:1) [120202-66-6].

» Clopidogrel Bisulfate contains not less than 97.0 percent and not more than 101.5 percent of  $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Clopidogrel Bisulfate RS

USP Clopidogrel Related Compound A RS

(+)-(S)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid.

USP Clopidogrel Related Compound B RS

Methyl (±)-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate, hydrochloride.

USP Clopidogrel Related Compound C RS

Methyl (–)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, hydrogen sulfate.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** It responds to the test for *Sulfate* (191).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Related compounds**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

*Phosphate buffer*, *Mobile phase*, and *System suitability solution*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound A RS, USP Clopidogrel Related Compound B RS, and USP Clopidogrel Related Compound C RS in methanol, and dilute with methanol to obtain a solution having known concentrations of about 20 µg per mL, 40 µg per mL, 120 µg per mL, and 200 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume, and mix to obtain a solution having final concentrations of about 0.5 µg per mL, 1 µg per mL, 3 µg per mL, and 5 µg per mL, respectively.

*Test solution*—Transfer about 100 mg of Clopidogrel Bisulfate, accurately weighed, to a 200-mL volumetric flask, dissolve in 5 mL of methanol and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and 4.6-mm × 15-cm column that contains packing L57. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for clopidogrel related compound A, 0.8 and 1.2 for the two enantiomers of clopidogrel related compound B, 1.0 for clopidogrel, and 2.0 for clopidogrel related compound C; and the resolution, *R*, between clopidogrel and the first enantiomer of clopidogrel related compound B is greater than 2.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 15% for each peak.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of clopidogrel related compound A and clopidogrel related compound C in the portion of Clopidogrel Bisulfate taken by the formula:

$$100(C_A / C_T)(r_U / r_S)$$

in which  $C_A$  is the concentration, in mg per mL, of the relevant clopidogrel related compound in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Clopidogrel Bisul-

fate in the *Test solution*;  $r_U$  is the peak response for the relevant clopidogrel related compound obtained from the *Test solution*; and  $r_S$  is the peak response for the relevant clopidogrel related compound obtained from the *Standard solution*.

Calculate the percentage of the first enantiomer of clopidogrel related compound B in the portion of Clopidogrel Bisulfate taken by the formula:

$$100 \times 0.5(C_B / C_T)(r_U / r_S)$$

in which  $C_B$  is the concentration, in mg per mL, of clopidogrel related compound B in the *Standard solution*; 0.5 is the correction for the content of the first enantiomer in clopidogrel related compound B;  $r_U$  and  $r_S$  are the peak responses of the first enantiomer of clopidogrel related compound B in the *Test solution* and *Standard solution*, respectively; and the other terms are as defined above.

Calculate the percentage of any impurity other than clopidogrel related compounds A, B, and C in the portion of Clopidogrel Bisulfate taken by the formula:

$$100(C / C_T)(r_U / r_S)$$

in which  $C$  is the concentration of clopidogrel bisulfate, in mg per mL, in the *Standard solution*;  $r_U$  is the peak response of any other impurity obtained from the *Test solution*;  $r_S$  is the peak response of the clopidogrel peak obtained from the *Standard solution*; and the other terms are as defined above: not more than 0.2% of clopidogrel related compound A is found; not more than 0.3% of the first enantiomer of clopidogrel related compound B is found; not more than 1.0% of clopidogrel related compound C is found; not more than 0.1% of any other impurity is found; and not more than 1.5% of total impurities is found. Disregard any peak observed in the blank.

**Assay**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

*Phosphate buffer*—Dissolve 1.36 g of monobasic potassium phosphate in about 500 mL of water, and dilute with water to 1000 mL.

*Mobile phase*—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in methanol to obtain a solution having a known concentration of about 1.0 mg of clopidogrel bisulfate per mL. Dilute a suitable portion of this solution, accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*System suitability solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and quantitatively dilute with methanol to obtain a solution having concentrations of about 100 µg per mL and 200 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Transfer about 100 mg of Clopidogrel Bisulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L57. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are

about 0.8 and 1.2 for enantiomers of clopidogrel related compound B, respectively, and 1.0 for clopidogrel; and the resolution,  $R$ , between clopidogrel and the first enantiomer of clopidogrel related compound B is greater than 2.5. Chromatograph the *Standard preparation*: the relative standard deviation for replicate injections determined from clopidogrel bisulfate is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the quantity, in mg, of  $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$  in the portion of Clopidogrel Bisulfate taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clopidogrel Bisulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clopidogrel Tablets

» Clopidogrel Tablets contain Clopidogrel Bisulfate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clopidogrel ( $C_{16}H_{16}ClNO_2S$ ).

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Clopidogrel Bisulfate RS

USP Clopidogrel Related Compound A RS

(+)-(S)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid.

USP Clopidogrel Related Compound B RS

Methyl ( $\pm$ )-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate, hydrochloride.

USP Clopidogrel Related Compound C RS

Methyl (–)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, hydrogen sulfate.

### Identification—

**A:** Ultraviolet Absorption (197U)—

*Spectral range:* 250 to 300 nm.

*Solution*—Use the test solution prepared as directed in the test for *Uniformity of dosage units*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* pH 2.0 hydrochloric acid buffer (see *Buffer Solutions* under *Reagents, Indicators, and Solutions*); 1000 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Standard solution*—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in 20.0 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration corresponding to that of the solution under test.

*Procedure*—Determine the amount of  $C_{16}H_{16}ClNO_2S$  dissolved by employing UV absorption at a wavelength of about 240 nm on filtered portions of the solution under test in comparison with the *Standard solution*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{16}ClNO_2S$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Using a suitable volumetric flask, place 1 Tablet in 50.0 mL of 0.1 N hydrochloric acid. Sonicate for 5 minutes, and cool. Quantitatively transfer 5.0 mL of this solution to the flask, and dilute with 0.1 N hydrochloric acid to 50.0 mL. Pass a portion of the solution through a suitable filter having a 0.45- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Determine the amount of clopidogrel by employing UV absorption at the wavelength of maximum absorbance at about 270 nm, in comparison with a *Standard solution* having a known concentration of USP Clopidogrel Bisulfate RS in 0.1 N hydrochloric acid.

**Related compounds**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

*Phosphate buffer and Mobile phase*—Prepare as directed in the *Assay under Clopidogrel Bisulfate*.

*System suitability solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and dilute with methanol to obtain a solution having concentrations of about 100  $\mu$ g per mL and 200  $\mu$ g per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Standard solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound A RS, and USP Clopidogrel Related Compound C RS in methanol to obtain a solution having known concentrations of about 40  $\mu$ g per mL, 250  $\mu$ g per mL, and 300  $\mu$ g per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume. This solution contains about 1  $\mu$ g of clopidogrel bisulfate per mL, 6  $\mu$ g of clopidogrel related compound A per mL, and 7.5  $\mu$ g of clopidogrel related compound C per mL.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (free base), to a 200-mL volumetric flask, add 5 mL of methanol, dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 minutes, and mix. Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate after discarding the first 5 mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and 4.6-mm  $\times$  15-cm column that contains packing L57. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 and 1.2 for the two enantiomers of clopidogrel related compound B and 1.0 for clopidogrel; and the resolution,  $R$ , between clopidogrel and the first enantiomer of clopidogrel related compound B is greater than 2.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for clopidogrel related compound A, 1.0 for clopidogrel and 2.0 for clopidogrel related compound C; and the relative standard deviation for replicate injections is not more than 15% for each peak.

*Procedure*—Inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of clopidogrel related



compounds A and C in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C/W)(r_U / r_S)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; C is the concentration, in  $\mu\text{g}$  per mL, of the relevant clopidogrel related compound in the *Standard solution*; W is the weight, in mg, of clopidogrel in the portion of Tablets used to prepare the *Test solution* based on the labeled quantity of clopidogrel per Tablet, Tablet weight, and the weight of the portion of Tablets used; and  $r_U$  and  $r_S$  are the peak responses of the corresponding related compounds obtained from the *Test solution* and the *Standard solution*, respectively.

Calculate the percentage of any other impurity (excluding clopidogrel related compound B) in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C_C / W)(r_U / r_S)$$

in which  $C_C$  is the concentration of clopidogrel bisulfate, in  $\mu\text{g}$  per mL, in the *Standard solution*;  $r_U$  is the peak response of any other impurity obtained from the *Test solution*;  $r_S$  is the peak response of clopidogrel peak obtained from the *Standard solution*; and the other terms are as defined above: not more than 1.2% of clopidogrel related compound A is found, not more than 1.5% of clopidogrel related compound C is found, not more than 0.2% of any other single impurity (excluding clopidogrel related compound B) is found, and not more than 2.5% of total impurities (excluding clopidogrel related compound B) is found.

**Assay**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

*Phosphate buffer, Mobile phase, and Chromatographic system*—Proceed as directed in the Assay under Clopidogrel Bisulfate.

*System suitability preparation*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and quantitatively dilute with methanol to obtain a solution having concentrations of about 100  $\mu\text{g}$  per mL and 200  $\mu\text{g}$  per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in methanol to obtain a solution having a known concentration of about 0.1 mg of clopidogrel bisulfate per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (base), to a 100-mL volumetric flask, and add 50 mL of methanol. Sonicate for 5 minutes, and stir for 30 minutes. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to the flask, dilute with methanol to 50.0 mL, and mix. Pass a portion of this solution through a filter having a 0.45- $\mu\text{m}$  or finer porosity, and use the filtrate after discarding the first 5 mL.

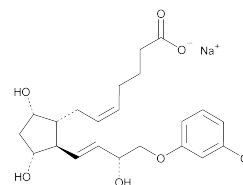
*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the analyte peaks. Calculate the quantity, in mg, of clopidogrel ( $\text{C}_{16}\text{H}_{16}\text{ClNO}_2\text{S}$ ) in the portion of Tablets taken by the formula:

$$1000(321.82/419.90)C(r_U / r_S)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; C is the concentration, in mg per mL, of USP Clopidogrel Bisulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the

peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cloprostenol Sodium



$\text{C}_{22}\text{H}_{28}\text{ClNaO}_6$  446.90

5-Heptenoic acid, 7-[2-[4-(3-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-, [1 $\alpha$ (Z),2 $\beta$ (1E,3R\*),3 $\alpha$ ,5 $\alpha$ ]-, sodium salt, ( $\pm$ )-. ( $\pm$ )-Sodium (Z)-7-[(1R\*,2R\*,3R\*,5S\*)-2-[(E)-(3R\*)-4-(m-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoate [55028-72-3].

» Cloprostenol Sodium contains not less than 97.5 percent and not more than 102.5 percent of  $\text{C}_{22}\text{H}_{28}\text{ClNaO}_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cloprostenol Sodium RS

**Identification**—

A: Infrared Absorption (197K).

B: It meets the requirements of the test for Sodium (191).

**Water, Method I** (921)—Use 50 mg dissolved in 1 mL of dehydrated alcohol: not more than 3.0%.

**Chromatographic purity**—

*Mobile phase*—Prepare a filtered and degassed mixture of the chromatographic solvent hexane, dehydrated alcohol, and glacial acetic acid (930:70:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Test solution*—Dissolve an accurately weighed quantity of Cloprostenol Sodium in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 20 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the Assay.

*Procedure*—Inject a volume (about 5  $\mu\text{L}$ ) of the *Test solution* into the chromatograph. Record the chromatogram for a total time of not less than twice the retention time of the peak due to cloprostenol, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Cloprostenol Sodium taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for each impurity, and  $r_S$  is the sum of the responses of all of the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.5% of total impurities is found. Disregard any peak below 0.05%.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of the chromatographic solvent hexane, dehydrated alcohol, and glacial acetic acid (900:100:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.8 mg per mL.

**Assay preparation**—Using a suitable quantity of Cloprostenol Sodium, accurately weighed, proceed as directed in the *Standard preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L3. The flow rate is about 1.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5 for the cloprostenol peak; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_{22}H_{28}ClNaO_6$  in the portion of Cloprostenol Sodium taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Cloprostenol RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Cloprostenol Sodium in the *Assay preparation*; and  $r_U$  and  $r_S$  are the cloprostenol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cloprostenol Injection

» Cloprostenol Injection is a sterile solution of Cloprostenol Sodium in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cloprostenol ( $C_{22}H_{29}ClO_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers protected from light. Store at controlled room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only, and to indicate the strength as the equivalent amount of cloprostenol per dose.

**USP Reference standards** <11>—

USP Cloprostenol Sodium RS

USP Endotoxin RS

USP Hydrocortisone Acetate RS

**Identification**—

**A:** The retention time of the cloprostenol peak in the chromatogram of the *Assay preparation* corresponds to that of the cloprostenol peak in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**B:** It meets the requirements of the test for *Sodium* <191>.

**Bacterial endotoxins** <85>—It contains not more than 2500 USP Endotoxin Units per mg of cloprostenol.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Related compounds**—

**Mobile phase and System suitability solution**—Prepare as directed in the *Assay*.

**Standard solution**—Prepare as directed for *Standard preparation* under *Assay*.

**Test solution**—Prepare as directed for *Assay preparation*.

**Chromatographic system** (see *Chromatography* <621>)—Prepare as directed in the *Assay*.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of cloprostenol in the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the peak response of cloprostenol obtained from the *Standard solution*: not more than 1.0% of any individual impurity is found, and not more than 2.5% of total impurities is found. Disregard any peak below 0.05%.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

**pH 2.5 Monobasic sodium phosphate solution**—Prepare an aqueous solution containing 2.4 mg of monobasic sodium phosphate dihydrate per mL of solution. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase**—Prepare a filtered and degassed mixture of pH 2.5 Monobasic sodium phosphate solution and acetonitrile (73:27). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS and USP Hydrocortisone Acetate RS in dehydrated alcohol, and dilute with *Mobile phase* to obtain a solution containing about 0.25 mg of cloprostenol sodium and 0.5 mg of hydrocortisone acetate per mL of solution.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Dilute a volume of Injection in dehydrated alcohol and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution containing 0.1 mg of cloprostenol per mL of solution, based on the label claim.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 5-mm × 25-cm column that contains packing L1. The flow rate is about 1.8 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the hydrocortisone acetate peak and the cloprostenol peak is not less than 6. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

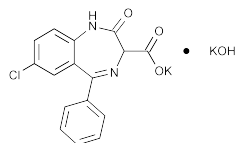
**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the cloprostenol peak. Calculate the percentage label claim of cloprostenol ( $C_{22}H_{29}ClO_6$ ) in the portion of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)(M_1 / M_2)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of cloprostenol in the

*Assay preparation*;  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively,  $M_1$  is the molecular weight of cloprostenol (424.92), and  $M_2$  is the molecular weight of cloprostenol sodium (446.90).

## Clorazepate Dipotassium



$C_{16}H_{11}ClK_2N_2O_4$  408.92

1*H*-1,4-Benzodiazepine-3-carboxylic acid, 7-chloro-2,3-dihydro-2-oxo-5-phenyl-, potassium salt compound with potassium hydroxide (1:1).

Potassium 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-carboxylate compound with potassium hydroxide (1:1) [57109-90-7].

» Clorazepate Dipotassium contains not less than 98.5 percent and not more than 101.5 percent of  $C_{16}H_{11}ClK_2N_2O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve under nitrogen in tight, light-resistant containers.

**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_9ClNO$  231.68

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.

$C_{15}H_{11}ClN_2O$  270.72

USP Clorazepate Dipotassium RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 7 µg per mL.

*Medium:* sodium hydroxide solution (1 in 2500).

**Loss on drying** (731)—Dry it in vacuum at 60° for 1 hour; it loses not more than 0.5% of its weight.

**Heavy metals, Method II** (231): 0.002%.

**Related compounds**—

TEST 1—

*Phosphate buffer solution*—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 2.5 N sodium hydroxide to a pH of 8.0, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Dissolve about 5 mL of 2,6-dimethylaniline in 50 mL of hexane, and carefully add dropwise hydrochloric acid to precipitate the amine hydrochloride. Filter through a sintered-glass funnel, wash the solid precipitate with hexane, and allow the precipitate to dry. Transfer about 50 mg of the dried precipitate of 2,6-dimethylaniline hydrochloride to a 100-mL volumetric flask, add 10.0 mL of *Phosphate buffer solution* and 40 mL of water, and dilute with acetonitrile to volume.

*Standard solution*—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to ob-

tain a solution having a known concentration of about 75 µg per mL. Transfer 4.0 mL of this solution to a 50-mL conical flask, add 4.0 mL of 0.7 M potassium carbonate, 2.0 mL of *Internal standard solution*, and 15.0 mL of water. Insert a stopper, and mix.

*Test solution*—Transfer an accurately weighed quantity of about 50 mg of Clorazepate Dipotassium to a 50-mL conical flask. Add 4.0 mL of 0.7 M potassium carbonate, and start stirring the solution. Add 2 mL of *Internal standard solution* and 19.0 mL of water. Stop stirring about 5 minutes after the addition of the 0.7 M potassium carbonate solution. [NOTE—Prepare fresh immediately before each injection.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*; the relative retention time for 2,6-dimethylaniline is about 0.8 and 1.0 for nordazepam; the relative standard deviation of the peak area ratio of nordazepam to 2,6-dimethylaniline for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of nordazepam in the portion of Clorazepate Dipotassium taken by the formula:

$$2500(C/W) (R_i / R_s)$$

in which *C* is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution*; *W* is the weight, in mg, of Clorazepate Dipotassium taken to prepare the *Test solution*;  $R_i$  is the peak area ratio of any impurity to 2,6-dimethylaniline obtained from the *Test solution*; and  $R_s$  is the peak area ratio of nordazepam to 2,6-dimethylaniline obtained from the *Standard solution*: not more than 0.5% of nordazepam is found and not more than 0.1% of any individual impurity is found.

TEST 2—

*Diluent*—Prepare a mixture of 0.001 N sodium hydroxide and acetonitrile (1:1).

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent*, to obtain a solution having a known concentration of about 0.0026 mg per mL.

*Test solution*—Transfer about 300 mg of Clorazepate Dipotassium, accurately weighed, to a glass test tube. Add 10.0 mL of *Diluent*, and vigorously mix on a vortex mixer for about 90 seconds. [NOTE—Prepare fresh immediately before each injection.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*; the relative standard deviation of the peak height for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity

in the portion of Clorazepate Dipotassium taken by the formula:

$$1000(C/W)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution*; W is the weight, in mg, of sample taken;  $r_i$  is the peak height of each impurity obtained from the *Test solution*; and  $r_s$  is the peak height of 2-amino-5-chlorobenzophenone obtained from the *Standard solution*: not more than 0.1% of 2-amino-5-chlorobenzophenone is found, not more than 0.1% of any other individual impurity is found, and not more than 1.0% of total impurities in *Test 1* and *Test 2* is found.

**Assay**—Transfer about 150 mg of Clorazepate Dipotassium, accurately weighed, to a 250-mL beaker, add 100 mL of glacial acetic acid, and stir until dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a 1 in 100 solution of lithium perchlorate in glacial acetic acid. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.63 mg of  $C_{16}H_{11}ClK_2N_2O_4$ .

## Clorazepate Dipotassium Tablets

» Clorazepate Dipotassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of clorazepate dipotassium ( $C_{16}H_{11}ClK_2N_2O_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_{10}ClNO$  231.68

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one.

$C_{15}H_{11}ClN_2O$  270.72

USP Clorazepate Dipotassium RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{11}ClK_2N_2O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Clorazepate Dipotassium RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{11}ClK_2N_2O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### PROCEDURE FOR CONTENT UNIFORMITY—

**Standard solution**—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.

**Test solution**—Transfer 1 Tablet to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Centrifuge a portion of this solution for 15 minutes, and filter the supernatant, discarding the first 20 mL. Dilute an accurately measured portion of the filtrate with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells at the wavelength of maximum absorbance at about 231 nm, with a suitable spectrophotometer, using 0.01 M sodium hydroxide as the blank.

### Related compounds—

#### METHOD I—

**Phosphate buffer solution**—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 1 N sodium hydroxide to a pH of 8.0, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 66 µg per mL. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of 0.7 M potassium carbonate and 3.0 mL of acetonitrile, dilute with water to volume, mix, and filter.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container. Add 5 mL of acetonitrile, 5 mL of 0.7 M potassium carbonate, and 15 mL of water, stir for 10 minutes, and filter. [NOTE—Prepare fresh before each injection, and use within 3 minutes.]

**Chromatographic system**—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for not less than twice the retention time of nordazepam, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$25C(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution*;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for nordazepam obtained from the *Standard solution*: not more than 2.0% of nordazepam is found.

#### METHOD II—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in acetonitrile to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute with water to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide

and acetonitrile (7:3) to volume, and mix. Transfer 15 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3) to volume, and mix.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container, add 10 mL of a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3), mix, shake by mechanical means for 10 minutes, and filter.

**Chromatographic system**—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$10C(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution*;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_s$  is the response of the 2-amino-5-chlorobenzophenone peak obtained from the *Standard solution*: the sum of all impurities, other than nordazepam, found in *Method I* and *Method II* is not more than 0.5%.

#### Assay—

**Buffer solution**—Transfer 5.0 mL of 1 M tetrabutylammonium hydroxide in methanol to a 1-L volumetric flask, dilute with water to volume, adjust with phosphoric acid to a pH of 7.5, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 60 µg per mL. Shake by mechanical means for 15 minutes, and filter.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clorazepate dipotassium, to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Transfer 15 mL of this solution to a 100-mL volumetric flask, dilute with 0.01 M sodium hydroxide to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

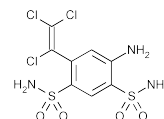
**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of clorazepate dipotassium ( $C_{16}H_{11}ClK_2N_2O_4$ ) in the portion of Tablets taken by the formula:

$$1333C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Clorazepate Dipotassium RS in the *Standard preparation*; and

$r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clorsulon



$C_8H_8Cl_3N_3O_4S_2$  380.66  
1,3-Benzenedisulfonamide, 4-amino-6-(trichloroethenyl)-  
4-Amino-6-(trichlorovinyl)-*m*-benzenedisulfonamide  
[60200-06-8].

» Clorsulon contains not less than 98.0 percent and not more than 101.0 percent of  $C_8H_8Cl_3N_3O_4S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** <11>—  
USP Clorsulon RS

#### Identification—

**A:** *Infrared Absorption* <197M>.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for clorsulon, the retention time of which corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**Melting range** <741>: between 197° and 203°.

**Loss on drying** <731>—Dry it in vacuum at 100° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method II** <231>: 0.003%.

**Chromatographic purity**—[NOTE—The *Standard solutions* and *Test solutions* should be stored in low-actinic glassware.] Prepare a solution of Clorsulon in methanol containing 10.0 mg per mL (*Test solution*). Prepare a solution of USP Clorsulon RS in methanol containing 10.0 mg per mL (*Standard solution A*). Transfer 1.0 mL of *Standard solution A* to a 100-mL volumetric flask, dilute with methanol to volume, and mix (*Standard solution B*). Apply 10-µL portions of the *Test solution* and of *Standard solution A*, and 5- and 10-µL portions of *Standard solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of chloroform and methanol (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the chromatograms show principal spots at about the same  $R_f$  value. Estimate the amounts of any additional spots observed in the chromatogram of the *Test solution* by comparing them with the spots in the two chromatograms obtained from *Standard solution B*, corresponding to 0.5% and 1.0% of impurity: no spot, other than the principal spot, in the chromatogram of the *Test solution* is larger or more intense than that of the principal spot in the chromatogram obtained from the 5-µL portion of *Standard solution B* (0.5%), and the sum of all such impurities is not more than 2.0%.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (70:30:0.1). Make

adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clorsulon RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL. Store the solution in low-actinic glassware.

**Assay preparation**—Transfer about 50 mg of Clorsulon, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a second 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Store the solution in low-actinic glassware.

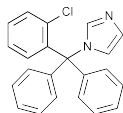
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 7400 theoretical plates; the tailing factor is not more than 1.4; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub> in the portion of Clorsulon taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Clorsulon RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the clorsulon peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clotrimazole



C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub> 344.84  
1*H*-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-;  
1-(*o*-Chloro- $\alpha,\alpha$ -diphenylbenzyl)imidazole [23593-75-1].

### DEFINITION

Clotrimazole contains NLT 98.0% and NMT 102.0% of C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>, calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197M>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 4.35 mg/mL of dibasic potassium phosphate  
**Mobile phase:** Acetonitrile and *Buffer* (3:1). Pass through a membrane filter having a 0.2-µm or finer pore size. The ratio of volumes may be changed to obtain the required resolution.

**Standard solution:** 0.5 mg/mL of USP Clotrimazole RS in methanol

**System suitability solution:** 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

**Sample solution:** 0.5 mg/mL of Clotrimazole in methanol

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub> in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** <281>: NMT 0.1%
- HEAVY METALS**, *Method II* <231>: NMT 10 ppm

#### Organic Impurities

##### PROCEDURE 1: LIMIT OF IMIDAZOLE

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Standard solution:** 500 µg/mL of USP Imidazole RS in chloroform

**Sample solution:** 100 mg/mL of Clotrimazole in chloroform

**Application volume:** 5 µL

**Developing solvent system:** Methanol and chloroform (3:2)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed for *Chromatography* <621>, *Thin-Layer Chromatography*. After air-drying the plate for 5 min, place it in a closed container with a dish containing 100 g of iodine in a shallow layer, and allow to remain for 60 min. Remove the plate from the container, and observe the chromatogram.

**Acceptance criteria:** Any brown spot from the *Sample solution* at an  $R_f$  value corresponding to the principal spot from the *Standard solution* is not greater in size or intensity than the principal spot from the *Standard solution*: NMT 0.5% of imidazole.

• **PROCEDURE 2: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**

**Buffer, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 50 µg/mL of USP Clotrimazole Related Compound A RS prepared by dissolving in methanol using about 75% of the final flask volume. Dilute with *Buffer* to volume.

**Sample solution:** Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, dilute with methanol to volume, and mix.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clotrimazole related compound A in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole related compound A from the *Sample solution*

$r_S$  = peak response of clotrimazole related compound A from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.5%

**SPECIFIC TESTS**

- **LOSS ON DRYING (731):** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS (o-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78
  - USP Imidazole RS

## Clotrimazole Cream

**DEFINITION**

Clotrimazole Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

**Buffer:** 4.35 mg/mL of dibasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (3:1)

[NOTE—The ratio of volumes may be changed to obtain the required resolution.]

**Standard solution:** 0.5 mg/mL of USP Clotrimazole RS in methanol

**System suitability solution:** 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

**Sample solution:** Transfer the equivalent of 25 mg of clotrimazole from the Cream to a 50-mL screw-capped centrifuge tube. Add 25.0 mL of methanol, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol-ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a 50-mL volumetric flask. Add 20.0 mL of methanol to the residue in the centrifuge tube, and repeat the ex-

traction starting with "heat at 50° in a water bath".

Transfer the supernatant to the volumetric flask containing the supernatant from the first extraction, dilute with methanol to volume, and mix.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{17}ClN_2$  in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, at a temperature between 2° and 30°.
- **LABELING:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Clotrimazole Vaginal Cream.
- **USP REFERENCE STANDARDS (11)**
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS (o-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78

## Clotrimazole Lotion

**DEFINITION**

Clotrimazole Lotion contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak for clotrimazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

**Buffer:** 4.35 g/L of dibasic potassium phosphate in water

**Mobile phase:** Methanol and *Buffer* (3:1). Pass through a filter of 0.5-µm or finer pore size.

**Internal standard solution:** 0.07 mg/mL of testosterone propionate in dehydrated alcohol

**Standard stock solution A:** 2 mg/mL of USP Clotrimazole RS in dehydrated alcohol

**Standard stock solution B:** 0.1 mg/mL of USP Clotrimazole Related Compound A RS in dehydrated alcohol

**Standard solution:** *Standard stock solution A, Standard stock solution B, and Internal standard solution (5.0: 5.0: 10.0)*

**Sample solution:** Nominally 1 mg/mL, prepared as follows. Transfer the equivalent of 10 mg of clotrimazole from freshly mixed Lotion to a screw-capped, 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution*, place the cap on the tube, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol-ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a test tube. Add 10.0 mL of dehydrated alcohol to the residue in the centrifuge tube, and repeat the extraction as directed above, beginning with "place the cap on the tube". Transfer the supernatant to the test tube containing the supernatant from the first extraction.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

#### Columns

**Guard:** 2.1-mm × 6-cm, 10-μm packing L2

**Analytical:** 3.9-mm × 30-cm; 10-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for clotrimazole related compound A, clotrimazole, and testosterone propionate are 0.9, 1.0, and 1.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between clotrimazole related compound A and clotrimazole, and NLT 1.9 between clotrimazole and testosterone propionate

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole to testosterone propionate from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole to testosterone propionate from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A

**Analysis:** Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the *Assay*, calculate the percentage of clotrimazole related compound A in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole related compound A in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 5%

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **PH** <791>: 5.0–7.0

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature between 2° and 30°.

• **USP REFERENCE STANDARDS** <11>

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

## Clotrimazole Lozenges

» Clotrimazole Lozenges contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in a suitable molded base.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Clotrimazole RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**B:** Place a quantity of finely powdered Lozenges, equivalent to about 50 mg of clotrimazole, into a screw-capped 50-mL test tube, add 20.0 mL of dichloromethane, and mix. Shake by mechanical means for 10 minutes, and allow the suspension to settle. Use the supernatant as the test solution. Separately apply 20 μL of this solution and 20 μL of a Standard solution of USP Clotrimazole RS in dichloromethane containing 2.5 mg per mL to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of diethyl ether and ammonium hydroxide (8:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution. Dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide in 10 mL of dilute hydrochloric acid (1 in 4), dilute with water to 100 mL, mix, and prepare a spray reagent by diluting 10 mL of this solution and 10 mL of dilute hydrochloric acid (1 in 4) with water to 100 mL. Spray the plate with the spray reagent, and visually locate the clotrimazole spots on the plate: the spots are orange.



**Dissolution** <711>—

*Medium:* 0.1 N hydrochloric acid; 500 mL, deaerated.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

Determine the amount of  $C_{22}H_{17}ClN_2$  dissolved by employing the following method.

*25 mM Phosphate buffer*—Dissolve 4.4 g of dibasic potassium phosphate in 1000 mL of water.

*100 mM Phosphate buffer*—Dissolve 17.4 g of dibasic potassium phosphate in 1000 mL of water.

*Diluent*—Prepare a filtered and degassed mixture of methanol and *100 mM Phosphate buffer* (60:40).

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *25 mM Phosphate buffer* (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard solution*—Dissolve an accurately weighed quantity of USP Clotrimazole RS, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a concentration of about 0.02 mg per mL.

*Working standard solution*—Transfer 5.0 mL of the *Standard solution* to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Test solution*—Withdraw 25 mL of the solution under test from the vessel. Pass through a 0.45- $\mu$ m polyvinylidene difluoride filter, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the filtrate to a 25-mL volumetric flask. Dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 3.9-mm  $\times$  7.5-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in percentage, of  $C_{22}H_{17}ClN_2$  released by the formula:

$$\frac{r_U \times C_S \times 500 \times D \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak responses for the *Test solution* and the *Working standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Working standard solution*; 500 is the volume, in mL, of *Medium*;  $D$  is the dilution factor of the *Test solution*; 100 is the conversion factor to percentage; and  $LC$  is the label claim, in mg.

*Tolerances*—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{22}H_{17}ClN_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

*Buffer solution*—Dissolve 1 g of ammonium carbonate in 1000 mL of water. Adjust with 10% sulfuric acid solution to a pH of 6.0.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Internal standard stock solution*—Dissolve an accurately weighed quantity of triphenylmethane in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a concentration of 5 mg per mL.

*Internal standard solution*—Pipet 10.0 mL of *Internal standard stock solution* into a 250-mL volumetric flask, and dilute with *Mobile phase* to volume.

*Standard preparation*—Transfer about 20 mg of USP Clotrimazole RS, accurately weighed, to a 100-mL volumetric flask. Add 4.0 mL of *Internal standard stock solution*, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Weigh and pulverize not fewer than 10 Lozenges. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of clotrimazole, to a 50-mL screw-capped centrifuge tube. Pipet 25 mL of *Internal standard solution* into the tube. Sonicate for 10 minutes, then shake for 10 minutes. Centrifuge at 2500 rpm for 30 minutes. Use the clear supernatant layer as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for clotrimazole and 1.4 for triphenylmethane; the resolution,  $R$ , between clotrimazole and triphenylmethane is not less than 1.5; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Lozenges taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Clotrimazole RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses for clotrimazole to triphenylmethane obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Clotrimazole Topical Solution**


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» Clotrimazole Topical Solution is a solution of Clotrimazole in a suitable nonaqueous, hydrophilic solvent. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**Packaging and storage**—Preserve in tight containers, at a temperature between 2° and 30°.

**USP Reference standards** <11>—

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS  
(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

**Change to read:**

**Identification**—• In a suitable chromatographic chamber, arranged for thin-layer chromatography (see *Chromatography* <621>) and containing 200 mL of ether, place a beaker containing 25 mL of ammonium hydroxide. Cover the chamber, and allow to equilibrate for 2 hours. Transfer a volume of Topical Solution, equivalent to about 10 mg of clotrimazole, to a screw-capped, 50-mL centrifuge tube, and add 5 mL of dilute ammonium hydroxide (1 in 100) and

10 mL of chloroform. Shake vigorously, and centrifuge to obtain a clear chloroform phase. Apply 20  $\mu$ L of the lower chloroform phase and 20  $\mu$ L of a solution of USP Clotrimazole RS in chloroform containing 1 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that obtained from the *Standard solution*. Dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide in 10 mL of dilute hydrochloric acid (1 in 4), dilute with water to 100 mL, mix, and prepare a spray reagent by diluting 10 mL of this solution and 5 mL of dilute hydrochloric acid (1 in 4) with water to 200 mL, and mixing. Spray the plate evenly with this spray reagent: the principal spots from the *Test solution* and the *Standard solution* are orange. • (ERR 1-May-2012)

#### Assay—

**Buffer**—Prepare as directed in the Assay under *Clotrimazole*.

**Mobile phase**—Methanol and Buffer (3:1)

**Internal standard solution**—Transfer about 66 mg of testosterone propionate to a 100-mL volumetric flask, dissolve in 75 mL of methanol, dilute with *Dibasic potassium phosphate solution* to volume, and mix.

**Standard preparation**—Transfer about 50 mg of USP Clotrimazole RS to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Resolution solution**—Prepare a solution of USP Clotrimazole Related Compound A RS in methanol having a concentration of about 0.2 mg per mL. Transfer 12 mL of this solution to a 25-mL volumetric flask, add 4 mL of *Dibasic potassium phosphate solution* and 3 mL of *Standard preparation*, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Topical Solution, equivalent to about 50 mg of clotrimazole, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 2.1-mm  $\times$  6-cm guard column that contains 10- $\mu$ m packing L7 and a 3.9-mm  $\times$  30-cm analytical column that contains 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for clotrimazole related compound A and 1.0 for clotrimazole; and the resolution,  $R$ , between clotrimazole related compound A and clotrimazole is not less than 1.2. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for clotrimazole and 1.5 for testosterone propionate; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in each mL of Topical Solution taken by the formula:

$$50(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Clotrimazole RS in the *Standard preparation*; V is the volume, in mL, of Topical Solution taken; and  $R_U$  and  $R_S$  are the peak response ratios of clotrimazole to testosterone propionate

obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clotrimazole Vaginal Inserts

» Clotrimazole Vaginal Inserts contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards <11>—

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

#### Change to read:

**Identification**—In a suitable chromatographic chamber, arranged for thin-layer chromatography (see *Chromatography* <621>) and containing 200 mL of ether, place a beaker containing 25 mL of ammonium hydroxide. Cover the chamber, and allow to equilibrate for 2 hours. Place an amount of finely powdered Vaginal Inserts, equivalent to about 50 mg of clotrimazole, in a screw-capped, 50-mL centrifuge tube. Add 10 mL of chloroform, and shake vigorously for about 2 minutes. Centrifuge to clarify. [NOTE—The supernatant may remain slightly turbid.] Apply 20  $\mu$ L of the lower chloroform phase and 20  $\mu$ L of a solution of USP Clotrimazole RS in chloroform containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that obtained from the *Standard solution*. Dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide in 10 mL of dilute hydrochloric acid (1 in 4), dilute with water to 100 mL, mix, and prepare a spray reagent by diluting 10 mL of this solution and 5 mL of dilute hydrochloric acid (1 in 4) with water to 200 mL, and mixing. Spray the plate evenly with this spray reagent: the principal spots from the *Test solution* and the *Standard solution* are orange. • (ERR 1-May-2012)

**Disintegration** <701>: 20 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Buffer**—Prepare as directed in the Assay under *Clotrimazole*.

**Mobile phase**—Methanol and Buffer (3:1)

**Internal standard solution**, **Standard preparation**, **Resolution solution**, and **Chromatographic system**—Prepare as directed in the Assay under *Clotrimazole Topical Solution*.

**Assay preparation**—Weigh and finely powder not fewer than 10 Vaginal Inserts. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of clotrimazole, to a 50-mL, screw-capped centrifuge tube, add 10.0 mL of *Internal standard solution* and 15 mL of *Mobile phase*, rotate for 15 minutes, and centrifuge for 10 minutes. Using a suitable syringe, transfer the supernatant to a 100-mL volumetric flask. Rinse the syringe with 25 mL of *Mobile phase*, adding the rinsings to the centrifuge tube.

Rotate the centrifuge tube for 15 minutes, and centrifuge for 10 minutes. Using a suitable syringe, transfer the supernatant to the 100-mL volumetric flask. Rinse the syringe with 25 mL of *Mobile phase*, and add the washings to the 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Vaginal Inserts taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Clotrimazole RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of clotrimazole to testosterone propionate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clotrimazole and Betamethasone Dipropionate Cream

### DEFINITION

Clotrimazole and Betamethasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) and an amount of betamethasone dipropionate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable cream base.

### IDENTIFICATION

- A.** The retention times of the major peaks for clotrimazole and betamethasone dipropionate of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay* for clotrimazole and betamethasone.

### ASSAY

#### PROCEDURE

**Buffer:** 6.6 g/L of dibasic ammonium phosphate in water

**Mobile phase:** Prepare a mixture of methanol and *Buffer* (7:3), and adjust with phosphoric acid to a pH of  $7.0 \pm 0.2$ . Pass through a membrane filter having a 0.45- $\mu$ m or finer pore size, and degas.

**Internal standard solution:** 0.15 mg/mL of progesterone in alcohol

**Clotrimazole stock solution:** 5 mg/mL of USP Clotrimazole RS in alcohol

**Betamethasone dipropionate stock solution:** 6.4/ mg/mL of USP Betamethasone Dipropionate RS in alcohol,  $I$  being the ratio (in mg/g) of betamethasone to clotrimazole in the Cream

**Clotrimazole related compound A stock solution:** 0.5 mg/mL of USP Clotrimazole Related Compound A RS in methanol

**Standard solution:** Transfer 1.0 mL of *Clotrimazole related compound A stock solution* to a suitable container, and evaporate to dryness in a water bath at room temperature under a stream of nitrogen. To the residue add 2.0 mL each of *Clotrimazole stock solution*, *Betamethasone dipropionate stock solution*, and *Internal standard solution*.

**Sample solution:** Weigh a portion of Cream equivalent to 10 mg of clotrimazole, and transfer to a screw-capped, 50-mL centrifuge tube. Add 2.0 mL of *Internal standard solution* and 4.0 mL of alcohol, place the cap on the tube, and heat at  $60^\circ$  in a water bath for 10 min, with occasional shaking. Remove the tube from the bath, cool in an ice bath for 20 min, and promptly

centrifuge. Transfer a portion of the supernatant to a test tube, and use as the *Sample solution*.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.7 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for betamethasone dipropionate, clotrimazole related compound A, progesterone, and clotrimazole are about 1.0, 1.2, 1.4, and 1.7, respectively.]

### Suitability requirements

**Resolution:** NLT 1.0 between betamethasone dipropionate and clotrimazole related compound A, NLT 1.5 between clotrimazole related compound A and progesterone, and NLT 1.8 between progesterone and clotrimazole

**Relative standard deviation:** NMT 2.0% determined from clotrimazole and betamethasone dipropionate and NMT 4.0% determined from clotrimazole related compound A

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Cream taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole to progesterone from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole to progesterone from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Clotrimazole stock solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Cream taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times (M_{r1} / M_{r2}) \times 100$$

$R_U$  = peak response ratio of betamethasone dipropionate to progesterone from the *Sample solution*

$R_S$  = peak response ratio of betamethasone dipropionate to progesterone from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Betamethasone dipropionate stock solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betamethasone, 392.46

$M_{r2}$  = molecular weight of betamethasone dipropionate, 504.60

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ); 90.0%–110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ )

### IMPURITIES

#### ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A

**Buffer, Mobile phase, Internal standard solution, Clotrimazole stock solution, Betamethasone dipropionate stock solution, Clotrimazole related compound A stock solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Use as directed in the *Assay*.

**Analysis:** Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the *Assay*, calcu-

late the percentage of clotrimazole related compound A in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = peak response ratio of clotrimazole related compound A to progesterone from the *Sample solution*  
 $R_S$  = peak response ratio of clotrimazole related compound A to progesterone from the *Standard solution*  
 $C_S$  = concentration of USP Clotrimazole Related Compound A RS in the *Clotrimazole related compound A stock solution* (mg/mL)  
 $C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)  
**Acceptance criteria:** NMT 5.0%

## PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

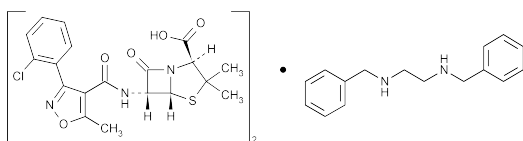
## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Betamethasone Dipropionate RS
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS
  - (*o*-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78

## Cloxacillin Benzathine



$(C_{19}H_{18}ClN_3O_5S)_2 \cdot C_{16}H_{20}N_2$  1112.11

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]-amino]-3,3-dimethyl-7-oxo-, [2*S*-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-, compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1). (2*S*,5*R*,6*R*)-6-[3-(*o*-Chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1) [23736-58-5].

» Cloxacillin Benzathine has a potency equivalent to not less than 704  $\mu$ g and not more than 821  $\mu$ g of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards** (11)—

USP Cloxacillin Benzathine RS  
 USP Cloxacillin Sodium RS

## Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

**Solutions:** Obtain the test solution as follows. To about 20 mg in a 50-mL conical flask, add 5 mL of 5 N sodium hydroxide, and heat on a steam bath for 20 minutes. Cool, transfer 1 mL of this solution to a separator containing 10 mL of 1.2 N sulfuric acid, and extract with 50 mL of ether. Wash the ether extract with 30 mL of water, and extract the ether layer with 50 mL of 0.1 N sodium hydroxide. Similarly prepare the Standard solution from about 15 mg of USP Cloxacillin Sodium RS.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 3.0 and 6.5, in a suspension containing 10 mg per mL.

**Sterility** (71)—Where the label states that Cloxacillin Benzathine is sterile, it meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, to use Soybean-Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and to shake the tubes once daily.

**Water, Method I** (921): not more than 5.0%.

## Assay—

**0.1 M Phosphate buffer**—Dissolve 55.2 g of monobasic sodium phosphate in water, and dilute with water to 4 L.

**Mobile phase**—Combine 1000 mL of acetonitrile and 3000 mL of 0.1 M Phosphate buffer. Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of  $4.6 \pm 0.2$ . Pass through a 0.45- $\mu$ m nylon filter, and degas. [NOTE—The retention time of cloxacillin is very sensitive to the acetonitrile content of the *Mobile phase*.]

**Diluent**—Transfer 13.8 g of monobasic sodium phosphate to a 2-L volumetric flask, mix, and dilute with water to volume. Combine 1800 mL of the resulting solution with 1200 mL of acetonitrile. Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.4.

**Standard preparations**—In duplicate, dissolve an accurately weighed quantity of USP Cloxacillin Sodium RS in *Diluent* to obtain solutions having known concentrations of about 112  $\mu$ g per mL.

**Assay preparations**—In duplicate, dissolve an accurately weighed quantity of Cloxacillin Benzathine in *Diluent* to obtain solutions having concentrations of about 128  $\mu$ g per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute and the column temperature is 40°. Chromatograph the *Standard preparations*, and record the peak areas as directed for *Procedure*: the tailing factor is less than 2.0; the peak areas of the two *Standard preparations* agree within 98% to 102%; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparations* and the *Assay preparations* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu$ g, of  $C_{19}H_{18}ClN_3O_5S$  in each mg of Cloxacillin Benzathine taken by the formula:

$$P(C_S / C_U)(r_U / r_S)$$

in which *P* is the assigned potency, in  $\mu$ g of cloxacillin per mg, of USP Cloxacillin Sodium RS;  $C_S$  and  $C_U$  are the concentrations, in  $\mu$ g per mL, of cloxacillin sodium and cloxacil-

lin benzathine in the *Standard preparations* and the *Assay preparations*, respectively; and  $r_U$  and  $r_S$  are the average peak areas of the cloxacillin peaks obtained from the *Assay preparations* and the *Standard preparations*, respectively.

## Cloxacillin Benzathine Intramammary Infusion

» Cloxacillin Benzathine Intramammary Infusion is a suspension of Cloxacillin Benzathine in a suitable oil vehicle. It has a potency equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

**Packaging and storage**—Preserve in disposable syringes that are well-closed containers, except that where the Intramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.

**Labeling**—Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.

### USP Reference standards (11)—

USP Cloxacillin Benzathine RS

USP Cloxacillin Sodium RS

**Identification, Infrared Absorption** (197K)—Obtain the test specimen as follows. Transfer a quantity of Intramammary Infusion, equivalent to about 500 mg of cloxacillin, to a 50-mL centrifuge tube, add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for about 30 seconds after each addition of toluene. Dry the residue in vacuum over silica gel.

**Sterility** (71) (where labeled as being sterile)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and to shake the tubes once daily.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

### Assay—

0.1M Phosphate buffer, Mobile phase, Diluent, *Standard preparations*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cloxacillin Benzathine*.

**Assay preparations**—In duplicate, quantitatively express the entire contents of a syringe of Cloxacillin Benzathine Intramammary Infusion into a 500-mL volumetric flask. Add about 300 mL of methanol, and stir for 45 minutes  $\pm$  1 minute. Dilute with methanol to volume, and stir for an additional 10 minutes  $\pm$  1 minute. Immediately transfer 45 mL of the resulting solution to a 50-mL polypropylene centrifuge tube, and centrifuge for 10 minutes. From the supernatant remove an aliquot, and dilute with a sufficient volume of *Diluent* to prepare a solution containing about 100  $\mu$ g of cloxacillin per mL.

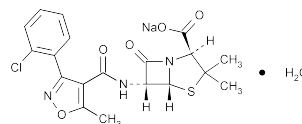
**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparations* and the *Assay preparations* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

$\mu$ g, of  $C_{19}H_{18}ClN_3O_5S$  in each syringe of Cloxacillin Benzathine Intramammary Infusion taken by the formula:

$$P(CD/1000)(r_U / r_S)$$

in which  $P$  is the assigned potency, in  $\mu$ g of cloxacillin per mg, of USP Cloxacillin Sodium RS;  $C$  is the concentration, in  $\mu$ g per mL, of cloxacillin sodium in the *Standard preparations*;  $D$  is the dilution factor used in preparing the *Assay preparations*; and  $r_U$  and  $r_S$  are the average peak areas of the cloxacillin peaks obtained from the *Assay preparations* and the *Standard preparations*, respectively.

## Cloxacillin Sodium



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$  475.88

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbon-yl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2S-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )].

Monosodium (2S,5R,6R)-6-[3-(o-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7081-44-9].

Anhydrous 457.87 [642-78-4].

» Cloxacillin Sodium contains the equivalent of not less than 825  $\mu$ g of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) per mg.

**Packaging and storage**—Preserve in tight containers, and store at a temperature not exceeding 25°.

**Labeling**—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

### USP Reference standards (11)—

USP Cloxacillin Sodium RS

### Identification—

**A: Infrared Absorption** (197K).

**B:** It responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**Sterility** (71)—Where the label states that Cloxacillin Sodium is sterile, it meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and to shake the tubes once daily.

**pH** (791): between 4.5 and 7.5, in a solution containing 10 mg per mL.

**Water, Method I** (921): between 3.0% and 5.0%.

**Dimethylaniline** (223): meets the requirement.

### Assay—

**Buffer**—Prepare a 0.02 M solution of monobasic potassium phosphate in water, and adjust with 2 N sodium hydroxide to a pH of 6.8.

**Mobile phase**—Prepare a mixture of *Buffer* and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Prepare a solution of USP Cloxacillin Sodium RS in *Buffer* having a known concentration of about 0.55 mg per mL.

**Assay preparation**—Transfer about 110 mg of Cloxacillin Sodium, accurately weighed, to a 200-mL volumetric flask, dilute with *Buffer* to volume, and mix. Stir with the aid of a magnetic stirrer for 5 minutes to dissolve.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) in each mg of Cloxacillin Sodium taken by the formula:

$$200(CE / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cloxacillin Sodium RS in the *Standard preparation*; E is the cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) equivalent, in µg per mg, of USP Cloxacillin Sodium RS; W is the weight, in mg, of Cloxacillin Sodium taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cloxacillin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cloxacillin Sodium Capsules

» Cloxacillin Sodium Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—  
USP Cloxacillin Sodium RS

**Dissolution** <711>—

*Medium*: 0.05 M pH 6.8 potassium phosphate buffer; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) dissolved by employing the method set forth in the *Assay*, in comparison with a *Standard solution* having a known concentration of USP Cloxacillin Sodium RS in the *Dissolution Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Water, Method I** <921>: not more than 5.0%.

**Assay**—

*Buffer, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cloxacillin Sodium*.

**Assay preparation**—Weigh and finely powder the contents of not fewer than 10 Capsules. Transfer an accurately weighed quantity of the powder, equivalent to about

100 mg of cloxacillin, to a 200-mL volumetric flask, dilute with *Buffer* to volume, mix, and stir for 10 minutes. Filter about 25 mL of this mixture, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cloxacillin Sodium*. Calculate the quantity, in mg, of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) in the portion of Capsule contents taken by the formula:

$$0.2CE(r_U / r_S)$$

in which the terms are as defined therein.

## Cloxacillin Sodium Intramammary Infusion

» Cloxacillin Sodium Intramammary Infusion is a suspension of Cloxacillin Sodium in a suitable natural or chemically modified vegetable oil vehicle with a suitable dispersing agent. It has a potency equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in disposable syringes that are well-closed containers, except that where the Intramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.

**USP Reference standards** <11>—  
USP Cloxacillin Sodium RS

**Labeling**—Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.

**Identification, Infrared Absorption** <197K>—Obtain the test specimen as follows. Transfer a quantity of Intramammary Infusion, equivalent to about 500 mg of cloxacillin, to a 50-mL centrifuge tube, add 15 mL of isooctane, mix, and centrifuge. Decant and discard the isooctane. Wash the residue with two 15-mL portions of isooctane and two 15-mL portions of ethyl ether, and discard the washings. Dry the residue in a current of air.

**Sterility** <71> (where labeled as being sterile)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, to use Soybean-Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and to shake the tubes once daily.

**Water, Method I** <921>: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed for cloxacillin under *Antibiotics—Microbial Assays* <81>, expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer No. 1* and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer No. 1* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the *Standard*.

## Cloxacillin Sodium for Oral Solution

» Cloxacillin Sodium for Oral Solution is a dry mixture of Cloxacillin Sodium and one or more suitable buffers, colors, flavors, and preservatives. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cloxacillin Sodium RS

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 7.5, in the solution constituted as directed in the labeling.

**Water, Method I** (921): not more than 1.0%.

**Assay**—

*Buffer, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Cloxacillin Sodium.

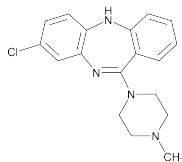
*Assay preparation*—Constitute Cloxacillin Sodium for Oral Solution as directed in the labeling. Transfer an accurately measured volume of the resulting solution, equivalent to about 125 mg of cloxacillin, to a 250-mL volumetric flask, dilute with *Buffer* to volume, mix, and stir for 15 minutes. Filter about 25 mL of this mixture, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Cloxacillin Sodium. Calculate the quantity, in mg, of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) in the portion of Cloxacillin Sodium for Oral Solution taken by the formula:

$$0.25CE(r_U / r_S)$$

in which the terms are as defined therein.

## Clozapine



$C_{18}H_{19}ClN_4$  326.82  
5*H*-Dibenzo[*b,e*][1,4]diazepine, 8-chloro-11-(4-methyl-1-piperazinyl)-;  
8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine [5786-21-0].

### DEFINITION

Clozapine contains NLT 98.0% and NMT 102.0% of clozapine ( $C_{18}H_{19}ClN_4$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol, triethylamine, and water (800: 0.75: 200)

**Diluent:** Methanol and water (80:20)

**Standard solution:** 0.1 mg/mL of USP Clozapine RS in *Diluent*

**System suitability stock solution:** Transfer 10 mg of USP Clozapine RS to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

**System suitability solution:** *Standard solution* and *System suitability stock solution* (1:1)

**Sample solution:** 0.1 mg/mL of Clozapine in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

**Run time:** 3 times the retention time of clozapine

**System suitability**

**Samples:** *Standard solution* and *System Suitability solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between clozapine and any other peak, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clozapine ( $C_{18}H_{19}ClN_4$ ) in the portion of Clozapine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of clozapine from the *Sample solution*

$r_S$  = peak response of USP Clozapine RS from the *Standard solution*

$C_S$  = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clozapine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS, Method II** (231): 20 ppm

#### • ORGANIC IMPURITIES

**Diluent:** Methanol and water (80:20)

**Buffer:** 2.0 g/L of monobasic potassium phosphate. Adjust with phosphoric acid (85%) to a pH of 2.4.

[NOTE—The pH of this solution must not be below 2.4.]

**Solution A:** Filtered and degassed mixture of acetonitrile, methanol, and *Buffer* (1:1:8)

**Solution B:** Filtered and degassed mixture of acetonitrile, methanol, and *Buffer* (4:4:2)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
24	0	100
29	0	100
40	100	0

**System suitability solution:** Dissolve 4 mg of USP Clozapine Resolution Mixture RS in 4 mL of methanol, add 1 mL of water, and dilute with *Diluent* to 10 mL.

**Standard solution:** 0.75 µg/mL of USP Clozapine RS in *Diluent*

**Sample solution:** 0.75 mg/mL prepared as follows. Transfer a suitable quantity of Clozapine to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are provided in *Table 2*.]

#### Suitability requirements

**Resolution:** NLT 2.5 between *Impurity C* and clozapine, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for clozapine, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Disregard any peak with an area less than 0.5 times the area of the clozapine peak from the *Standard solution*.]

Calculate the percentage of each related compound and any unknown impurity in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of any impurity from the *Sample solution*

$r_S$  = peak response of clozapine from the *Standard solution*

$C_S$  = concentration of USP Clozapine RS from the *Standard solution* (mg/mL)

$C_U$  = concentration of Clozapine from the *Sample solution* (mg/mL)

$F$  = relative response factor of the impurity (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity C <sup>a</sup>	0.9	1.0	0.3
Clozapine	1.0	—	—
Impurity D <sup>b</sup>	1.1	0.35	0.2
Impurity A <sup>c</sup>	1.6	1.2	0.1
Impurity B <sup>d</sup>	1.7	1.0	0.2
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

<sup>a</sup> 8-Chloro-11-(piperazin-1-yl)-5H-dibenzo[*b,e*][1,4]diazepine.

<sup>b</sup> 4-Chloro-*N*¹-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.

<sup>c</sup> 8-Chloro-5,10-dihydro-11H-dibenzo[*b,e*][1,4]diazepin-11-one.

<sup>d</sup> 11,11'-(Piperazine-1,4-diyl)bis(8-chloro-5H-dibenzo[*b,e*][1,4]diazepine).

#### SPECIFIC TESTS

- LOSS ON DRYING (731):** Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### USP REFERENCE STANDARDS (11)

USP Clozapine RS

USP Clozapine Resolution Mixture RS

Contains the following components:

Clozapine.

Impurity A: 8-chloro-5,10-dihydro-11H-dibenzo[*b,e*][1,4]diazepin-11-one.

Impurity B: 11,11'-(piperazine-1,4-diyl)bis(8-chloro-5H-dibenzo[*b,e*][1,4]diazepine).

Impurity C: 8-chloro-11-(piperazin-1-yl)-5H-dibenzo[*b,e*][1,4]diazepine.

Impurity D: 4-chloro-*N*¹-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.

## Clozapine Tablets

#### DEFINITION

Clozapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clozapine (C<sub>18</sub>H<sub>19</sub>ClN<sub>4</sub>).

#### IDENTIFICATION

- A.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to those of the principal spots of the *Standard solutions*, as obtained in the test for *Organic Impurities*.
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Methanol, triethylamine, and water (800:0.75:200)

**Standard solution:** 0.125 mg/mL of USP Clozapine RS prepared as follows. Transfer the required amount of USP Clozapine RS to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol. [NOTE—Dissolve the Reference Standard in methanol, and dilute with water to obtain the final concentration. The final solvent composition of methanol and water is about 8:2.]

**System suitability stock solution:** Transfer 10 mg of clozapine to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

**System suitability solution:** *Standard solution* and *System suitability stock solution* (1:1)

**Sample solution:** Transfer 125 mg of clozapine from a quantity of finely powdered Tablets (NLT 20) to a 1-L volumetric flask. Dissolve in 640 mL of methanol, sonicate for 10 min, dilute with water to volume, mix, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.0-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the clozapine peak and any other peak, *System suitability solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*



**Relative standard deviation:** NMT 2.0% for replicate injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of clozapine ( $C_{18}H_{19}ClN_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clozapine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** pH 4.0 acetate buffer prepared as follows. Dissolve 2 g of sodium hydroxide in 450 mL of water. Adjust with glacial acetic acid to a pH of 4.0. Dilute to 1 L; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:** USP Clozapine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

##### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** 290 nm

##### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of clozapine ( $C_{18}H_{19}ClN_4$ ) dissolved.

**Tolerances:** NLT 85% (Q) of the labeled amount of clozapine ( $C_{18}H_{19}ClN_4$ ) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent:** Chloroform and methanol (4:1)

**Standard stock solution:** 5.0 mg/mL of USP Clozapine RS in *Diluent*

**Standard solutions:** Dilute portions of the *Standard stock solution* with *Diluent* to obtain the following solutions.

Standard solution	Dilution	Concentration ( $\mu\text{g/mL}$ of RS)	Percentage (for comparison with Sample)
A	1 in 200	25	0.5
B	1 in 250	20	0.4
C	1 in 333	15	0.3
D	1 in 500	10	0.2
E	1 in 1000	5	0.1

**Sample solution:** Transfer an equivalent to 125 mg of clozapine from a portion of finely powdered Tablets (NLT 20) to a 25-mL volumetric flask. Dissolve in 20 mL of *Diluent*, shake by mechanical means for 15 min, dilute with *Diluent* to volume, and filter.

##### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20  $\mu\text{L}$

**Developing solvent system:** *n*-Heptane, chloroform, dehydrated alcohol, and ammonium hydroxide (30:30:30:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots of the *Sample solution* with those of the principal spots of the *Standard solutions*.

**Acceptance criteria:** No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (NMT 0.5%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 2.0%.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Clozapine RS

## Coal Tar

» Coal Tar is the tar obtained as a by-product during the destructive distillation of bituminous coal at temperatures in the range of 900° to 1100°. It may be processed further either by extraction with alcohol and suitable dispersing agents and maceration times or by fractional distillation with or without the use of suitable organic solvents.

**Packaging and storage**—Preserve in tight containers.

**Residue on ignition** <281>: not more than 2.0%, from 100 mg.

## Coal Tar Ointment

» Prepare Coal Tar Ointment as follows.

Coal Tar . . . . .	10 g
Polysorbate 80 . . . . .	5 g
Zinc Oxide Paste . . . . .	985 g
to make . . . . .	1000 g

Blend the Coal Tar with the Polysorbate 80, and incorporate the mixture with the Zinc Oxide Paste.

**Packaging and storage**—Preserve in tight containers.

## Coal Tar Topical Solution

» Prepare Coal Tar Topical Solution as follows.

Coal Tar .....	200 g
Polysorbate 80 .....	50 g
Alcohol, a sufficient quantity, to make .....	1000 mL

Mix the Coal Tar with 500 g of washed sand (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and add the Polysorbate 80 and 700 mL of Alcohol. Macerate the mixture for 7 days in a closed vessel with frequent agitation. Filter, and rinse the vessel and the filter with sufficient Alcohol to make the product measure 1000 mL.

**Packaging and storage**—Preserve in tight containers.

**Alcohol content** (611): between 81.0% and 86.0% of  $C_2H_5OH$ .

## Cyanocobalamin Co 57 Capsules

Vitamin  $B_{12-^{57}Co}$ .

Vitamin  $B_{12-^{57}Co}$  [41559-38-0; 13115-03-2].

» Cyanocobalamin Co 57 Capsules contain Cyanocobalamin in which a portion of the molecules contain radioactive cobalt ( $^{57}Co$ ) in the molecular structure. Each Capsule contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{57}Co$  as cyanocobalamin expressed in megabecquerels (microcuries) at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount.

**Specific activity:** not less than 0.02 MBq (0.5  $\mu Ci$ ) per  $\mu g$  of cyanocobalamin.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store in a cold place.

**Labeling**—Label the Capsules to include the following: the date of calibration; the amount of cyanocobalamin expressed in  $\mu g$  per Capsule; the amount of  $^{57}Co$  as cyanocobalamin expressed in megabecquerels (microcuries) per Capsule at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{57}Co$  is 270.9 days.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

**Radionuclide identification**—A solution of 1 or more Capsules in water responds to the test for *Radionuclide identification* under *Cyanocobalamin Co 57 Oral Solution*.

**Disintegration** (701): 30 minutes, testing 1 Capsule in 1 N hydrochloric acid maintained at  $37 \pm 2^\circ$  as the immersion fluid.

**Uniformity of dosage units:** meet the requirements.

**Procedure for content uniformity**—Determine the instrument response of each of 10 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the average radioactivity per Capsule. The radioactivity of none of the Capsules differs by more than 10% from the average. The relative standard deviation is less than 3.5%.

**Radiochemical purity**—Dissolve the contents of 1 Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant as the *Test solution*. It meets the requirements of the test for *Radiochemical purity* under *Cyanocobalamin Co 57 Oral Solution*.

**Radionuclidic purity**—Dissolve the contents of 1 Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant. It meets the requirements of the test for *Radionuclidic purity* under *Cyanocobalamin Co 57 Oral Solution*.

**Content of cyanocobalamin**—Determine the content, in  $\mu g$  per Capsule, of cyanocobalamin as directed under *Vitamin  $B_{12}$  Activity Assay* (171).

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu Ci$ ) per Capsule, of Cyanocobalamin Co 57 Capsules by use of a calibrated system as directed under *Radioactivity* (821).

## Cyanocobalamin Co 57 Oral Solution

Vitamin  $B_{12-^{57}Co}$ .

Vitamin  $B_{12-^{57}Co}$  [41559-38-0; 13115-03-2].

» Cyanocobalamin Co 57 Oral Solution is a solution suitable for oral administration, containing Cyanocobalamin in which a portion of the molecules contain radioactive cobalt ( $^{57}Co$ ) in the molecular structure. Cyanocobalamin Co 57 Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{57}Co$  as cyanocobalamin expressed in megabecquerels (microcuries) per mL at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. Cyanocobalamin Co 57 Oral Solution contains a suitable antimicrobial agent.

**Specific activity:** not less than 0.02 MBq (0.5  $\mu Ci$ ) per  $\mu g$  of cyanocobalamin.

**Packaging and storage**—Preserve in tight containers, protected from light, and store in a cold place.

**Labeling**—Label it to include the following: the date of calibration; the amount of  $^{57}Co$  as cyanocobalamin expressed as total megabecquerels (microcuries) and as megabecquerels (microcuries) per mL at the time of calibration; the amount of cyanocobalamin expressed in  $\mu g$  per mL; the name and quantity of the added preservative; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{57}Co$  is 270.9 days, and directs that the Oral Solution be protected from light.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of

$^{57}\text{Co}$  of known purity that exhibits a major photopeak having an energy of 0.122 MeV.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 5.5.

**Radiochemical purity**—

*Mobile phase*—Prepare a solution of 10.0 g of dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Prepare a mixture of this solution and methanol (73.5:26.5), mix, and degas. Use within 2 days.

*Test solution*—Use the Oral Solution.

*Standard solution*—Transfer about 10 mg of cyanocobalamin, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 361-nm detector, a gamma detector adjusted for  $^{57}\text{Co}$  and a 4.6-mm  $\times$  25-cm stainless steel column that contains 5- $\mu\text{m}$  packing L7. The flow rate is about 1 mL per minute.

*Procedure*—Inject about 100  $\mu\text{L}$  of the *Standard solution* into the chromatograph, record the chromatogram for 30 minutes, and note the retention time of the cyanocobalamin peak. Inject 100  $\mu\text{L}$  of the *Test solution* into the chromatograph, and record the chromatogram for three times the retention time of cyanocobalamin. Measure the peak areas using the gamma detector, and calculate the percentage of cyanocobalamin present as cyanocobalamin  $^{57}\text{Co}$  in the portion of Oral Solution taken by the formula:

$$100(r_U / r_T)$$

in which  $r_U$  is the peak response for cyanocobalamin  $^{57}\text{Co}$  obtained from the *Test solution*; and  $r_T$  is the total of all the peak area responses in the radiochromatogram obtained from the *Test solution*. Not less than 90% of the total radioactivity is found as cyanocobalamin  $^{57}\text{Co}$ .

**Radionuclidic purity**—Using a suitable calibrated instrument (see *Radioactivity* (821)) and standardized solutions of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$ , record the gamma spectrum of the Oral Solution. The spectrum does not differ significantly from that of the standardized  $^{57}\text{Co}$  solution. Determine the relative amounts of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$  present. Cobalt 58 has a half-life of 70.9 days, and its presence is shown by 0.511-MeV and 0.811-MeV gamma photons. Cobalt 60 has a half-life of 5.27 years, and its presence is shown by 1.173-MeV and 1.333-MeV gamma photons. Not more than 1% of the total radioactivity is due to  $^{60}\text{Co}$ ; and not more than 2% of the total radioactivity is due to  $^{58}\text{Co}$ ,  $^{60}\text{Co}$ , and other radionuclidic impurities.

**Content of cyanocobalamin**—Determine the content, in  $\mu\text{g}$  per mL, of cyanocobalamin as directed under *Vitamin B<sub>12</sub> Activity Assay* (171).

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly under Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Oral Solution by use of a calibrated system as directed under *Radioactivity* (821).

## Cyanocobalamin Co 58 Capsules

Vitamin B<sub>12</sub>-  $^{58}\text{Co}$

» Cyanocobalamin Co 58 Capsules contain Cyanocobalamin in which a portion of the molecules contain radioactive cobalt ( $^{58}\text{Co}$ ) in the molecular structure. Each Capsule contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{58}\text{Co}$  as cyanocobalamin expressed in megabecquerels (or microcuries) at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount.

**Specific activity:** not less than 0.02 MBq (or 0.5  $\mu\text{Ci}$ ) per  $\mu\text{g}$  of cyanocobalamin.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store in a cold place.

**Labeling**—Label it to include the following: the date of calibration; the amount of cyanocobalamin expressed in  $\mu\text{g}$  per Capsule; the amount of  $^{58}\text{Co}$  as cyanocobalamin expressed in MBq (or  $\mu\text{Ci}$ ) per Capsule at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{58}\text{Co}$  is 70.9 days.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

**Disintegration** (701): 30 minutes, testing one Capsule in 1 N hydrochloric acid maintained at  $37 \pm 2^\circ$  as the immersion fluid.

**Radionuclide identification** (821)—

**A:** Its gamma-ray spectrum is identical to that of a specimen of  $^{58}\text{Co}$  that exhibits major photopeaks at 0.511 MeV (annihilation radiation) and 0.811 MeV.

**B:** The retention time of the major peak in the radiochromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Radiochemical purity*.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Determine the instrument response of each of 10 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the average radioactivity per Capsule. The radioactivities of none of the Capsules differ by more than 10% from the average. The relative standard deviation is less than 3.5%.

**Radiochemical purity**—

*Mobile phase*—Prepare a solution of 10.0 g of dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Prepare a mixture of the solution so obtained and methanol (73.5:26.5), mix, and degas. Use within 2 days. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard solution*—Transfer about 10 mg of USP Cyanocobalamin RS, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Test solution*—Dissolve the contents of one Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 361-nm detector,

a gamma detector adjusted for  $^{58}\text{Co}$ , and a 4.6-mm  $\times$  25-cm stainless steel column that contains 5- $\mu\text{m}$  packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*.

**Procedure**—Inject about 100  $\mu\text{L}$  of the *Standard solution* into the chromatograph, record the chromatogram for 30 minutes, and note the retention time of the cyanocobalamin peak. Inject 100  $\mu\text{L}$  of the *Test solution* into the chromatograph, record the chromatogram for three times the retention time of cyanocobalamin, and measure the peak areas using the gamma detector. Calculate the percentage of cyanocobalamin present as cyanocobalamin  $^{58}\text{Co}$  in the portion of Capsules taken by the formula:

$$100(r_U / r_S)$$

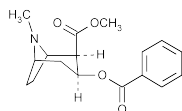
in which  $r_U$  is the peak area for cyanocobalamin  $^{58}\text{Co}$  obtained from the *Test solution*; and  $r_S$  is the sum of all the peak areas in the radiochromatogram obtained from the *Test solution*: not less than 90% of the total radioactivity is found as cyanocobalamin  $^{58}\text{Co}$ .

**Radionuclidic purity**—Using a suitable, calibrated instrument (see *Radioactivity* <821>) and standardized solutions of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$ , record the gamma spectrum. The spectrum does not differ significantly from that of the standardized  $^{58}\text{Co}$  solution. Determine the relative amounts of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$  present. Cobalt 57 has a half-life of 270.9 days, and its presence is shown by 0.122 MeV gamma photons. Cobalt 60 has a half-life of 5.27 years and its presence is shown by 1.173 MeV and 1.333 MeV gamma photons. Not more than 1% of the total radioactivity is due to  $^{60}\text{Co}$ ; and not more than 2% of the total radioactivity is due to  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ , and other radionuclidic impurities.

**Content of cyanocobalamin**—Determine the content, in  $\mu\text{g}$  per Capsule, of cyanocobalamin as directed under *Vitamin B<sub>12</sub> Activity Assay* <171>.

**Assay for radioactivity** <821>—Using a suitable counting assembly (see *Selection of a Counting Assembly*) and calibrated system, determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ) per Capsule, of Cyanocobalamin Co 58 Capsules.

## Cocaine



$\text{C}_{17}\text{H}_{21}\text{NO}_4$  303.35

8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, [1*R*-(*exo*,*exo*)]-.

Methyl 3 $\beta$ -hydroxy-1 $\alpha$ H,5 $\alpha$ H-tropane-2 $\beta$ -carboxylate benzoate (ester) [50-36-2].

» Cocaine, dried over phosphorus pentoxide for 3 hours, contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_{17}\text{H}_{21}\text{NO}_4$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** <11>—  
USP Cocaine Hydrochloride RS

### Identification—

**A: Ultraviolet Absorption** <197U>—

*Solution:* 15  $\mu\text{g}$  per mL.

*Medium:* dilute hydrochloric acid (1 in 120).

Absorptivities at 233 nm, calculated on the dried basis, do not differ by more than 3.0%.

**B:** It meets the requirements under *Identification—Organic Nitrogenous Bases* <181>, USP Cocaine Hydrochloride RS being used, and sodium carbonate TS being used in place of sodium hydroxide TS.

**C:** Dissolve about 100 mg in a mixture of 0.4 mL of dilute hydrochloric acid (1 in 12) and water to make 5 mL, and add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken. Add 1 mL of hydrochloric acid: a permanent, orange-colored, crystalline precipitate is formed.

**D:** Dissolve about 10 mg in 1 mL of dilute hydrochloric acid (1 in 600), and evaporate on a steam bath just to dryness. Dissolve the residue in 2 drops of water, and add 1 mL of potassium permanganate solution (1 in 300): a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic violet-red crystalline aggregates under the low power of a microscope, similar to those obtained from USP Cocaine Hydrochloride RS.

**Melting range, Class I** <741>: between 96° and 98°.

**Loss on drying** <731>—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Readily carbonizable substances** <271>—Dissolve about 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

**Limit of cinnamyl-cocaine and other reducing substances**—Dissolve about 300 mg of finely powdered Cocaine in 1 mL of dilute hydrochloric acid (1 in 12) with the aid of heat, if necessary, and dilute with water to 15 mL. Mix 5 mL of this solution with 0.3 mL of dilute sulfuric acid (1 in 35) and 0.1 mL of potassium permanganate solution (1 in 300): the violet color does not disappear entirely within 30 minutes.

**Limit of isotropyl-cocaine**—Dilute in a beaker 5 mL of the solution of Cocaine prepared in the test for *Cinnamyl-cocaine and other reducing substances* with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, and stir the solution vigorously for 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.

**Assay**—Dissolve about 600 mg of Cocaine, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.34 mg of  $\text{C}_{17}\text{H}_{21}\text{NO}_4$ .

## Cocaine Hydrochloride

$\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HCl}$  339.81

8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, hydrochloride, 1*R*-(*exo*,*exo*)-.

Methyl 3 $\beta$ -hydroxy-1 $\alpha$ H,5 $\alpha$ H-tropane-2 $\beta$ -carboxylate, benzoate (ester) hydrochloride [53-21-4].

» Cocaine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** <11>—  
USP Cocaine Hydrochloride RS

**Identification—**

**A:** It meets the requirements under *Identification—Organic Nitrogenous Bases* (181), sodium carbonate TS being used in place of 1 N sodium hydroxide.

**B:** To 5 mL of a solution (1 in 50) add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken gently. Add 1 mL of hydrochloric acid: a permanent, orange-colored crystalline precipitate is formed.

**C:** To a solution of about 10 mg in 2 drops of water add 1 mL of 0.1 N potassium permanganate: a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic, violet-red crystalline aggregates under the low power of a microscope.

**D:** It responds to the tests for *Chloride* (191).

**Specific rotation** (7815): between  $-71^{\circ}$  and  $-73^{\circ}$ .

*Test solution:* 20 mg, previously dried, per mL, in water.

**Acidity—**Dissolve 500 mg in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required to produce a yellow color.

**Loss on drying** (731)—Dry it over silica gel for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Readily carbonizable substances** (271)—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid F*.

**Limit of cinnamyl-cocaine and other reducing substances—**To 5 mL of a solution (1 in 50) add 0.3 mL of 1 N sulfuric acid and 0.10 mL of 0.10 N potassium permanganate: the violet color does not disappear entirely within 30 minutes.

**Limit of isotropyl-cocaine—**Dilute 5 mL of a solution (1 in 50) in a beaker with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, stir the solution vigorously during 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.

**Assay—**Dissolve about 500 mg of Cocaine Hydrochloride, accurately weighed, in a mixture of 40 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 2 drops of quinaldine red TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.98 mg of  $C_{17}H_{21}NO_4 \cdot HCl$ .

## Cocaine Hydrochloride Tablets for Topical Solution

» Cocaine Hydrochloride Tablets for Topical Solution contain not less than 91.0 percent and not more than 109.0 percent of the labeled amount of  $C_{17}H_{21}NO_4 \cdot HCl$ .

**Packaging and storage—**Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—  
USP Cocaine Hydrochloride RS

**Identification—**

**A:** Add 5 drops of chromium trioxide solution (1 in 20) to 5 mL of a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 50): a yellow precipitate is formed and it redissolves when the mixture is shaken. On the addition of 1 mL of hydrochloric acid, a permanent, yellowish orange, crystalline precipitate is formed.

**B:** Dissolve a portion of powdered Tablets, equivalent to about 10 mg of cocaine hydrochloride, in 1 mL of water, filter, and add 2 mL of 0.1 N potassium permanganate: a red-purple, crystalline precipitate, which appears brown when collected on a filter, is formed, and it shows characteristic, crystalline aggregates under the low power of a microscope.

**C:** Add silver nitrate TS, dropwise, to a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 20): a white precipitate is formed, and it is insoluble in nitric acid.

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity—*Place 1 Tablet in a 100-mL volumetric flask, add 50 mL of water, and shake the flask until the tablet is dissolved. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate, if necessary, with water to provide a solution containing approximately 80  $\mu$ g of cocaine hydrochloride per mL. Concomitantly determine the absorbances of this test solution and a Standard solution of USP Cocaine Hydrochloride RS in the same medium having a known concentration of about 80  $\mu$ g per mL, in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{17}H_{21}NO_4 \cdot HCl$  in the Tablet by the formula:

$$(T/D)C(A_U/A_S)$$

in which *T* is the labeled quantity, in mg, of cocaine hydrochloride in the Tablet; *D* is the concentration, in  $\mu$ g per mL, of cocaine hydrochloride in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; *C* is the concentration, in  $\mu$ g per mL, of USP Cocaine Hydrochloride RS in the Standard solution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay—**Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 60 mg of cocaine hydrochloride, in 10 mL of water, render the solution slightly alkaline with 6 N ammonium hydroxide, and completely extract the cocaine with small successive portions of ether. Evaporate the combined ether extracts on a steam bath to one-half their volume, transfer the remaining liquid to a separator, and wash with three 5-mL portions of water. Shake the water washings with a small portion of ether, and add the ether washing to the combined ether extracts. Add 10.0 mL of 0.05 N sulfuric acid VS to the ether solution, agitate the mixture thoroughly, and draw off the acidified water layer into a beaker. Wash the ether with two small portions of water, add the washings to the acid liquid, and titrate the excess acid with 0.02 N sodium hydroxide VS, using methyl red TS as the indicator. Each mL of 0.05 N sulfuric acid is equivalent to 16.99 mg of  $C_{17}H_{21}NO_4 \cdot HCl$ .

## Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution

**DEFINITION**

Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution contains NLT 3.6 g and NMT 4.4 g of cocaine hydrochloride ( $C_{17}H_{21}NO_4 \cdot HCl$ ), NLT 0.90 g and NMT 1.10 g of tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ), and NLT 20 mg and NMT 30 mg of epinephrine ( $C_9H_{13}NO_3$ ) in each 100 mL of Topical Solution.

Prepare Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Cocaine Hydrochloride	4.0 g
Tetracaine Hydrochloride	1.0 g
Epinephrine Injection (1:1000)	25.0 mL
Benzalkonium Chloride	10 mg
Edetate Disodium	6.4 mg
Sodium Chloride Injection (0.9%)	35 mL
Purified Water, a sufficient quantity to make	100 mL

Dissolve the *Cocaine Hydrochloride* and *Tetracaine Hydrochloride* in 25 mL of *Purified Water*, and add the *Epinephrine Injection* (1:1000). Separately dissolve *Edetate Disodium* in *Sodium Chloride Injection* (0.9%), and dilute quantitatively and stepwise, if necessary, with *Sodium Chloride Injection* (0.9%) to obtain 35 mL of a solution containing 6.4 mg of *Edetate Disodium*. Similarly, and separately, dissolve *Benzalkonium Chloride* in *Purified Water* (or use *Benzalkonium Chloride Solution*), and dilute quantitatively and stepwise, if necessary, with *Purified Water* to obtain 10 mL of a solution containing 10 mg of *Benzalkonium Chloride*. Combine the three solutions, add sufficient *Purified Water* to bring to final volume, and mix well.

## ASSAY

### • TETRACAINE HYDROCHLORIDE

**Solution A:** 6.3 g/L of monobasic potassium phosphate containing 0.55 g/L of sodium 1-octanesulfonate. Adjust with phosphoric acid to a pH of 2.5.

**Solution B:** Acetonitrile and *Solution A* (10:90). Pass through a suitable filter of 0.5-μm or finer pore size, and degas.

**Solution C:** Acetonitrile and *Solution A* (30:70). Pass through a suitable filter of 0.5-μm or finer pore size, and degas.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
10	0	100
24	0	100
25	100	0
75	100	0

**Standard solution:** 0.5 mg/mL of USP Tetracaine Hydrochloride RS

**Sample solution:** Transfer 0.5 mL of Topical Solution to a 10-mL volumetric flask, dilute with water to volume, and mix.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 30-cm; 10-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5 for the analyte peak

**Relative standard deviation:** NMT 2.0% for replicate injections

## Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in g, of tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of tetracaine hydrochloride in the *Standard solution* (mg/mL)

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

$F$  = conversion factor,  $10^{-3}$  g/mg

**Acceptance criteria:** 0.90–1.10 g

### • COCAINE HYDROCHLORIDE

**Solution A, Solution B, Solution C, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay for Tetracaine Hydrochloride*.

**Standard solution:** 2 mg/mL of USP Cocaine Hydrochloride RS

## Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in g, of cocaine hydrochloride ( $C_{17}H_{21}NO_4 \cdot HCl$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of cocaine hydrochloride in the *Standard solution* (mg/mL)

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

$F$  = conversion factor,  $10^{-3}$  g/mg

**Acceptance criteria:** 3.6–4.4 g

### • EPINEPHRINE

**Solution A, Solution B, Solution C, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay for Tetracaine Hydrochloride*.

**Standard solution:** Transfer 3 mg of USP Epinephrine Bitartrate RS to a 25-mL volumetric flask, and dilute with water to volume. Transfer 4.0 mL of the resultant solution to a 25-mL volumetric flask, and dilute with water to volume.

## Analysis

**Samples:** *Sample solution* and *Standard solution*  
Calculate the quantity, in mg, of epinephrine ( $C_9H_{13}NO_3$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D \times V$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of epinephrine bitartrate in the *Standard solution* (mg/mL)

$M_{r1}$  = molecular weight of epinephrine, 183.20

$M_{r2}$  = molecular weight of epinephrine bitartrate, 333.29

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

**Acceptance criteria:** 20–30 mg

## SPECIFIC TESTS

- **pH** (791): 4.0–6.0

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in sterile, tight, light-resistant containers. Store in a refrigerator.
- **LABELING:** Label it to indicate that it is intended for external use only and that it is not to be used if a precipitate is present. The label states that it is to be protected from light.

- **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded
- **USP REFERENCE STANDARDS** (11)
  - USP Cocaine Hydrochloride RS
  - USP Epinephrine Bitartrate RS
  - USP Tetracaine Hydrochloride RS

## Cod Liver Oil

### DEFINITION

Cod Liver Oil is the partially destearinated fixed oil obtained from fresh livers of *Gadus morrhua* L. and other species of Fam. Gadidae. Cod Liver Oil contains, in each g, NLT 180 µg (600 USP Units) and NMT 750 µg (2500 USP Units) of vitamin A and NLT 1.5 µg (60 USP Units) and NMT 6.25 µg (250 USP Units) of vitamin D.

Cod Liver Oil may be flavored by the addition of NMT 1% of a suitable flavor or a mixture of flavors. A suitable antioxidant may be added.

### IDENTIFICATION

#### A. PRESENCE OF VITAMIN A

**Sample solution:** 25 mg/mL of Cod Liver Oil in chloroform

**Analysis:** To 1 mL of the *Sample solution* add 10 mL of antimony trichloride TS.

**Acceptance criteria:** A blue color results immediately.

#### B. FATTY ACID PROFILE

**Antioxidant solution:** 0.05 mg/mL of butylated hydroxytoluene in hexanes

**System suitability solution:** Prepare a mixture containing equal amounts of methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate in *Antioxidant solution*.

**Standard stock solution:** 45 mg/mL of USP Cod Liver Oil RS in *Antioxidant solution*

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* into a quartz tube, and evaporate with a gentle stream of nitrogen. Add 1.5 mL of a 2% solution of sodium hydroxide in methanol, cap tightly with a polytetrafluoroethylene-lined cap, mix, and heat in a water bath for 7 min. Cool, add 2 mL of a 120 mg/mL solution of boron trichloride in methanol, cover with nitrogen, cap tightly, mix, and heat in a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and mix in a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and mix in a vortex mixer or shake thoroughly for at least 15 s. Allow the upper layer to become clear, and transfer to a separate tube. Shake the methanol layer once more with 1 mL of isooctane, and combine the isooctane extracts. Wash the combined extracts twice with 1 mL of water, and dry over anhydrous sodium sulfate.

**Sample solution:** Proceed as directed for the *Standard solution*, except replace USP Cod Liver Oil RS with Cod Liver Oil.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm × 30-m fused silica capillary column bonded with a 0.25-µm film of phase G16

**Temperature**

**Injector:** 250°

**Detector:** 280°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	1	225	20

**Carrier gas:** Helium

**Split flow ratio:** 200:1

**Injection size:** 1 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Chromatogram similarity:** The chromatogram from the *Standard solution* is similar to the reference chromatogram supplied with USP Cod Liver Oil RS. Identify the retention times of the relevant fatty acid methyl esters by comparing the chromatogram of the *Standard solution* with the reference chromatogram supplied with USP Cod Liver Oil RS.

**Resolution:** NLT 1.3 between methyl oleate and methyl *cis*-vaccinate, and that between methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement, *Standard solution*

**Theoretical area percentages:** 24.4 ± 1 for methyl palmitate, 24.8 ± 1 for methyl stearate, 25.2 ± 1 for methyl arachidate, and 25.6 ± 1 for methyl behenate, *System suitability solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution*.

Determine the number of fatty acid methyl esters in the *Sample solution*. The number of fatty acid methyl ester peaks exceeding 0.05% of the total area of fatty acid methyl esters is at least 24, and the 24 largest peaks of the methyl esters account for more than 90% of the total area. (These correspond to the following, in common elution order: 14:0, 15:0, 16:0, 16:1 *n*-7, 16:4 *n*-1, 18:0, 18:1 *n*-9, 18:1 *n*-7, 18:2 *n*-6, 18:3 *n*-3, 18:4 *n*-3, 20:1 *n*-11, 20:1 *n*-9, 20:1 *n*-7, 20:2 *n*-6, 20:4 *n*-6, 20:3 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:1 *n*-11, 22:1 *n*-9, 21:5 *n*-3, 22:5 *n*-3, and 22:6 *n*-3.)

Calculate the area percentage for each fatty acid methyl ester in the portion of Cod Liver Oil taken:

$$\text{Result} = (r_A/r_B) \times 100$$

$r_A$  = peak area of each individual fatty acid methyl ester

$r_B$  = total area from all peaks, except the solvent peak and butylated hydroxytoluene

**Acceptance criteria:** The *Sample solution* meets the limits described in *Table 2*.

**Table 2**

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
<b>Saturated fatty acids</b>			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
<b>Monounsaturated fatty acids</b>			
Palmitoleic acid	16:1 <i>n</i> -7	4.5	11.5
<i>cis</i> -Vaccenic acid	18:1 <i>n</i> -7	2.0	7.0
Oleic acid	18:1 <i>n</i> -9	12.0	21.0
Gadoleic acid	20:1 <i>n</i> -11	1.0	5.5

Table 2 (Continued)

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Gondoic acid	20:1 <i>n</i> -9	5.0	17.0
Erucic acid	22:1 <i>n</i> -9	0	1.5
Cetoleic acid	22:1 <i>n</i> -11	5.0	12.0
<b>Polyunsaturated fatty acids</b>			
Linoleic acid	18:2 <i>n</i> -6	0.5	3.0
$\alpha$ -Linolenic acid	18:3 <i>n</i> -3	0	2.0
Morotic acid	18:4 <i>n</i> -3	0.5	4.5
Eicosapentaenoic acid	20:5 <i>n</i> -3	7.0	16.0
Docosahexaenoic acid	22:6 <i>n</i> -3	6.0	18.0

**ASSAY****• VITAMIN A**

**Sample:** 500 mg to 1 g of Cod Liver Oil

**Analysis:** Proceed as directed under *Vitamin A Assay* (571).

**Acceptance criteria:** 180  $\mu$ g (600 USP Units) to 750  $\mu$ g (2500 USP Units) of vitamin A per g of Cod Liver Oil

**• VITAMIN D**

**Solution A:** *n*-Amyl alcohol and dehydrated hexane (3:997)

**Solution B:** Acetonitrile, water, and phosphoric acid (96:3.8:0.2)

**Butylated hydroxytoluene solution:** 10 mg/mL of butylated hydroxytoluene in chromatographic hexane

**Aqueous potassium hydroxide solution:** Dissolve 800 mg of potassium hydroxide in 1000 mL of freshly boiled water, mix, and cool. [NOTE—Prepare this solution fresh daily.]

**Alcoholic potassium hydroxide solution:** Dissolve 3 g of potassium hydroxide in 50 mL of freshly boiled water, add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare this solution fresh daily.]

**Ascorbic acid solution:** 100 mg/mL of ascorbic acid in water. [NOTE—Prepare this solution fresh daily.]

**Internal standard solution:** 5  $\mu$ g/mL of USP Ergocalciferol RS in alcohol

**Standard stock solution:** 5  $\mu$ g/mL of USP Cholecalciferol RS in alcohol

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution* to a round-bottomed flask. Proceed as directed for *Sample solution 1* beginning with "Add 5 mL of..."

**Sample solution 1:** Transfer 4.00 g of Cod Liver Oil to a round-bottomed flask. Add 5 mL of *Ascorbic acid solution*, 100 mL of alcohol, and 10 mL of *Aqueous potassium hydroxide solution*, and mix. Reflux the mixture on a steam bath for 30 min. Add 100 mL of a 10 mg/mL sodium chloride solution. Cool rapidly under running water, and transfer the saponified mixture to a 500-mL separator, rinsing the saponification flask with 75 mL of a 10 mg/mL sodium chloride solution, and then with 150 mL of a mixture of ether and hexane (1:1). Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Discard the lower layer. Wash the ether-hexane extracts by shaking vigorously with 50 mL of *Alcoholic potassium hydroxide solution*, and then washing with three 50-mL portions of a 10 mg/mL sodium chloride solution. Filter the upper layer through 5 g of anhydrous sodium sulfate on a fast filter paper into a 250-mL flask suitable for a rotary evaporator. Wash the filter with 10 mL of a mixture of ether and hexane (1:1), and combine with the extract. Evaporate the solvent at reduced pressure at a temperature not exceeding 30°, and fill with nitrogen when the evaporation is complete. Alternatively evaporate the solvent under a

gentle stream of nitrogen at a temperature not exceeding 30°. Dissolve the residue in 1.5 mL of *Butylated hydroxytoluene solution*. [NOTE—Gentle heating in an ultrasonic bath may be required. A large fraction of the white residue is cholesterol.]

**Sample solution 2:** To 4.00 g of Cod Liver Oil add 2.0 mL of *Internal standard solution*, and proceed as directed for *Sample solution 1* beginning with "Add 5 mL of..."

**Clean-up chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Mobile phase:** *Solution A*

**Clean-up column:** 4.6-mm  $\times$  25-cm stainless steel; packing L10

**Injection size:** 350  $\mu$ L

**Analysis (clean-up)**

**Samples:** *Standard solution*, *Sample solution 1*, and *Sample solution 2*

Collect separately the eluates from 2 min before to 2 min after the retention time of cholecalciferol in a glass tube containing 1 mL of *Butylated hydroxytoluene solution* and fitted with a hermetic closure. Evaporate each tube under a stream of nitrogen at a temperature not exceeding 30°. Dissolve each residue in 1.5 mL of acetonitrile, and inject into the analytical chromatographic system below.

**Analytical chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Mobile phase:** *Solution B*

**Analytical column:** 4.6-mm  $\times$  15-cm stainless steel; 5- $\mu$ m packing L1

**Injection size:** 200  $\mu$ L

**System suitability**

**Sample:** *Standard solution* (after the clean-up)

**Suitability requirements**

**Resolution:** NLT 1.4 between cholecalciferol and ergocalciferol

**Relative standard deviation:** NMT 2.0% for the cholecalciferol peak from replicate injections

**Analysis**

**Samples:** *Standard solution*, *Sample solution 1*, and *Sample solution 2* (after the clean-up)

Calculate the content of vitamin D, in  $\mu$ g/g, in the portion of Cod Liver Oil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

$R_U$  = peak response of cholecalciferol relative to the corrected internal standard in the *Sample solution 2*, as calculated below

$R_S$  = peak response of cholecalciferol relative to the internal standard in the *Standard solution*

$C_S$  = concentration of USP Cholecalciferol RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of Cod Liver Oil in *Sample solution 2* (g/mL)

$$R_U = r_{U2}/[r_{IS2} - (r_{IS1} \times r_{U2}/r_{U1})]$$

$r_{U2}$  = peak response for cholecalciferol from *Sample solution 2*

$r_{IS2}$  = peak response for the internal standard from *Sample solution 2*

$r_{IS1}$  = peak response for the internal standard from *Sample solution 1*

$r_{U1}$  = peak response for cholecalciferol from the *Sample solution 1*

**Acceptance criteria:** 1.5  $\mu$ g (60 USP Units) to 6.25  $\mu$ g (250 USP Units) of vitamin D per g of Cod Liver Oil

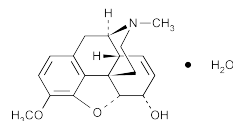


**SPECIFIC TESTS**

- **SPECIFIC GRAVITY** (841): 0.918–0.927
- **COLOR:** When viewed transversely in a tall, cylindrical, standard oil-specimen bottle of colorless glass of about 120-mL capacity, the color of the Cod Liver Oil is not more intense than that of a mixture of cobaltous chloride CS, ferric chloride CS, and water (11:76:33), in a similar bottle of the same internal diameter.
- **NONDESTEARINATED COD LIVER OIL**  
**Sample:** Cod Liver Oil  
**Analysis:** Fill a tall, cylindrical, standard oil-specimen bottle of 120-mL capacity with the *Sample* at a temperature between 23° and 28°, insert the stopper, and immerse the bottle in a mixture of ice and water for 3 h.  
**Acceptance criteria:** The oil remains clear and does not deposit stearin.
- **FATS AND FIXED OILS, Unsaponifiable Matter** (401): NMT 1.30%
- **FATS AND FIXED OILS, Acid Value** (401)  
**Sample solution:** Mix 15 mL of alcohol with 15 mL of ether, add 5 drops of phenolphthalein TS, and neutralize with 0.1 N sodium hydroxide. Dissolve 2.0 g of Cod Liver Oil in the mixture, and boil the oil solution gently under a reflux condenser for 10 min.  
**Analysis:** Cool, and titrate the mixture with 0.1 N sodium hydroxide VS to the production of a pink color that persists after shaking for 30 s.  
**Acceptance criteria:** NMT 1.0 mL of 0.1 N sodium hydroxide is required.
- **FATS AND FIXED OILS, Iodine Value** (401): 145–180
- **FATS AND FIXED OILS, Saponification Value** (401): 180–192  
 [NOTE—If carbon dioxide has been used as a preservative, expose the Cod Liver Oil in a shallow dish in a vacuum desiccator for 24 h before weighing the specimen for determination of the saponification value.]
- **FATS AND FIXED OILS, Anisidine Value** (401): NMT 30

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. It may be bottled or otherwise packaged in containers from which air has been expelled by the production of a vacuum or by an inert gas.
- **LABELING:** The vitamin A potency and vitamin D potency, when designated on the label, are expressed in USP Units/g of oil. The potencies may be expressed also in metric units, on the basis that 1 USP Vitamin A Unit equals 0.3 µg and 40 USP Vitamin D Units equals 1 µg. Where the content of docosahexaenoic acid or eicosapentaenoic acid is claimed, state the concentration in mg/g.
- **USP REFERENCE STANDARDS** (11)  
 USP Cholecalciferol RS  
 USP Cod Liver Oil RS  
 USP Ergocalciferol RS

**Codeine**

$C_{18}H_{21}NO_3 \cdot H_2O$  317.38  
 Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, monohydrate, (5 $\alpha$ ,6 $\alpha$ )-  
 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol monohydrate [6059-47-8].  
 Anhydrous 299.37 [76-57-3].

» Codeine, dried at 80° for 4 hours, contains not less than 98.5 percent and not more than 100.5 percent of  $C_{18}H_{21}NO_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Codeine Sulfate RS

**Identification**—

**A: Infrared Absorption** (197K)—Proceed as directed with the codeine test specimen and the codeine standard specimen obtained from 50 mg of USP Codeine Sulfate RS dissolved in 15 mL of water, then rendered alkaline with 6 N ammonium hydroxide and extracted with several 10-mL portions of chloroform, followed by evaporation of the combined chloroform extracts on a steam bath to dryness, and drying at 80° for 4 hours.

**B: Ultraviolet Absorption** (197U)—

*Solution:* 100 µg per mL.

*Medium:* 0.1 N sulfuric acid.

Absorptivity at 284 nm, calculated on the dried basis, is between 112.9% and 119.9% of that of USP Codeine Sulfate RS.

**Melting range** (741)—When previously dried, it melts between 154° and 158°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it at 80° for 4 hours: it loses not more than 6.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Readily carbonizable substances** (271)—Dissolve 10 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid S*.

**Chromatographic purity**—Prepare a solution of it in dehydrated alcohol containing 40 mg per mL (*Solution A*). Dilute 2.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution B*). Dilute 1.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution C*). Apply separate 10-µL volumes of *Solution A*, *Solution B*, and *Solution C* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of dehydrated alcohol, cyclohexane, and ammonium hydroxide (72:30:6) until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow the solvent to evaporate. Spray the plate with a reagent prepared by mixing 3 mL of chloroplatinic acid solution (1 in 10) with 97 mL of water, followed by the addition of 100 mL of potassium iodide solution (6 in 100), and examine the chromatogram: no spot obtained from *Solution A*, other than the principal spot and any spot observed at the origin, is more intense than the principal spot obtained from *Solution B* (2%); and not more than one such spot having an  $R_f$  greater than that of the principal spot is more intense than the principal spot obtained from *Solution C* (1%).

**Limit of morphine**—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a neutral or slightly acid solution of Codeine (1 in 100) prepared with the aid of sulfuric acid: no blue color is produced immediately.

**Assay**—Dissolve about 400 mg of Codeine, previously dried and accurately weighed, by warming it in 30.0 mL of 0.1 N sulfuric acid VS. Cool, and add 10 mL of water. Add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N sulfuric acid is equivalent to 29.94 mg of  $C_{18}H_{21}NO_3$ .

## Codeine Phosphate

$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  406.37

Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5 $\alpha$ ,6 $\alpha$ )-, phosphate (1:1) (salt), hemihydrate. 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol phosphate (1:1) (salt) hemihydrate [41444-62-6]. Anhydrous 397.37 [52-28-8].

» Codeine Phosphate contains not less than 99.0 percent and not more than 101.5 percent of  $C_{18}H_{21}NO_3 \cdot H_3PO_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store up to 40° as permitted by the manufacturer.

### USP Reference standards (11)—

USP Codeine Phosphate RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** Neutralize a solution (1 in 50) with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in 2 N nitric acid and in 6 N ammonium hydroxide.

**Acidity**—Dissolve 100 mg in 20 mL of water, and titrate with 0.010 N sodium hydroxide to a pH of 5.4, using a pH meter: not more than 1.0 mL of 0.010 N sodium hydroxide is required.

**Water**, *Method I* (921): not more than 3.0%.

**Chloride**—To 10 mL of a solution (1 in 100), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

**Sulfate**—To 10 mL of a solution (1 in 100) add a few drops of barium chloride TS: no turbidity is produced immediately.

**Limit of morphine**—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a solution of Codeine Phosphate (1 in 100): no blue color is produced immediately.

**Chromatographic purity**—Using Codeine Phosphate, proceed as directed in the test for *Chromatographic purity* under *Codeine*, except to use a mixture of 0.01 N hydrochloric acid and dehydrated alcohol (4:1), instead of dehydrated alcohol, to prepare *Solution A*, *Solution B*, and *Solution C*.

**Assay**—Dissolve about 1 g of Codeine Phosphate, accurately weighed, in 50 mL of glacial acetic acid, warming slightly if necessary to effect solution, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 39.74 mg of  $C_{18}H_{21}NO_3 \cdot H_3PO_4$ .

## Codeine Phosphate Injection

» Codeine Phosphate Injection is a sterile solution of Codeine Phosphate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

NOTE—Do not use the Injection if it is more than slightly discolored or contains a precipitate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Codeine Phosphate RS

USP Endotoxin RS

### Identification—

**A:** Dilute a volume of Injection, equivalent to about 90 mg of codeine phosphate, with water to about 10 mL, add 1 drop of hydrochloric acid, and extract with three 10-mL portions of chloroform, discarding the chloroform extracts. Add 6 N ammonium hydroxide until the solution is alkaline, and extract with several 10-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness, and dry at 80° for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as that of the codeine obtained by similarly treating 1 mL of a solution of USP Codeine Phosphate RS (1 in 100).

**B:** A volume of Injection, equivalent to about 60 mg of codeine phosphate, responds to *Identification test B* under *Codeine Phosphate*.

**Bacterial endotoxins** (85)—It contains not more than 5.8 USP Endotoxin Units per mg of codeine phosphate.

**pH** (791): between 3.0 and 6.0.

**Limit of morphine**—Diluted with water to a concentration of 5 mg of codeine phosphate per mL, it meets the requirements of the test for *Limit of morphine* under *Codeine Phosphate*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 75 mg of codeine phosphate, to a small separator, and add about 15 mL of water. Add 2 drops of phosphoric acid, and extract with four 10-mL portions of chloroform, collecting the chloroform extracts in a separator. Wash the combined chloroform extracts with 10 mL of water, and add the water wash to the first separator containing the sample. Discard the chloroform extracts. Proceed as directed in the *Assay* under *Codeine Phosphate Tablets*, beginning with "render the solution alkaline with 6 N ammonium hydroxide." Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

### Add the following:

## ▲Codeine Phosphate Oral Solution

### DEFINITION

Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

Prepare Codeine Phosphate Oral Solution 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Codeine Phosphate	300 mg
Purified Water	1.3 mL
Vehicle: Ora-Sweet <sup>a</sup> , a sufficient quantity to make	100 mL

<sup>a</sup>Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place *Codeine Phosphate* powder in a suitable calibrated container. Add *Purified Water* and mix to dissolve. Add the *Ora-Sweet* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Buffer:** 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5

**Mobile phase:** Methanol and *Buffer* (30:70). Filter and degas.

**Internal standard solution:** 0.5 mg/mL of theophylline

**Standard stock solution:** 1.0 mg/mL of USP Codeine Phosphate RS

**Standard solution:** Pipet 0.5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 0.6 mL of the *Internal standard solution*, dilute with water to volume, and pass through a membrane filter of 0.2-μm pore size to obtain a solution with nominal concentrations of 5 μg/mL of codeine phosphate and 3 μg/mL of theophylline.

**Sample solution:** Shake the Oral Solution thoroughly by hand. Transfer 2 mL of Oral Solution from each bottle into a 50-mL volumetric flask, and dilute with water to volume. Transfer 4.17 mL of the diluted sample to a 100-mL volumetric flask, add 0.6 mL of *Internal standard solution*, dilute with water to volume, and filter to obtain a solution with nominal concentrations of 5 μg/mL of codeine phosphate and 3 μg/mL of theophylline.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention times for codeine phosphate and theophylline are about 1.9 and 3.8 min, respectively.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of codeine phosphate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of codeine phosphate to the internal standard from the *Standard solution*

$C_S$  = concentration of codeine phosphate in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of codeine phosphate in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS****• pH <791>:** 3.7–4.7**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

**• LABELING:** Label it to indicate the *Beyond-Use Date*, and that it is to be well-shaken before use.

**• BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at controlled room temperature

**• USP REFERENCE STANDARDS <11>**

USP Codeine Phosphate RS▲<sub>USP36</sub>

**Codeine Phosphate Tablets**

» Codeine Phosphate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards <11>—**

USP Codeine Phosphate RS

**Identification—**

**A:** Digest a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, with 15 mL of water and 5 mL of 2 N sulfuric acid for 1 hour. Filter, if necessary, and wash any undissolved residue with a few mL of water. Render the filtrate alkaline with 6 N ammonium hydroxide, extract with several small portions of chloroform, and proceed as directed in *Identification* test A under *Codeine Phosphate Injection*, beginning with “Evaporate the combined chloroform extracts.” The specified results are observed.

**B:** To a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, add 10 mL of water and 2 drops of 2 N sulfuric acid. Digest, with frequent shaking, for 15 minutes, and filter. Neutralize 5 mL of the filtrate with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in diluted nitric acid and in 6 N ammonium hydroxide.

**Dissolution <711>—**

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Codeine Phosphate RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  is dissolved in 45 minutes.

**Uniformity of dosage units <905>:** meet the requirements.

**Procedure for content uniformity**—Transfer 1 Tablet, previously crushed or finely powdered, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve. Dilute with water to volume, and filter, if necessary, discarding the first 20 mL of the filtrate. Transfer an aliquot of the filtrate, equivalent to about 6 mg of codeine phosphate, to a 50-mL volumetric flask containing 2 mL of 3 N hydrochloric acid, and dilute with water to volume. Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 120 μg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 284 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  in the Tablet taken by the formula:

$$2.5(C/V)(A_U/A_S)(406.37/397.37)$$

in which C is the concentration, in μg per mL, of USP Codeine Phosphate RS in the Standard solution; V is the volume, in mL, of the aliquot taken of the solution of the Tablet;  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively; and 406.37

and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively.

**Limit of morphine**—A 1-mL portion of the filtrate from Identification test B meets the requirements of the test for Limit of morphine under Codeine Phosphate.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 150 mg of codeine phosphate, and transfer to a 100-mL volumetric flask. Add 20 mL of 0.5 N sulfuric acid, and shake the mixture occasionally during 2 hours. Add water to volume, mix, and filter through a filtering crucible. Transfer to a separator an accurately measured portion of the filtrate, equivalent to not less than 75 mg of codeine phosphate, render the solution alkaline with 6 N ammonium hydroxide, and completely extract the alkaloid with successive 15-mL portions of chloroform. Evaporate the combined chloroform solution on a steam bath nearly to dryness. Dissolve the residue in about 2 mL of methanol, heating, if necessary, add methyl red TS, and titrate with 0.02 N sulfuric acid VS to a faint pink color. Add about 40 mL of freshly boiled, cooled water, and complete the titration with 0.02 N sulfuric acid VS. Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

## Codeine Sulfate

$(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$  750.85

$(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4$  696.82

Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5 $\alpha$ ,6 $\alpha$ )-, sulfate (2:1) (salt), trihydrate; 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol sulfate (2:1) (salt) trihydrate [6854-40-6]. Anhydrous [1420-53-7].

### DEFINITION

Codeine Sulfate, dried at 105° for 3 h, contains NLT 98.0% and NMT 102.0% of  $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)
 

Sample solution: 100  $\mu$ g/mL in water

Acceptance criteria: Absorptivities at 284 nm do not differ by more than 3.0%, calculated on the dried basis.
- **C. IDENTIFICATION TESTS—GENERAL, Sulfate** (191): Meets the requirements

### ASSAY

#### PROCEDURE

**Buffer:** Dissolve 4.0 g of potassium phosphate monobasic in 2000 mL of water, and adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Solution A:** 5 mM sodium heptane sulfonate in methanol and Buffer (3:7). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of Mobile phase produced, and filter.]

**Solution B:** 5 mM sodium heptane sulfonate in methanol and Buffer (11:9). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of Mobile phase produced, and filter.]

**Diluent:** Solution A

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
16	0	100
16.5	100	0
24	100	0

**Standard solution:** 1 mg/mL of USP Codeine Sulfate RS in Diluent

**Sample solution:** 1 mg/mL of Codeine Sulfate in Diluent

### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Sample:** Standard solution

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of codeine sulfate

$[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$  in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of codeine sulfate from the Sample solution

$r_S$  = peak area of codeine sulfate from the Standard solution

$C_S$  = concentration of USP Codeine Sulfate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Codeine Sulfate in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Organic Impurities

#### PROCEDURE 1

**Buffer, Solution A, Solution B, Diluent, Mobile phase, and Chromatographic system:** Proceed as directed in the Assay.

**Standard stock solution:** 1 mg/mL of USP Codeine Sulfate RS in Diluent

**Standard solution:** 0.01 mg/mL of USP Codeine Sulfate RS in Diluent from Standard stock solution

**Sensitivity solution:** 0.5  $\mu$ g/mL of USP Codeine Sulfate RS in Diluent from Standard stock solution

**Sample solution:** 1 mg/mL of Codeine Sulfate in Diluent

### System suitability

**Samples:** Standard solution and Sensitivity solution

### Suitability requirements

**Tailing factor:** NLT 0.5 and NMT 1.5, Standard solution

**Relative standard deviation:** NMT 5.0%, Standard solution

**Signal-to-noise ratio:** NLT 10, Sensitivity solution

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each individual impurity in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the Sample solution

$r_S$  = peak response of codeine sulfate from the Standard solution

$C_S$  = concentration of USP Codeine Sulfate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Codeine Sulfate in the Sample solution (mg/mL)

$F$  = relative response factor of the related compounds (see Impurity Table 1)

**Acceptance criteria**Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.5%.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
10-Hydroxy-codeine <sup>a</sup>	0.81	1.35	0.15
Codeine- <i>N</i> -oxide <sup>b</sup>	0.90	1.0	0.15
Codeine sulfate	1.00	—	—
Norcodeine <sup>c</sup>	1.09	1.0	0.15
Codeinone <sup>d</sup>	1.16	1.0	0.15
Codeine methyl ether <sup>e</sup>	1.34	1.0	1.0
Individual unspecified impurities	—	—	0.10

<sup>a</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ ,10-diol.<sup>b</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol *N*-oxide.<sup>c</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxymorphinan-6 $\alpha$ -ol.<sup>d</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -one.<sup>e</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3,6 $\alpha$ -dimethoxy-17-methylmorphinan.**PROCEDURE 2: LIMIT OF MORPHINE****Analysis:** Dissolve 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a 10 mg/mL solution of Codeine Sulfate.**Acceptance criteria:** No blue color is produced immediately.**SPECIFIC TESTS****OPTICAL ROTATION, Specific Rotation (781S)****Sample solution:** 20 mg/mL, in water**Acceptance criteria:**  $-112.5^{\circ}$  to  $-115.0^{\circ}$ **ACIDITY****Analysis:** Dissolve 500 mg in 15 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide.**Acceptance criteria:** NMT 0.30 mL is required for neutralization.**WATER DETERMINATION, Method III (921):** Dry 500 mg at  $105^{\circ}$  for 3 h: it loses between 6.0% and 7.5% of its weight.**READILY CARBONIZABLE SUBSTANCES TEST (271)****Sample solution:** Dissolve 10 mg in 5 mL of sulfuric acid.**Acceptance criteria:** The solution has no more color than *Matching Fluid S*.**ADDITIONAL REQUIREMENTS****PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**USP REFERENCE STANDARDS (11)**

USP Codeine Sulfate RS

**Codeine Sulfate Tablets****DEFINITION**Codeine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of  $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$ .**IDENTIFICATION****A. INFRARED ABSORPTION (197K)****Standard solution:** 50 mg of USP Codeine Sulfate RS dissolved in 15 mL of water, then rendered alkaline with 6 N ammonium hydroxide and extracted with several10-mL portions of chloroform, followed by evaporation of the combined chloroform extracts on a steam bath to dryness, drying at  $80^{\circ}$  for 4 h**Sample solution:** Digest an equivalent to 50 mg of codeine sulfate, from finely powdered Tablets, with 15 mL of water and 5 mL of 2 N sulfuric acid for 1 h. Filter, if necessary, and wash any undissolved residue with a few mL of water. Render the filtrate alkaline with 6 N ammonium hydroxide, extract with several small portions of chloroform, and evaporate the chloroform solution on a steam bath to dryness, drying at  $80^{\circ}$  for 4 h.**B. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** A filtered solution of Tablets meets the requirements.**ASSAY****PROCEDURE****Diluent:** 0.5% of phosphoric acid (5 mL of concentrated phosphoric acid and 1000 mL of water)**Solution A:** Acetonitrile and 0.1% ammonium hydroxide (1.0 mL of concentrated ammonium hydroxide and 1000 mL of water) (1:19)**Solution B:** Acetonitrile and 0.1% ammonium hydroxide (9:11)**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
41	100	0
50	100	0

[NOTE—The *Standard* and *Sample solutions*, for the degradation products, are stable for 4 days when stored at room temperature in amber vials.]**Standard solution:** 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent***Sensitivity solution:** 0.6  $\mu$ g/mL of USP Codeine Sulfate RS from the *Standard solution* in *Diluent***Sample solution:** 1.2 mg/mL of codeine sulfate in *Diluent*. [NOTE—Dissolve 20 Tablets in 80% of the flask volume of *Diluent*, and sonicate for 15–30 min with occasional swirling before diluting with *Diluent* to volume.]**Chromatographic system**(See *Chromatography (621)*, *System Suitability*.)**Mode:** LC**Detector:** UV 282 nm**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L1**Column temperature:**  $40^{\circ}$ **Flow rate:** 1.2 mL/min**Injection size:** 40  $\mu$ L**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Relative standard deviation:** NMT 2.0%, *Standard solution***Tailing factor:** NMT 2.0, *Standard solution***Column efficiency:** NLT 10,000 theoretical plates, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak area of codeine sulfate from the *Sample solution* $r_S$  = peak area of codeine sulfate from the *Standard solution* $C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

- $C_U$  = concentration of codeine sulfate in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of codeine sulfate, trihydrate, 750.87  
 $M_{r2}$  = molecular weight of codeine sulfate, anhydrous, 696.82

Acceptance criteria: 93.0%–107.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

Medium: Water; 500 mL

Apparatus 2: 25 rpm

Time: 45 min

Detector: UV, maxima at about 284 nm

Cell: 1 cm

Blank: *Medium*

Standard solution: USP Codeine Sulfate RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.8- $\mu$ m pore size.

Tolerances: NLT 75% (Q) of the labeled amount of  $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$  is dissolved.

### • UNIFORMITY OF DOSAGE UNITS <905>

Procedure for content uniformity

Standard solution: 110  $\mu$ g/mL of USP Codeine Sulfate RS in 0.2 N sulfuric acid

Sample solution: Transfer 1 Tablet to a 50-mL volumetric flask. Add 20 mL of 0.5 N sulfuric acid and 10 mL of water. Shake until the Tablet is disintegrated, and allow to stand for 16 h. Dilute with water to volume, and filter, discarding the first few mL of the filtrate. Dilute the resulting filtrate with 0.2 N sulfuric acid to obtain a solution containing nominally 120  $\mu$ g/mL of codeine sulfate (trihydrate).

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

Mode: UV-Vis

Analytical wavelength: About 284 nm

Cell: 1 cm

Blank: 0.2 N sulfuric acid

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*  
 Calculate the percentage of  $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$  in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* ( $\mu$ g/mL)  
 $C_U$  = nominal concentration of codeine sulfate trihydrate in the *Sample solution* ( $\mu$ g/mL)  
 $M_{r1}$  = molecular weight of codeine sulfate trihydrate, 750.87  
 $M_{r2}$  = molecular weight of anhydrous codeine sulfate, 696.82

Acceptance criteria: Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response of each individual impurity from the *Sample solution*  
 $r_S$  = peak response of codeine sulfate from the *Standard solution*

- $C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of codeine sulfate in the *Sample solution* (mg/mL)  
 $F$  = relative response factor of the related compounds (see *Impurity Table 1*)

### Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Codeine-N-oxide <sup>a</sup>	0.39	1.25	0.2
Codeine sulfate	1.00	—	—
Codeinone <sup>b</sup>	1.10	1.0	0.3
Individual unspecified degradant	—	—	0.2

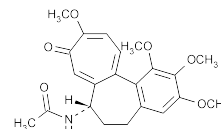
<sup>a</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol N-oxide.

<sup>b</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -one.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS <11>**  
 USP Codeine Sulfate RS

## Colchicine



$C_{22}H_{25}NO_6$  399.44

Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-, (S)-.

Colchicine [64-86-8].

» Colchicine is an alkaloid contained in various species of *Colchicum* and in other genera. It contains not less than 94.0 percent and not more than 101.0 percent of  $C_{22}H_{25}NO_6$ , calculated on the anhydrous, solvent-free basis.

*Caution—Colchicine is extremely poisonous.*

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards <11>**—

USP Colchicine RS

**Identification, Infrared Absorption <197K>**—[NOTE—Disregard any peak occurring at 1735  $cm^{-1}$ .]

**Specific rotation <781S>**: between  $-240^\circ$  and  $-250^\circ$ , calculated on the anhydrous, solvent-free basis.

*Test solution*: 10 mg per mL, in alcohol.

**Water, Method I <921>**: not more than 2.0%.

**Limit of colchicine**—To 5 mL of a solution (1 in 100) add 2 drops of ferric chloride TS: no definite green color is produced.

**Limit of ethyl acetate—**

*Internal standard solution*—Dilute 0.5 mL of *n*-propyl alcohol with water to 100.0 mL.

*Standard solution*—Pipet 1 mL of ethyl acetate and 0.5 mL of *n*-propyl alcohol into a 1000-mL volumetric flask, add water to volume, and mix. Each mL of *Standard solution* contains 0.90 mg of ethyl acetate.

*Test solution*—Place about 250 mg of Colchicine, accurately weighed, in a 10-mL volumetric flask, dissolve in about 8 mL of water, and add 1.0 mL of *Internal standard solution*. Add water to volume, and mix.

*Procedure*—Determine appropriate sensitivity settings on a gas chromatograph (see *Chromatography* (621)) fitted with a 4-mm × 1.5-m column packed with 20% (w/v) phase G14 on support S1, maintaining the column temperature at 75°, using nitrogen as the carrier gas, and using a flame-ionization detector. Inject the *Standard solution* and the *Test solution*, determine the peak height for ethyl acetate relative to the peak height for *n*-propyl alcohol, and calculate the percentage, by weight, of ethyl acetate in the portion of Colchicine taken: not more than 8.0% is found.

**Chromatographic purity**—The sum of the responses of any peaks other than that due to colchicine, eluting within 1.5 times the retention time for colchicine, is not more than 5.0% of the sum of all responses, obtained as directed in the *Assay*.

**Residual solvents** (467): meets the requirements, except that the limit of chloroform is 100 ppm.

**Assay**—[NOTE—Perform all dilutions in low-actinic glassware.]

*Mobile phase*—Dilute 45 mL of 0.5 M monobasic potassium phosphate with water to 450 mL. Add about 530 mL of methanol, cool to room temperature, and add methanol to bring the volume to 1000 mL. Adjust with 0.5 M phosphoric acid to a pH of 5.5 ± 0.05, and pass through a 0.45-μm membrane filter.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Colchicine RS in a mixture of methanol and water (1:1), and dilute quantitatively and stepwise with the same mixture to obtain a solution having a known concentration of about 6 μg per mL. This solution is stable for 4 months when stored tightly stoppered and in the dark.

*Assay preparation*—[NOTE—Prepare immediately before use.] Transfer about 60 mg of Colchicine, accurately weighed, to a 500-mL volumetric flask, dissolve in a mixture of methanol and water (1:1), dilute with the same mixture to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with the same mixture to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4500 theoretical plates, the retention time for colchicine is between 5.5 and 9.5 minutes, and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all peaks recorded during 1.5 times the retention time for colchicine. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in the Colchicine taken by the formula:

$$10C(r_U / r_S)$$

in which *C* is the concentration, in μg per mL, of the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the colchicine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Colchicine Injection**

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» Colchicine Injection is a sterile solution of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in Water for Injection, prepared from Colchicine with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub>.

*Caution*—Colchicine is extremely poisonous.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Colchicine RS

USP Endotoxin RS

**Identification**—

**A:** Transfer a volume of Injection, equivalent to about 2 mg of colchicine, to a separator. Add 5 mL of water, and extract with 15 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.

**B:** The UV absorption spectrum of the Injection, diluted with water to a concentration of about 10 μg of colchicine per mL, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Colchicine RS, concomitantly measured.

**Bacterial endotoxins** (85)—It contains not more than 166.7 USP Endotoxin Units per mg of colchicine.

**pH** (791): between 6.0 and 7.2, in a solution of Injection containing 1.0 mg of potassium chloride in each mL.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—[NOTE—Perform all dilutions in low-actinic glassware.]

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under Colchicine.

*Assay preparation*—[NOTE—Prepare immediately before use.] Transfer an accurately measured volume, *V* mL, of Injection, equivalent to about 1 mg of colchicine, to a 50-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Pipet 30 mL of this solution into a 100-mL volumetric flask, dilute with the same mixture to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under Colchicine, and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in each mL of the Injection taken by the formula:

$$(C / 6V)(r_U / r_S)$$

in which *C* is the concentration, in μg per mL, of the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Colchicine Tablets**

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» Colchicine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub>.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Colchicine RS

**Identification**—Weigh a portion of ground Tablets, equivalent to about 20 mg of colchicine, triturate with 20 mL of water, allow the solids to settle, and filter the supernatant into a separator. Extract with 30 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.

**Dissolution**, *Procedure for a Pooled Sample* (711)—[NOTE—Conduct this procedure without delay, under subdued light, and using low-actinic glassware.]

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

*Procedure*—Determine the amount of  $C_{22}H_{25}NO_6$  dissolved, employing the procedure set forth in the *Assay* under *Colchicine*, making any necessary modifications.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{22}H_{25}NO_6$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—[NOTE—Perform all dilutions in low-actinic glassware.]

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Colchicine*.

*Assay preparation*—[NOTE—Prepare immediately before use.] Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.6 mg of colchicine, to a 100-mL volumetric flask, add about 50 mL of a mixture of methanol and water (1:1), and shake by mechanical means for 15 minutes, rinsing down the walls of the flask at about 8 minutes. Dilute with the same mixture to volume, and pass through a 0.45- $\mu$ m membrane filter.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Colchicine*, and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of  $C_{22}H_{25}NO_6$  in the portion of Tablets taken by the formula:

$$0.1 C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Colestipol Hydrochloride

» Colestipol Hydrochloride is an insoluble, high molecular weight basic anion-exchange copolymer of diethylenetriamine and 1-chloro-2,3-epoxypropane with approximately one out of five amino nitrogens protonated. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as cholate binding capacity.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Colestipol Hydrochloride RS

**Identification**—*Infrared Absorption* (197K)—Prepare the test specimen and the Standard specimen by mixing about 3 to 4 mg of Colestipol Hydrochloride and of USP Colestipol

Hydrochloride RS, respectively, with about 150 mg of potassium bromide.

**pH** (791)—Prepare a 10% (w/w) suspension of it in deionized water in a clean vial. Insert the stopper, shake at approximately 10-minute intervals for 1 hour, and centrifuge. Transfer a portion of the clear supernatant to a suitable container, and record the pH as soon as the reading has stabilized: the pH is between 6.0 and 7.5.

**Loss on drying** (731)—Dry it in vacuum at a pressure of about 5 mm of mercury at 75° for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.3%.

**Heavy metals**, *Method II* (231): not more than 0.002%.

**Content of chloride**—

*Test preparation*—Using about 20 mg of Colestipol Hydrochloride, accurately weighed, proceed as directed under *Oxygen Flask Combustion* (471), 10 mL of 0.05 N sodium hydroxide being used as the absorbing liquid. Do not allow the paper specimen wrapper to come in contact with the liquid, and ignite the paper with an IR igniter. After combustion is complete, shake the flask vigorously, and allow to stand, with frequent shaking, for about 40 minutes or until no cloudiness is present. Transfer the solution to a 50-mL beaker. Wash the flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding each washing to the beaker, and add 0.2 mL of nitric acid.

*Reagent blank preparation*—Using a paper specimen wrapper, complete the combustion, and allow the mixture to stand for about 40 minutes or until no cloudiness is present, as directed under *Test preparation*. Transfer the solution so obtained to a 50-mL beaker. Wash the combustion flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding the washings to the beaker, and add 0.2 mL of nitric acid.

*Procedure*—Titrate the *Test preparation* and the *Reagent blank preparation* with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride electrode and a glass reference electrode (see *Titrimetry* (541)). Determine the volume of 0.05 N silver nitrate VS consumed by the test specimen taken by the formula:

$$V - V_B$$

in which V and  $V_B$  are the volumes, in mL, of titrant used for the *Test preparation* and the *Reagent blank preparation*, respectively. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of Cl: the chloride content is between 6.5% and 9.0%, calculated on the dried basis.

**Water absorption**—Transfer about 5 g of Colestipol Hydrochloride, accurately weighed, to a dry, 100-mL plastic container, and add about 80 g of water, accurately weighed. Cover the container, and allow the suspension to equilibrate for 72 hours. With the aid of vacuum, filter the slurry transferred to a medium-porosity, fritted-glass funnel, and collect the filtrate in a tared plastic container. Disconnect the vacuum 2 minutes after the collection of the last portion of the filtrate. Weigh the container and the filtrate, and determine the weight, in g, of the filtrate. Determine the amount of water absorbed by subtracting the weight of the filtrate from the weight of water taken for the test, and divide the weight, in g, of the absorbed water by the weight, in g, of Colestipol Hydrochloride taken: each g absorbs between 3.3 g and 5.3 g of water.

**Cholate binding capacity**—

*Cholate solution*—Dissolve accurately weighed quantities of sodium cholate and sodium chloride in water, and quantitatively dilute with water to obtain a solution having known concentrations of 10.0 mg of sodium cholate per mL and 9.0 mg of sodium chloride per mL. Adjust the solution by the dropwise addition of hydrochloric acid to a pH of 6.4  $\pm$  0.1.



**Test preparation**—Transfer  $1.0 \pm 0.01$  g of Colestipol Hydrochloride to a glass-stoppered, 125-mL conical flask. Add 100.0 mL of *Cholate solution*, insert the stopper securely in the flask, shake vigorously for 90 minutes with the flask positioned horizontally on a platform shaker, remove the flask from the shaker, and allow the solids to settle for 5 minutes.

**Procedure**—Transfer 20.0 mL of supernatant from the *Test preparation* to a 40-mL beaker, transfer 20.0 mL of *Cholate solution* to a second 40-mL beaker, and adjust both solutions by the dropwise addition of 1 N sodium hydroxide to a pH of  $10.5 \pm 0.5$ . Titrate both solutions with 0.1 N hydrochloric acid VS, determining the endpoints potentiometrically, and measure the titrant volume corresponding to the difference between the midpoints of the two inflections in the titration curves obtained for each solution (see *Titrimetry* (541)). Determine the volume of titrant equivalent to the bound cholate by subtracting the volume of 0.1 N hydrochloric acid VS used in titrating the *Test preparation* from that used in titrating the *Cholate solution*. Calculate the *Cholate binding capacity*, in mEq per g, taken by the formula:

$$5VN / W$$

in which  $V$  is the volume, in mL, of titrant equivalent to the bound cholate;  $N$  is the normality of the 0.1 N hydrochloric acid VS; and  $W$  is the weight, in g, of Colestipol Hydrochloride taken for the *Test preparation*. The *Cholate binding capacity* is between 1.1 mEq per g and 1.6 mEq per g.

**Water-soluble substances**—Transfer 5.0 g of Colestipol Hydrochloride, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at  $37 \pm 1^\circ$ . Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice—first through a premoistened 0.45- $\mu$ m nylon membrane filter and then through a 0.45- $\mu$ m PVDF filter, collecting the filtrate in a tared 125-mL conical flask. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or nitrogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at  $75^\circ$  for 1 hour, allow to cool in a desiccator, and weigh: not more than 0.5% of water-soluble substances is found in the portion of Colestipol Hydrochloride taken.

#### Colestipol exchange capacity—

**Resin base preparation**—Combine not less than 2 g of Colestipol Hydrochloride and 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, insert a stopper in the flask, secure the flask on a platform shaker, and shake the mixture for 3 to 4 hours. Filter the suspension through a coarse-porosity, fritted-glass funnel, and wash the resin with 500 mL of water. Transfer the resin to a 1000-mL beaker, add 200 mL of water, allow to stand for 10 minutes, filter the suspension, and check the pH of the filtrate. Repeat the washing procedure with 200-mL portions of water until the pH of the filtrate is below 8 (as much as 5000 mL of water may be required). Dry the colestipol base resin so obtained and the funnel at a pressure of about 5 mm of mercury at  $60^\circ$  for 16 hours. Break up any aggregates, and store the *Resin base preparation* in a desiccator.

**Procedure**—Transfer about 1.0 g of the *Resin base preparation* to a 125-mL conical flask, add 100.0 mL of 0.20 N hydrochloric acid, insert a stopper in the flask, and shake the mixture by mechanical means for 2.5 hours. Filter a portion of the suspension through a pledget of glass wool, and transfer 8.0 mL of the filtrate (*test preparation*) to a 25-mL beaker. Transfer 5.0 mL of the same 0.20 N hydrochloric acid that was used to equilibrate the resin to a second 25-mL beaker, and add 5.0 mL of water. Titrate both solutions with 0.2 N sodium hydroxide VS, determining the endpoints potentiometrically (see *Titrimetry* (541)), and cal-

culate the *Colestipol exchange capacity*, in mEq per g, taken by the formula:

$$(100N / W)[(V_b / 5) - (V_a / 8)]$$

in which  $N$  is the normality of the sodium hydroxide VS;  $W$  is the weight, in g, of the *Resin base preparation* taken;  $V_b$  is the volume, in mL, of titrant used to neutralize the 5.0-mL aliquot of 0.20 N hydrochloric acid; and  $V_a$  is the volume, in mL, of titrant used to neutralize the residual acid in the test preparation. Each g exchanges not less than 9.0 mEq and not more than 11.0 mEq of sodium hydroxide, calculated as colestipol exchange capacity.

## Colestipol Hydrochloride for Oral Suspension

» Colestipol Hydrochloride for Oral Suspension is a mixture of Colestipol Hydrochloride with a suitable flow-promoting agent. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as the cholate binding capacity.

**Packaging and storage**—Preserve in tight, single-dose or multiple-dose containers.

**USP Reference standards** (11)—

USP Colestipol Hydrochloride RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen and the Standard specimen by mixing about 5 mg of Colestipol Hydrochloride for Oral Suspension and 5 mg of USP Colestipol Hydrochloride RS, respectively, with about 100 to 125 mg of potassium bromide.

**Minimum fill** (755): meets the requirements for powders.

**Water-soluble substances**—Transfer 5.0 g of Colestipol Hydrochloride for Oral Suspension, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at  $37 \pm 1^\circ$ . Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice—first through a premoistened 0.45- $\mu$ m nylon membrane filter and then through a 0.45- $\mu$ m PVDF filter, collecting the filtrate in a tared 100-mL fused quartz crucible. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or nitrogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at  $75^\circ$  for 1 hour, allow to cool in a desiccator, and weigh. Calculate the initial percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken. Again heat the residue in a muffle furnace maintained at  $800 \pm 25^\circ$  for 4 hours, allow to cool in a desiccator, and weigh. Calculate the percentage of inert ingredients present. Calculate the actual percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken by subtracting the percentage of inert ingredients from the initial percentage of water-soluble substances found. Not more than 0.5% of water-soluble substances is found.

**Other requirements**: meets the requirements of the tests for *Cholate binding capacity* and *pH* under *Colestipol Hydrochloride*.

## Colestipol Hydrochloride Tablets

» Colestipol Hydrochloride Tablets contain Colestipol Hydrochloride. Each Tablet binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate per g of the labeled amount of colestipol hydrochloride, calculated as cholate binding capacity.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Colestipol Hydrochloride RS

**Identification**, *Infrared Absorption* (197K)—

**Test specimen**—Completely remove the coating film from a Tablet with a suitable implement, and grind the contents into fine powder. To about 30 to 40 mg of the powder, add about 15 mL of methanol, shake vigorously for 3 minutes, then sonicate for 10 minutes, and shake for another 3 minutes. Pass through a suitable paper filter, wash the residue 3 times, each time with 10 mL of methanol. [NOTE—A qualitative paper filter, with a coarse porosity and a particle retention 20–25  $\mu\text{m}$ , is suitable.] Dry the residue at 60° in vacuum for 2 hours. Mix about 4 mg of the dried sample with about 150 mg of potassium bromide.

**Standard specimen**—Mix about 3 to 4 mg of USP Colestipol Hydrochloride RS with about 150 mg of potassium bromide.

**Uniformity of dosage units**—

*Sodium chloride solution, Cholate solution, 0.09 M Buffer solution pH 2.5, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the test for *Cholate binding capacity*.

**Test solution**—Transfer 1 Tablet to a 100-mL volumetric flask, dilute with *Cholate solution* to volume, and stir for 120 minutes. Let the sample settle down for at least 10 minutes, and filter a portion using a 0.45- $\mu\text{m}$  PVDF filter, discarding the first 5 mL of the filtrate.

**Procedure**—Proceed as directed in the test for *Cholate binding capacity*, except to inject the *Test solution* instead of the *Test preparation*.

Select not fewer than 30 Tablets. Test 10 Tablets individually as directed above. The requirements are met if the cholate binding capacity in each of the 10 Tablets lies within the range of 1.15 to 1.55 mEq per g of the labeled amount of colestipol hydrochloride, and the relative standard deviation is not more than 6.0%.

If 1 Tablet is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g, or if the relative standard deviation is greater than 6.0%, or if both conditions prevail, test 20 additional Tablets. The requirements are met if not more than 1 Tablet of the 30 is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g of the labeled amount of colestipol hydrochloride, and the relative standard deviation for 30 Tablets does not exceed 7.8%.

**pH** (791)—Transfer 5 g of ground Tablets to a suitable flask, add 50 mL of deionized water, close the flask with a stopper, and stir for about 30 minutes or until the Tablets completely disintegrate. Centrifuge to obtain a clear supernatant: the pH is between 5.5 and 7.5.

**Cholate binding capacity**—

*0.09 M Buffer solution pH 2.5*—Dissolve 12.5 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

*Mobile phase*—Prepare a mixture of *0.09 M Buffer solution pH 2.5*, acetonitrile, and methanol (50:36:14), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Sodium chloride solution*—Prepare a solution in water containing 9.0 mg of sodium chloride per mL.

*Cholate solution*—Transfer an accurately weighed quantity of sodium cholate to a suitable volumetric flask, add *Sodium chloride solution* to about 80% of the final volume, sonicate to dissolve, and dilute with *Sodium chloride solution* to volume to obtain a solution having a known concentration of 10.0 mg of sodium cholate per mL on the anhydrous basis. [NOTE—Determine the water content of sodium cholate titrimetrically at the time of use.] Adjust the solution by the dropwise addition of 0.5 N hydrochloric acid to a pH of  $6.45 \pm 0.05$ . [NOTE—Do not allow the pH to go below 6.40 at any time.] Use this solution as soon as possible after preparation.

**Test preparation**—Transfer 10 Tablets to a glass-stoppered 1.5-L flask. Add 1000.0 mL of *Cholate solution*, insert the stopper securely in the flask, and stir for 120 minutes. Filter a portion using a 0.45- $\mu\text{m}$  PVDF filter, discarding the first 5 mL of the filtrate.

**Standard preparation**—Dilute a portion of the *Cholate solution* with *Sodium chloride solution*, to obtain a solution having a known concentration of about 4.0 mg of sodium cholate per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu\text{m}$  packing L7. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates; the tailing is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the concentration of the unbound cholate,  $C_T$ , in the *Test preparation* by the formula:

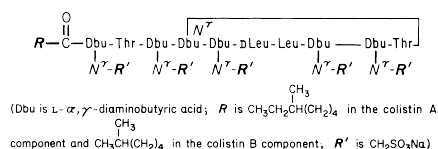
$$C_S \times (r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of sodium cholate in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cholate peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively. Calculate the cholate binding capacity, in mEq per g of the labeled amount of colestipol hydrochloride, by the formula:

$$(1000 / 430.6)(C_{CH} - C_T) / NL$$

in which 1000 is a conversion coefficient to g; 430.6 is the molecular weight of sodium cholate;  $C_{CH}$  is the concentration, in mg per mL, of sodium cholate in the *Cholate solution*;  $N$  is the number of Tablets taken to prepare the *Test preparation*; and  $L$  is the labeled amount of colestipol hydrochloride, in g per Tablet.

## Colistimethate Sodium



$\text{C}_{58}\text{H}_{105}\text{N}_{16}\text{Na}_5\text{O}_{28}\text{S}_5$  (colistin A component) 1749.82  
 $\text{C}_{57}\text{H}_{103}\text{N}_{16}\text{Na}_5\text{O}_{28}\text{S}_5$  (colistin B component) 1735.79  
 Colistimethate sodium.

Pentasodium colistinmethanesulfonate [8068-28-8; 21362-08-3].

» Colistimethate Sodium has a potency equivalent to not less than 390 µg of colistin per mg.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** ⟨11⟩—

USP Colistimethate Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

**Identification, Infrared Absorption** ⟨197K⟩.

**pH** ⟨791⟩: between 6.5 and 8.5, in a solution containing 10 mg per mL.

**Loss on drying** ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 7.0% of its weight.

**Heavy metals, Method II** ⟨231⟩: not more than 0.003%.

**Free colistin**—Dissolve 80 mg in 3 mL of water, and add 0.05 mL of silicotungstic acid solution (1 in 10): no immediate precipitate is formed.

**Other requirements**—Where the label states that Colistimethate Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Colistimethate for Injection*. Where the label states that Colistimethate Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Colistimethate for Injection*.

**Assay**—

**Assay preparation**—Dissolve a suitable quantity of Colistimethate Sodium, accurately weighed, in 2.0 mL of water, add a sufficient accurately measured volume of *Buffer No. 6* to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Colistimethate for Injection

» Colistimethate for Injection contains an amount of Colistimethate Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

**USP Reference standards** ⟨11⟩—

USP Colistimethate Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

**Bacterial endotoxins** ⟨85⟩—It contains not more than 2.0 USP Endotoxin Units per mg of colistin.

**Sterility** ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Other requirements**—It responds to the *Identification* test and meets the requirements for *pH*, *Loss on drying*, *Heavy metals*, and *Free colistin* under *Colistimethate Sodium*. It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Constituted Solutions* and *Labeling* under *Injections* ⟨1⟩.

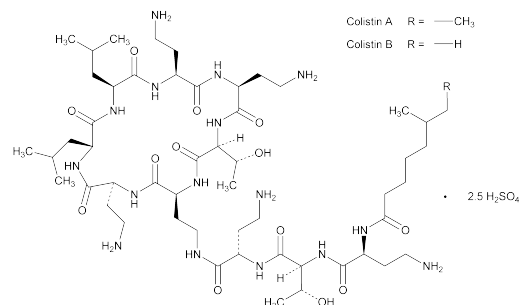
**Assay**—

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer No. 6* to obtain a solution having a convenient concentration.

**Assay preparation 2** (where the label states the quantity of colistin equivalent in a given volume of constituted solution)—Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Buffer No. 6* to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Colistin Sulfate



Colistin, sulfate.

Colistins sulfate [1264-72-8].

» Colistin Sulfate is the sulfate salt of an antibacterial substance produced by the growth of *Bacillus polymyxa* var. *colistinus*. It has a potency equivalent to not less than 500 µg of colistin per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** ⟨11⟩—

USP Colistin Sulfate RS

**Identification**—

**A:** Dissolve about 20 mg in 2 mL of buffer solution, prepared by adjusting 50 mL of 1 M monobasic potassium phosphate with 1 N sodium hydroxide to a pH of 7.0, diluting with water to 100 mL, and mixing. Add 0.2 mL of ninhydrin solution (1 in 200) and boil: a purple color is produced.

**B:** It meets the requirements of the tests for *Sulfate* ⟨191⟩.

**C: Liquid Chromatographic Identification Test**—

**Mobile phase**—Prepare a mixture of 0.1 M tribasic sodium phosphate and acetonitrile (77:23), and adjust with

phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Prepare a solution of USP Colistin Sulfate RS in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

**Test solution**—Prepare a solution of Colistin Sulfate in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 212-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. The chromatogram obtained from the *Test solution* corresponds qualitatively with that obtained from the *Standard solution*, exhibiting a major peak corresponding to colistin A and minor peaks at relative retention times of about 0.55 (colistin B) and 0.8.

**pH** <791>: between 4.0 and 7.0, in a solution containing 10 mg per mL.

**Loss on drying** <731>—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 7.0% of its weight.

**Assay**—Proceed as directed for Colistin under *Antibiotics—Microbial Assays* <81>.

## Colistin Sulfate for Oral Suspension

» Colistin Sulfate for Oral Suspension is a dry mixture of Colistin Sulfate with or without one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** <11>—

USP Colistin Sulfate RS

**Uniformity of dosage units** <905>—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>: meets the requirements.

**pH** <791>: between 5.0 and 6.0, in the suspension constituted as directed in the labeling.

**Loss on drying** <731>—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 3.0% of its weight.

**Assay**—Constitute Colistin Sulfate for Oral Suspension as directed in the labeling. Proceed as directed under *Antibiotics—Microbial Assays* <81> using an accurately measured volume of this suspension, freshly mixed and free from air bubbles, diluted quantitatively with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension

» Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is a sterile suspension containing the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of colistin, not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate (C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>). It contains one or more suitable buffers, detergents, dispersants, and preservatives.

**NOTE**—Where Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is prescribed, without reference to the quantity of colistin, neomycin, or hydrocortisone acetate contained therein, a product containing 3.0 mg of colistin, 3.3 mg of neomycin, and 10 mg of hydrocortisone acetate per mL shall be dispensed.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Colistin Sulfate RS

USP Neomycin Sulfate RS

USP Hydrocortisone Acetate RS

**Sterility** <71>: meets the requirements, 0.25 mL from each container being transferred directly to 90 mL of each medium.

**pH** <791>: between 4.8 and 5.2.

**Assay for colistin**—Proceed as directed under *Antibiotics—Microbial Assays* <81>, using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for neomycin**—Proceed as directed under *Antibiotics—Microbial Assays* <81>, using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for hydrocortisone acetate**—

**Reagent blank**—Dilute 200 mL of 22 N sulfuric acid with 100 mL of dehydrated alcohol.

**Phenylhydrazine reagent**—Dissolve 43.33 mg of phenylhydrazine hydrochloride in 100 mL of *Reagent blank*.

**Standard preparation**—Dissolve a suitable quantity of USP Hydrocortisone Acetate RS, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a solution having a known concentration of about 10 μg per mL.

**Assay preparation**—Transfer 5.0 mL of freshly mixed Otic Suspension to a 125-mL separator. Extract with three 20-mL portions of chloroform, filtering each chloroform extract through a pledget of cotton previously saturated with chloroform, collect the filtrates in a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 20 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix.

**Procedure**—Pipet 50 mL each of the *Standard* and the *Assay preparation* into separate 125-mL separators, add 2 mL of 0.1 N sodium hydroxide to each separator, shake, and allow the layers to separate. Filter both chloroform layers through glass wool, and collect the filtrates in separate beakers. Pipet two 20-mL portions of each chloroform filtrate into separate 125-mL separators. Add 25.0 mL of *Phenylhydrazine reagent* to one separator each of the filtrates from the *Standard preparation* and the *Assay preparation*, respectively, and add 25.0 mL of *Reagent blank* to the remaining two separators. Shake all four separators well, allow the layers to separate, and discard the chloroform layers. Drain the aqueous layers into separate centrifuge tubes, and centrifuge for 2 minutes. Pipet 10 mL of each solution into separate glass-stoppered test tubes. Place the tubes in a water bath maintained at a temperature of 60° for 30 minutes, then cool the solution to room temperature. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using water to set the instrument. Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in each mL of the Otic Suspension taken by the formula:

$$C(A_U - A_{UB} / A_S - A_{SB})$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*;  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* treated with *Phenylhydrazine reagent*, respectively; and  $A_{UB}$  and  $A_{SB}$  are the absorbances of the solution from the *Assay preparation* and the *Standard preparation* treated with the *Reagent blank*, respectively.

## Collodion

### DEFINITION

Collodion contains NLT 5.0%, by weight, of pyroxylin.

Pyroxylin	40 g
Ether	750 mL
Alcohol	250 mL
To make about	1000 mL

To the *Pyroxylin* in a suitable container add the *Alcohol* and *Ether*, and insert the stopper into the container. Shake the mixture occasionally until the *Pyroxylin* is dissolved.

[**CAUTION**—Collodion is highly flammable.]

### IDENTIFICATION

#### A.

**Analysis:** Expose a thin layer to air, leaving a transparent, tenacious film.

**Acceptance criteria:** The film of pyroxylin so obtained burns rapidly with a yellow flame.

#### B.

**Analysis:** Mix with an equal volume of water.

**Acceptance criteria:** A viscid, stringy mass of pyroxylin is produced.

### ASSAY

#### PROCEDURE

**Sample:** 10 mL

**Analysis:** Quickly pour the *Sample* into a tared flask, insert the stopper, and weigh the *Assay charge* accurately. Remove the stopper, warm on a steam bath, and

add 10 mL of water dropwise, with constant stirring. Evaporate the mixture on a steam bath, and dry the residue at 105° for 4 h.

**Acceptance criteria:** NLT 5.0%, by weight

### OTHER COMPONENTS

#### ALCOHOL DETERMINATION (611)

**Internal standard solution:** Acetone and 1,2-dichloroethane (20:80) in a glass-stoppered graduated cylinder  
**Standard stock solutions:** Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.

**Standard solutions:** Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate glass-stoppered, 50-mL graduated cylinders.

**Sample solution:** To 10 mL of Collodion in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 1.8-m  $\times$  3.5-mm glass; packing S3

**Temperatures**

**Column:** 150°

**Injection port:** 200°

**Detector:** 250°

**Carrier gas:** Helium

**Flow rate:** 50 mL/min

**Injection volume:** 4  $\mu\text{L}$

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Calculate the relative response factor,  $F$ , for each *Standard solution* taken:

$$F = C_S / R_S$$

$C_S$  = concentration of alcohol in the *Standard solution*, as a percentage (v/v)

$R_S$  = peak response ratio of alcohol to acetone from the respective *Standard solution*

Calculate the percentage of alcohol ( $C_2H_5OH$ ) in the portion of Collodion taken:

$$\text{Result} = R_U \times F_a$$

$R_U$  = peak response ratio of alcohol to acetone from the *Sample solution*

$F_a$  = average of the individual  $F$  values

**Acceptance criteria:** 22.0%–26.0%

### SPECIFIC TESTS

#### SPECIFIC GRAVITY (841): 0.765–0.775

#### ACIDITY

**Sample:** 5 mL

**Analysis:** Add the *Sample* to 5 mL of water.

**Acceptance criteria:** The liquid separated from the pyroxylin is not acid to litmus.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.

• **LABELING:** The label bears a caution statement to the effect that Collodion is highly flammable.

## Flexible Collodion

### DEFINITION

Prepare Flexible Collodion as follows.

Camphor	20 g
Castor Oil	30 g
Collodion, a sufficient quantity to make	1000 g

Weigh the ingredients, successively, into a dry, tared bottle. Insert the stopper in the bottle, and shake the mixture until the *Camphor* is dissolved.

### IDENTIFICATION

- **A.**  
**Analysis:** Expose a thin layer to air, leaving a transparent, tenacious film.  
**Acceptance criteria:** The film exhibits a distinct odor of camphor. The film of pyroxylin so obtained burns rapidly with a yellow flame.
- **B.**  
**Analysis:** Mix with an equal volume of water.  
**Acceptance criteria:** A viscid, stringy mass of pyroxylin is produced.

### ASSAY

- **ALCOHOL DETERMINATION (611)**  
**Internal standard solution:** Acetone and 1,2-dichloroethane (20:80) in a glass-stoppered, graduated cylinder  
**Standard stock solutions:** Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.  
**Standard solutions:** Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate, glass-stoppered, 50-mL graduated cylinders.  
**Sample solution:** To 10 mL of Flexible Collodion in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.  
**Chromatographic system**  
 (See *Chromatography (621)*, *System Suitability*).  
**Mode:** GC  
**Detector:** Thermal conductivity  
**Column:** 1.8-m × 3.5-mm glass; support S3  
**Temperatures**  
**Column:** 150°  
**Injection port:** 200°  
**Detector:** 250°  
**Carrier gas:** Helium  
**Flow rate:** 50 mL/min  
**Injection volume:** 4 µL  
**Analysis**  
**Samples:** *Standard solutions* and *Sample solution*  
 Calculate the relative response factor, *F*, for each *Standard solution* taken:

$$F = C_S/R_S$$

- $C_S$  = concentration of alcohol in the *Standard solution*, as a percentage (v/v)  
 $R_S$  = peak response ratio of alcohol to acetone from the respective *Standard solution*  
 Calculate the percentage of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the portion of Flexible Collodion taken:

$$\text{Result} = R_U \times F_A$$

- $R_U$  = peak response ratio of alcohol to acetone from the *Sample solution*  
 $F_A$  = average of the individual *F* values

Acceptance criteria: 21.0%–25.0%

### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.770–0.790

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.
- **LABELING:** The label bears a caution statement to the effect that Flexible Collodion is highly flammable.

## Colloidal Oatmeal

### DEFINITION

Colloidal Oatmeal is the powder resulting from the grinding and further processing of whole oat grain meeting U.S. Standards for Number 1 or Number 2 oats (7 CFR 810.1001).

### IDENTIFICATION

- **A.**  
**Analysis:** Prepare a smooth mixture of 10 g of Colloidal Oatmeal and 100 mL of warm water. Stir for 10 min.  
**Acceptance criteria:** The resulting slurry has a characteristic slippery feel and shows the development of slimy, viscous strands.
- **B.** A water slurry is colored reddish violet to deep blue by iodine TS.

### IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN, Total Ash (561):** NMT 2.5% on the dried basis

### SPECIFIC TESTS

- **NITROGEN DETERMINATION Method I (461):** NLT 2.0%
- **MICROBIAL ENUMERATION TESTS (61)** and **TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 10<sup>4</sup> cfu/g; the total combined molds and yeasts count does not exceed 150 cfu/g.
- **LOSS ON DRYING (731):** Dry a sample at 120° for 4 h: it loses NMT 10% of its weight.
- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING (786):** NMT 3% of the total particles exceed 150 µm in size and NMT 20% of the total particles exceed 75 µm in size.
- **FAT CONTENT**  
**Sample:** 4 g  
**Analysis:** Dry the *Sample* in vacuum at 100° for about 5 h. Extract the fat from it with anhydrous ethyl ether, using a continuous extraction apparatus, the extraction period being 4 h at a condensation rate of 5–6 drops/s. Evaporate the ether from the extract, transfer it to a tared beaker, and dry at 100° to constant weight. Perform a blank determination. Calculate the percentage of fat found, corrected for the blank.  
**Acceptance criteria:** NLT 0.2%
- **ROTATIONAL RHEOMETER METHODS (912)**

**Sample solution:** Transfer 25 g of Colloidal Oatmeal in small portions, with stirring at 1000 rpm over a 1-min period, to 500 mL of water contained in a beaker, maintained at 45° and equipped with a variable speed mixer. Stir for 5 min after the addition of the last portion of oatmeal. Allow the suspension to stand for 90 min, and equilibrate to ambient temperature. Stir the suspension at 800 rpm for 1 min.

**Apparatus:** Equip a suitable rotational viscometer with a spindle having a cylinder 1.88 cm in diameter and 6.25 cm high attached to a shaft 0.32 cm in diameter, the distance from the top of the cylinder to the lower tip of the shaft being 0.75 cm, and the immersion depth being 8.15 cm (No. 1 spindle).

**Analysis:** Determine and record the viscosity of the suspension, with the spindle rotating at 60 rpm. Convert

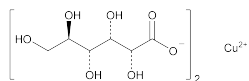
to centipoise by multiplying the reading by the constant for the viscometer spindle and speed employed.

**Acceptance criteria:** The average of three viscosities obtained is greater than 1 and less than 100 centipoises.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

### Copper Gluconate



$C_{12}H_{22}CuO_{14}$  453.84  
Copper, bis(D-gluconato-O<sup>1</sup>,O<sup>2</sup>)-;  
Copper D-gluconate (1:2) [527-09-3].

#### DEFINITION

Copper Gluconate contains NLT 98.0% and NMT 102.0% of copper gluconate ( $C_{12}H_{22}CuO_{14}$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Copper <191>:** A 50-mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**  
**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS  
**Sample solution:** 10 mg/mL of Copper Gluconate, heating in a water bath at 60°, if necessary, to dissolve  
**Chromatographic system**  
(See *Chromatography* <621>, *Thin-Layer Chromatography*.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel  
**Application volume:** 5 µL  
**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)  
**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.  
**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

#### ASSAY

- **PROCEDURE**  
**Sample:** 1.5 g of Copper Gluconate  
**Blank:** 100 mL of water  
**Titrimetric system**  
(See *Titrimetry* <541>.)  
**Mode:** Indirect titration  
**Titrant:** 0.1 N sodium thiosulfate VS  
**Endpoint detection:** Visual  
**Analysis:** Dissolve the *Sample* in 100 mL of water. Add 2 mL of glacial acetic acid and 5 g potassium iodide, mix, and titrate with *Titrant* to a light yellow color. Add 2 g of ammonium thiocyanide, and mix. Add 3 mL of starch TS, and continue titrating to a milk-white endpoint. Perform the *Blank* determination.

Calculate the percentage of copper gluconate ( $C_{12}H_{22}CuO_{14}$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $N$  = actual normality of the *Titrant* (mEq/mL)  
 $F$  = equivalency factor, 453.8 mg/mEq  
 $W$  = *Sample* weight (mg)  
**Acceptance criteria:** 98.0%–102.0%

#### IMPURITIES

- **CHLORIDE AND SULFATE, Chloride <221>**  
**Standard solution:** 1.0 mL of 0.020 N hydrochloric acid  
**Sample:** 1.0 g  
**Acceptance criteria:** NMT 0.07%
- **CHLORIDE AND SULFATE, Sulfate <221>**  
**Standard solution:** 1.0 mL of 0.020 N sulfuric acid  
**Sample:** 2.0 g  
**Acceptance criteria:** NMT 0.05%
- **ARSENIC, Method I <211>**  
**Test preparation:** 1.0 g in 35 mL of water  
**Acceptance criteria:** NMT 3 ppm
- **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

**Standard stock solution:** Transfer 10.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in *Heavy Metals* <231>, to a 100-mL volumetric flask. Add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume.

**Standard solution:** Transfer 0.40 mL of *Standard stock solution* to a 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.04 µg/mL of lead.

**Sample stock solution:** Transfer 4 g of Copper Gluconate to a 100-mL volumetric flask. Add 50 mL of water and 5 mL of nitric acid, and sonicate to dissolve the specimen. Dilute with water to volume. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.

**Blank:** Transfer 1.2 mL of nitric acid to a 100-mL volumetric flask and dilute with water to volume.

**Sample solution A:** Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of *Blank*. This solution contains 0.00 µg/mL of added lead from the *Standard solution*.

**Sample solution B:** Mix 10.0 mL of the *Sample stock solution* with 4.0 mL of the *Standard solution* and 6.0 mL of *Blank*. This solution contains 0.008 µg/mL of added lead from the *Standard solution*.

**Sample solution C:** Mix 10.0 mL of the *Sample stock solution* with 7.0 mL of the *Standard solution* and 3.0 mL of *Blank*. This solution contains 0.014 µg/mL of added lead from the *Standard solution*.

**Sample solution D:** Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of the *Standard solution*. This solution contains 0.020 µg/mL of added lead from the *Standard solution*.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Graphite furnace atomic absorption spectrophotometry

**Analytical wavelength:** 283.3 nm  
**Lamp:** Lead hollow-cathode  
**Argon flow rate:** 3 L/min, or as noted  
**Graphite tube temperature:** See Table 1.

Table 1

Temperature (°)	Time (s)
70	10
90	60
120	15
250 (no gas flow)	5
250	10
250 (no gas flow)	2
2000	3.2

**Injection volume:** 20 µL

#### Analysis

**Samples:** Blank and Sample solutions A, B, C, and D

The graphite tube is temperature-programmed to reach 2000° in about 2 min, as shown in Table 1. When the temperature reaches 2000°, determine the absorbance at 283.3 nm, corrected for background absorption. Inject the Sample solutions and Blank, and determine the absorbances. Correct the absorbance values from the Sample solutions by subtracting from each the absorbance value from the Blank. Plot the corrected absorbances of the Sample solutions versus their added lead concentrations, in µg/mL. Draw the straight line best fitting the four points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the concentration, C, in µg/mL, of lead in Sample solution A.

Calculate the content of lead in the portion of Copper Gluconate taken:

$$\text{Result} = (C \times V)/W$$

- C = concentration of lead in the Sample solution A (µg/mL), determined from the intercept of the linear regression line  
 V = volume of solvent taken to prepare the Sample solution A (mL)  
 W = weight of Calcium Gluconate taken to prepare the Sample solution A (g)

**Acceptance criteria:** NMT 25 µg/g

#### • REDUCING SUBSTANCES

**Sample:** 1.0 g of Copper Gluconate

**Blank:** 10 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back-titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the Sample to a 250-mL conical flask, add 10 mL of water to dissolve the Sample, then add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of Titrant, and 10 mL of 3 N hydrochloric acid, and titrate with Back-titrant, adding 3 mL of starch TS as the endpoint is approached. Perform the Blank determination.

Calculate the percentage of reducing substances (as dextrose) in the Sample taken:

$$\text{Result} = \{[(V_b - V_s) \times N \times F]/W\} \times 100$$

V<sub>b</sub> = Back-titrant volume consumed by the Blank (mL)

V<sub>s</sub> = Back-titrant volume consumed by the Sample (mL)

N = actual normality of the Back-titrant (mEq/mL)

F = equivalency factor, 27 mg/mEq

W = Sample weight (mg)

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Potassium Gluconate RS

## Corticotropin Injection

Corticotropin.

Corticotropin [9002-60-2].

» Corticotropin Injection is a sterile solution, in a suitable diluent, of the material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by man. Its potency is not less than 80.0 percent and not more than 125.0 percent of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a cold place.

**Labeling**—If the labeling of Injection recommends intravenous administration, include specific information on dosage.

**USP Reference standards** (11)—

USP Ascorbic Acid RS

USP Corticotropin RS

USP Endotoxin RS

USP Vasopressin RS

**Bacterial endotoxins** (85)—It contains not more than 3.1 USP Endotoxin Units per USP Corticotropin Unit.

**Vasopressin activity**—

**Phosphate buffer**—Dissolve 6.6 g of dibasic ammonium phosphate in about 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of Phosphate buffer and acetonitrile (87:13). [NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

**Standard solution**—Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of Phosphate buffer, and dilute with Phosphate buffer to obtain a final solution containing 0.1 USP Vasopressin Unit per mL.

**Test solution**—Dissolve the entire contents of a vial of the Injection in a known volume of Phosphate buffer, and dilute with Phosphate buffer to obtain a final solution containing 2.0 USP Corticotropin Units per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a variable wavelength detector set at 220 nm and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the vasopressin activity.



ity in USP Vasopressin Unit per USP Corticotropin Unit by the formula:

$$C(r_U / r_S)/2$$

in which  $C$  is the concentration, in USP Vasopressin Units per mL, of the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. The vasopressin activity is not more than 0.05 USP Vasopressin Unit per USP Corticotropin Unit.

**pH** (791): between 3.0 and 7.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Standard preparation**—Pipet 2.5 mL of gelatin TS into an opened container of USP Corticotropin RS, and mix, to obtain a solution having a concentration of 2.0 USP Corticotropin Units per mL. Using gelatin TS as a diluent, prepare three diluted standard solutions such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10 to 300 milli-units.

**Assay preparation**—In the same manner, using the same diluent, dilute the Injection to give three test solutions corresponding to those of the standard.

**The animals**—Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats with ether, and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 hours after the operation, select those rats weighing between 80 and 180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of not less than 6 rats each, and assign at random one of the three diluted standard solutions or of the three test solutions to each group.

**Procedure**—Inject all rats of each group subcutaneously with the assigned test doses. Three hours after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and comminute the glands as by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

**Ascorbic acid determination**—Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol-acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed in the next paragraph, calculate the amount of ascorbic acid in terms of mg of ascorbic acid in each 100 g of adrenal gland tissue.

Prepare a standard concentration-absorbance curve, using three standard solutions containing in each mL, respectively, 6.0, 8.0, and 10.0 µg of USP Ascorbic Acid RS in metaphosphoric acid solution (1 in 40). Pipet into each of 3 suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol-acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance in the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration-absorbance values, and draw the straight line best fitting the 3 plotted points.

**Calculation**—Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by

the symbol  $y$ , for each dosage group of  $f$  rats. If the data from one or more rats are missing, adjust to groups of equal size by suitable means (see *Replacement of Missing Values* (111)). Total the values of  $y$  in each group, and designate each total as  $T$ , subscripts 1 to 3 for the three successive dosage levels and subscripts  $S$  and  $U$  for the Standard and the Injection, respectively. Test both the agreement in slope of the dosage-response lines for the Standard and for the Injection, and the lack of curvature, as directed for a 3-dose balanced assay (see *Tests of Assay Validity* (111)). If the combined discrepancy as measured by  $F_3$  exceeds its tabular value in Table 9 (see *Combination of Independent Assays* (111)), regard these data as preliminary and repeat the assay.

Determine the logarithm of potency of the Injection by the formula:

$$M = (4iT_a / 3T_b) + \log R,$$

in which  $T_a = \Sigma(T_U - T_S)$ ;  $T_b = \Sigma(T_3 - T_1)$ ;  $i$  is the interval between successive log doses of both the *Standard preparation* and the *Assay preparation*; and  $R = v_S / v_U$  is the ratio of the high dose of the Standard in USP Units ( $v_S$ ) to the high dose of the Injection in mL ( $v_U$ ).

Compute the log confidence interval  $L$  (see *Confidence Intervals for Individual Assays* (111)).

**Replication**—Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Combination of Independent Assays* (111)). Calculate the weighted mean log-potency  $\bar{M}$  and its confidence interval,  $L_c$  (see *Confidence Intervals for Individual Assays* (111)). The potency,  $P$ , is satisfactory if  $P = \text{antilog } \bar{M}$  is not less than 80% and not more than 125% of the labeled potency and if the confidence interval does not exceed 0.40.

## Corticotropin for Injection

Corticotropin.

Corticotropin [9002-60-2].

» Corticotropin for Injection is the sterile, dry material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by man. Its potency is not less than 80.0 percent and not more than 125.0 percent of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent and suitable diluents and buffers.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**Labeling**—If the labeling of Corticotropin for Injection recommends intravenous administration, include specific information on dosage.

#### USP Reference standards (11)—

USP Ascorbic Acid RS  
USP Corticotropin RS  
USP Endotoxin RS  
USP Vasopressin RS

**Bacterial endotoxins** (85)—It contains not more than 3.1 USP Endotoxin Units per USP Corticotropin Unit.

#### Vasopressin activity—

*Phosphate buffer, Mobile phase, Standard solution, and Chromatographic system*—Prepare as directed for *Vasopressin activity* under *Corticotropin Injection*.

**Test solution**—Dissolve the entire contents of a vial of Corticotropin for Injection in a known volume of *Phosphate buffer*, and dilute with *Phosphate buffer* to obtain a final solution containing 2.0 USP Corticotropin Units per mL.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the vasopressin activity in USP Vasopressin Unit per USP Corticotropin Unit by the formula:

$$C(r_u / r_s)/2$$

in which C is the concentration, in USP Vasopressin Units per mL, of the *Standard solution*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. The vasopressin activity is not more than 0.05 USP Vasopressin Unit per USP Corticotropin Unit.

**pH** (791): between 2.5 and 6.0, in a solution constituted as directed in the labeling supplied by the manufacturer.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and *Constituted Solutions and Labeling under Injections* (1).

**Assay**—Proceed with the constituted solution of Corticotropin for Injection as directed in the Assay under *Corticotropin Injection*.

## Repository Corticotropin Injection

Corticotropin.  
Corticotropin [9002-60-2].

» Repository Corticotropin Injection is corticotropin in a solution of partially hydrolyzed gelatin. Its potency is not less than 80.0 percent and not more than 125.0 percent of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Ascorbic Acid RS  
USP Corticotropin RS  
USP Endotoxin RS  
USP Vasopressin RS

**Bacterial endotoxins** (85)—It contains not more than 3.1 USP Endotoxin Units per USP Corticotropin Unit.

**Vasopressin activity, pH, and Assay**—It meets the requirements for *Vasopressin activity*, *pH*, and *Assay* under *Corticotropin Injection*.

**Other requirements**—It meets the requirements under *Injections* (1).

## Corticotropin Zinc Hydroxide Injectable Suspension

Corticotropin zinc hydroxide.  
Corticotropin zinc hydroxide [9050-75-3].

» Corticotropin Zinc Hydroxide Injectable Suspension is a sterile suspension of corticotropin adsorbed on zinc hydroxide. Its potency is not less than 80.0 percent and not more than

125.0 percent of the potency stated on the label in USP Corticotropin Units. It contains not less than 1800  $\mu$ g and not more than 2200  $\mu$ g of zinc, and not less than 604  $\mu$ g and not more than 776  $\mu$ g of anhydrous dibasic sodium phosphate, for each 40 USP Corticotropin Units.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.

**Labeling**—Label it to indicate that it is not recommended for intravenous use and that the suspension is to be well shaken before use. The container label and the package label state the potency in USP Corticotropin Units in each mL.

**USP Reference standards** (11)—

USP Ascorbic Acid RS  
USP Corticotropin RS  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 3.1 USP Endotoxin Units per USP Corticotropin Unit.

**pH** (791): between 7.5 and 8.5, determined potentiometrically.

**Zinc**—Pipet a volume of the well-shaken Injectable Suspension, equivalent to about 6 mg of zinc, into a 125-mL conical flask, and add 2 mL of a buffer mixture containing 5.4 g of ammonium chloride and 26 mL of ammonium hydroxide in each 100 mL. Add 10 mL of water and 2 drops of eriochrome black TS, and titrate with 0.005 M edetate disodium VS to a clear blue endpoint. Perform a blank titration, and make any necessary correction. Each mL of 0.005 M edetate disodium is equivalent to 0.327 mg of Zn.

**Anhydrous dibasic sodium phosphate**—

**Ammonium molybdate reagent**—Dissolve 6.4 g of ammonium molybdate in 40 mL of water, and add 50 mL of 10 N sulfuric acid. Mix, and dilute with water to 100 mL. This solution is stable for about 2 weeks.

**Stannous chloride reagent**—Dissolve 1 g of stannous chloride in 5 mL of hydrochloric acid. Just prior to use, dilute 1 mL of this solution with water to 100 mL.

**Standard preparation**—Weigh accurately about 275 mg of anhydrous dibasic sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) into a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Dilute 10 mL of this solution with water to 100 mL.

**Test preparation**—Pipet 1 mL of the well-shaken Injectable Suspension into a 25-mL volumetric flask, add 0.1 mL of 10 N sulfuric acid, mix, dilute with water to volume, and again mix.

**Procedure**—Into separate 100-mL volumetric flasks pipet duplicate 10-mL portions of *Test preparation*, duplicate 10-mL portions of *Standard preparation*, and 10 mL of water to provide a blank. Treat each flask as follows. Dilute the contents with water to 60 mL, then add 10 mL of 10 N sulfuric acid, and mix. Add 10 mL of *Ammonium molybdate reagent*, mix, add 10 mL of water, and again mix. Add slowly, with mixing, 5 mL of *Stannous chloride reagent*, dilute with water to volume, and mix. Measure the absorbances of the solutions at 10 minutes, accurately timed, after the first addition of the *Stannous chloride reagent*, at a wavelength of 710 nm, with a suitable spectrophotometer, relative to the blank. Calculate the quantity, in  $\mu$ g, of anhydrous dibasic sodium phosphate in the portion of Injectable Suspension taken by the formula:

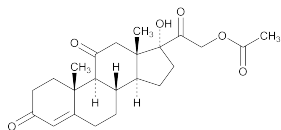
$$25C(A_u / A_s)$$

in which C is the concentration, in  $\mu$ g per mL, of  $\text{Na}_2\text{HPO}_4$  in the *Standard preparation*; and  $A_u$  and  $A_s$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Add sufficient 0.1 N hydrochloric acid to Injectable Suspension to effect complete solution, and using this in making the *Assay preparation*, proceed as directed in the Assay under *Corticotropin Injection*.

## Cortisone Acetate



$C_{23}H_{30}O_6$  402.48  
Pregn-4-ene-3,11,20-trione, 21-(acetyloxy)-17-hydroxy-;  
17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate  
[50-04-4].

### DEFINITION

Cortisone Acetate contains NLT 97.0% and NMT 102.0% of cortisone acetate ( $C_{23}H_{30}O_6$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Dissolve in methanol, evaporate the methanol on a steam bath, and dry at 105° for 30 min.

**Acceptance criteria:** Meets the requirements

#### • B. ULTRAVIOLET ABSORPTION (197U)

**Analytical wavelength:** 238 nm

**Medium:** Methanol

**Sample solution:** 10 µg/mL

**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (45:55)

**Buffer:** Transfer 20 mL of 1 N hydrochloric acid, 150 mL of 0.5 N potassium chloride, and 50 mL of 0.5 M sodium acetate to a 1-L volumetric flask, and dilute with water to volume.

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 0.1 mg/mL of USP Cortisone Acetate RS in *Diluent*, prepared as follows. Transfer 25 mg of USP Cortisone Acetate RS to a 250-mL volumetric flask, and dissolve in 100 mL of *Diluent*. Sonicate until a clear solution is obtained, and dilute with *Diluent* to volume.

**Sample solution:** 0.1 mg/mL of Cortisone Acetate in *Diluent*, prepared as follows. Transfer 25 mg of Cortisone Acetate to a 250-mL volumetric flask, and dissolve in 100 mL of *Diluent*. Sonicate until a clear solution is obtained, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; 10-µm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 2.0

**Capacity factor, *k'*:** NLT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cortisone Acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION (281):** Negligible, from 100 mg

#### • ORGANIC IMPURITIES

**Solution A:** Acetonitrile and water (3:7)

**Solution B:** Acetonitrile and water (7:3)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
25	10	90
30	10	90
31	90	10
51	90	10

**Diluent:** A filtered mixture of acetonitrile, glacial acetic acid, and water (7: 0.1: 3)

**Standard solution:** 20 µg/mL of USP Cortisone Acetate RS in *Diluent*

**Sample solution:** 2.5 mg/mL of Cortisone Acetate in *Diluent*. Sonicate to dissolve.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 5.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of the major peak from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cortisone Acetate in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Any individual impurity:** NMT 1.5%

**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

#### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 10 mg/mL in dioxane

**Acceptance criteria:** +208° to +217°

#### • LOSS ON DRYING (731)

**Sample:** Dry a sample at 105° for 30 min.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)  
USP Cortisone Acetate RS

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**Cortisone Acetate Injectable Suspension**


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» Cortisone Acetate Injectable Suspension is a sterile suspension of Cortisone Acetate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—  
USP Cortisone Acetate RS

**Identification**—Mix 25 mL of water with a volume of Injectable Suspension equivalent to about 25 mg of cortisone acetate. Centrifuge, or allow the insoluble material to settle, then decant and discard the supernatant. Add 20 mL of methanol and, using agitation and warming as necessary, dissolve the residue. Evaporate the solvent on a steam bath with the aid of a current of air, then dry the residue at 105° for 30 minutes; the residue so obtained responds to *Identification test A* under *Cortisone Acetate*.

**pH** (791): between 5.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase*—Prepare as directed in the *Assay* under *Cortisone Acetate Tablets*.

*Internal standard solution*—Prepare a solution of prednisone in *Mobile phase* having a concentration of 0.5 mg per mL.

*Standard preparation*—Transfer about 12 mg of USP Cortisone Acetate RS, accurately weighed, to a stoppered, 50-mL conical flask. Add 20.0 mL of *Internal standard solution*, and sonicate for 5 minutes. Pass a portion through a polytetrafluoroethylene syringe filter, then combine 1 mL of the filtrate and 4 mL of *Mobile phase* to obtain the *Standard preparation*.

*Resolution solution*—Dissolve a quantity of hydrocortisone acetate in the *Standard preparation* to obtain a solution containing about 0.1 mg of hydrocortisone acetate per mL.

*Assay preparation*—Using a pipet calibrated “to contain,” transfer 2.0 mL of freshly mixed Injectable Suspension to a volumetric flask of a size to give a cortisone acetate concentration of 2 mg per mL when diluted to volume. Rinse the suspension remaining in the pipet into the flask with isopropyl alcohol, dilute with isopropyl alcohol to volume, and sonicate for 3 minutes. Deliver a 3.0-mL aliquot of this solution to a stoppered, 25-mL conical flask, and evaporate on a steam bath with the aid of a current of air to dryness. Add 10.0 mL of *Internal standard solution*, insert the stopper, and sonicate for 5 minutes. Pass a portion through a polytetrafluoroethylene syringe filter, then combine approximately 1 mL of the filtrate and 4 mL of *Mobile phase* to obtain the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the *Assay* under *Cortisone Acetate Tablets*. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cortisone acetate and hydrocortisone acetate is not less than 2.2 (if necessary, add equal parts of *n*-butyl chlo-

ride and water-saturated *n*-butyl chloride to the *Mobile phase* to meet this requirement). Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are 0.6 for cortisone acetate and 1.0 for prednisone; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Proceed as directed in the *Assay* under *Cortisone Acetate Tablets*. Calculate the quantity, in mg, of cortisone acetate ( $C_{23}H_{30}O_6$ ) in each mL of the Injectable Suspension taken by the formula:

$$W(V/12)(R_U/R_S)$$

in which  $V$  is the capacity, in mL, of the volumetric flask used for the *Assay preparation*; and the other terms are as defined therein.

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**Cortisone Acetate Tablets**


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**DEFINITION**

Cortisone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Powder a number of Tablets equivalent to 25 mg of cortisone acetate. Add 25 mL of solvent hexane, and extract for 15 min with occasional agitation. Decant and discard the supernatant, then extract the residue with 5 mL of chloroform, with frequent agitation, for 5 min. Filter, add 10 mL of methanol to the filtrate, mix, evaporate the solvent on a steam bath with the aid of a current of air, then dry the residue at 105° for 30 min. Use the residue.

**Acceptance criteria:** Meet the requirements

**ASSAY**

- **PROCEDURE**

**Mobile phase:** *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

**Internal standard solution:** 0.04 mg/mL of methylparaben in *Mobile phase*

**Standard stock solution:** Transfer 12 mg of USP Cortisone Acetate RS to a glass-stoppered, 50-mL conical flask, add 25.0 mL of the *Internal standard solution*, and sonicate for 5 min.

**Standard solution:** Nominally 0.12 mg/mL of USP Cortisone Acetate RS, prepared by combining 1 mL of the *Standard stock solution* with 3 mL of *Mobile phase*

**System suitability solution:** 0.1 mg/mL of hydrocortisone acetate in the *Standard solution*

**Sample stock solution:** Weigh, then finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 12 mg of cortisone acetate, to a stoppered conical flask. Add 25.0 mL of the *Internal standard solution*, insert the stopper into the flask, and sonicate vigorously for 5 min. Pass a portion through a polytetrafluoroethylene syringe filter.

**Sample solution:** 0.12 mg/mL of cortisone acetate, prepared by combining 1 mL of the *Sample stock solution* filtrate and 3 mL of *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 1 mL/min

**Injection volume:** 15  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for methylparaben and cortisone acetate are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*. If necessary, add equal parts of *n*-butyl chloride and water-saturated *n*-butyl chloride to the *Mobile phase* to meet this requirement.

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cortisone acetate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cortisone acetate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cortisone acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISSOLUTION** <711>

**Medium:** 0.5% sodium lauryl sulfate solution; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** 5.55  $\mu$ g/mL of USP Cortisone Acetate RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 242 nm

**Cell size:** 1 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 75% (Q) of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905)

**Procedure for content uniformity**

**Mobile phase, Internal standard solution, Standard solution, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample stock solution:** Place 1 Tablet in a stoppered, 50-mL conical flask, deposit 0.25 mL of water onto the Tablet, insert the stopper into the flask, and allow to stand for 30 min. Add 2.5 mL of isopropyl alcohol, and place the unstoppered flask on a steam bath. Boil gently, if necessary, until the Tablet disintegrates, then evaporate the solvent with the aid of a current of air. Remove from the steam bath, add 10.0 mL of the *Internal standard solution* for each 5 mg of cortisone acetate declared, insert the stopper, and sonicate vigorously for 10 min. Pass a portion through a polytetrafluoroethylene syringe filter.

**Sample solution:** Combine 1 mL of the *Sample stock solution* filtrate and 3 mL of *Mobile phase*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cortisone acetate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cortisone acetate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cortisone acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** <11>  
USP Cortisone Acetate RS

## Purified Cotton

» Purified Cotton is the hair of the seed of cultivated varieties of *Gossypium hirsutum* Linné, or of other species of *Gossypium* (Fam. Malvaceae), freed from adhering impurities, deprived of fatty matter, bleached, and sterilized in its final container.

**Packaging and storage**—Package it in rolls of not more than 500 g of a continuous lap, with a light-weight paper running under the entire lap, the paper being of such width that it may be folded over the edges of the lap to a distance of at least 25 mm, the two together being tightly and evenly rolled, and enclosed and sealed in a well-closed container. It may be packaged also in other types of containers if these are so constructed that the sterility of the product is maintained.

**Labeling**—Its label bears a statement to the effect that the sterility cannot be guaranteed if the package bears evidence of damage or if the package has been opened previously.

**Sterility** <71>: meets the requirements.

**Acidity or alkalinity**—Thoroughly saturate about 10 g with 100 mL of recently boiled and cooled water, then with the aid of a glass rod press out two 25-mL portions of the water into white porcelain dishes. To one portion add 3 drops of phenolphthalein TS, and to the other portion add 1 drop of methyl orange TS: no pink color develops in either portion.

**Residue on ignition** <281>—Place about 5 g, accurately weighed, in a porcelain or platinum dish, and moisten with 2 N sulfuric acid. Gently heat the cotton until it is charred, then ignite more strongly until the carbon is completely consumed: not more than 0.20% of residue remains.

**Water-soluble substances**—Place 10 g, accurately weighed, in a beaker containing 1000 mL of water, and boil gently for 30 minutes, adding water as required to maintain the volume. Pour the water through a funnel into another vessel, and press out the excess water from the cotton with a glass rod. Wash the cotton in the funnel with two 250-mL portions of boiling water, pressing the cotton after each washing. Filter the combined extract and washings, and wash the filter thoroughly with hot water. Evaporate the combined extract and washings to a small volume, transfer to a tared porcelain or platinum dish, evaporate to dryness,

and dry the residue at 105° to constant weight: the residue weighs not more than 35 mg (0.35%).

**Fatty matter**—Pack 10 ± 0.01 g in a Soxhlet extractor provided with a tared receiver, and extract with ether for 5 hours at a rate such that the ether siphons over not less than four times per hour. The ether solution in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at 105° for 1 hour: the weight of the residue does not exceed 70 mg (0.7%).

**Dyes**—Pack about 10 g in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL: when observed downward through a column 20 cm in depth, the percolate may show a yellowish color, but neither a blue nor a green tint.

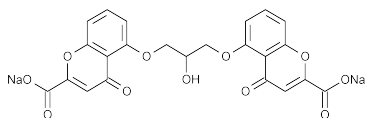
**Other foreign matter**—The pinches of it taken for the determination of *Fiber length* contain no oil stains or metallic particles.

**Fiber length and Absorbency**—Remove it from its wrappings, and condition it for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° (70 ± 2°F), before determining the *Fiber length* and *Absorbency*.

**Fiber length**—Determine the fiber length of Purified Cotton as directed under *Cotton—Fiber Length* (691): not less than 60% of the fibers, by weight, are 12.5 mm or greater in length, and not more than 10% of the fibers, by weight, are 6.25 mm or less in length.

**Absorbency**—Proceed as directed under *Cotton—Absorbency Test* (691): submersion is complete in 10 seconds at a temperature of 25°, and the cotton retains not less than 24 times its weight of water.

## Cromolyn Sodium



C<sub>23</sub>H<sub>14</sub>Na<sub>2</sub>O<sub>11</sub> 512.33

4*H*-1-Benzopyran-2-carboxylic acid, 5,5'-[(2-hydroxy-1,3-propanediyl)bis(oxy)bis[4-oxo-, disodium salt].

Disodium 5,5'-[(2-hydroxytrimethylene)dioxy]bis[4-oxo-4*H*-1-benzopyran-2-carboxylate] [15826-37-6].

» Cromolyn Sodium contains not less than 98.0 percent and not more than 101.0 percent of C<sub>23</sub>H<sub>14</sub>Na<sub>2</sub>O<sub>11</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cromolyn Sodium RS

**Identification**—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it, previously dried in vacuum at 105° to constant weight, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cromolyn Sodium RS.

**B:** The UV absorption spectrum of a 1 in 40,000 solution in *pH 7.4 sodium phosphate buffer* prepared as directed in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Cromolyn Sodium RS, concomitantly measured.

**Acidity or alkalinity**—Dissolve 1.0 g in 25 mL of carbon dioxide-free water, and add two drops of bromothymol blue TS. If the solution is yellow, not more than 0.25 mL of 0.1 N sodium hydroxide is required to produce a blue color. If the solution is blue, not more than 0.25 mL of 0.1 N hydrochloric acid is required to produce a yellow color.

**Water**, *Method I* (921): not more than 10.0%.

**Heavy metals**, *Method II* (231): 0.002%.

**Limit of oxalate**—Dissolve 100 mg in 20 mL of water, add 5.0 mL of iron salicylate TS, and dilute with water to 50 mL. Determine the absorbance of the solution at 480 nm against a water blank. The absorbance is not less than that of a solution containing 350 µg of oxalic acid prepared in the same manner (0.35%).

**Related compounds**—Dissolve 100 mg of Cromolyn Sodium in 10.0 mL of a mixture of water, stabilizer-free tetrahydrofuran, and acetone (6:4:1). Similarly prepare a solution of USP Cromolyn Sodium RS in the same solvent mixture having a concentration of 10 mg per mL (*Standard solution A*). Quantitatively dilute a volume of *Standard solution A* with the same solvent mixture to obtain a diluted standard solution having a concentration of 0.05 mg per mL (*Standard solution B*). Apply 10-µL portions of the test solution, *Standard solution A*, and *Standard solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (9:9:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the *R<sub>f</sub>* value of the principal spot obtained from the test solution corresponds to that obtained from *Standard solution A*. Any spot in the chromatogram obtained from the test solution moving ahead of the principal spot is not more intense than the spot in the chromatogram obtained from *Standard solution B* (0.5%).

**Assay**—

*pH 7.4 Sodium phosphate buffer*—Dissolve 70 g of anhydrous dibasic sodium phosphate in 900 mL of water. Adjust to a pH of 7.4 by the addition of dilute phosphoric acid (1 in 10). Dilute with water to 1000 mL, and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cromolyn Sodium RS in water to obtain a solution having a known concentration of about 250 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix. The final concentration is about 25 µg per mL.

**Assay preparation**—Transfer about 100 mg of Cromolyn Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in about 100 mL of water, dilute with water to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a 1 in 100 solution of *pH 7.4 Sodium phosphate buffer* as the blank. Calculate the quantity, in mg, of C<sub>23</sub>H<sub>14</sub>Na<sub>2</sub>O<sub>11</sub> in the Cromolyn Sodium taken by the formula:

$$4C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Cromolyn Sodium RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Cromolyn Sodium Inhalation Powder

» Cromolyn Sodium Inhalation Powder is a mixture of equal parts of Lactose and Cromolyn Sodium contained in a hard gelatin capsule. It contains not less than 95.0 percent and not more than 125.0 percent of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature. Avoid excessive heat.

### USP Reference standards (11)—

USP Cromolyn Sodium RS

**Identification**—It meets the requirements of *Identification test B* under *Cromolyn Sodium*.

**Uniformity of dosage units** (905): meets the requirements.

### Assay—

*pH 7.4 Sodium phosphate buffer*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Assay preparation*—Remove and accurately weigh the contents of not fewer than 20 capsules of Cromolyn Sodium Inhalation Powder, and transfer the combined contents to a 250-mL volumetric flask. Dissolve in 100 mL of water, dilute with water to volume, and mix. Transfer an aliquot of this solution, equivalent to 8 mg of cromolyn sodium, to a 250-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

*Standard preparation*—Dissolve a suitable quantity of USP Cromolyn Sodium RS, previously dried in vacuum at 105° to constant weight and accurately weighed, in water to obtain a solution having a known concentration of about 350 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix. The final concentration is about 35 µg per mL.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a solution of *pH 7.4 Sodium phosphate buffer* (1 in 250) as the blank. Calculate the quantity, in mg, of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ) in the aliquot taken by the formula:

$$0.25C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Cromolyn Sodium RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Cromolyn Sodium Inhalation Solution

» Cromolyn Sodium Inhalation Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{23}H_{14}Na_2O_{11}$ .

**Packaging and storage**—Preserve in single-unit double-ended glass ampuls or in low-density polyethylene ampuls.

**Labeling**—The label indicates that the Inhalation Solution is not to be used if it contains a precipitate.

### USP Reference standards (11)—

USP Cromolyn Sodium RS

**Identification**—The UV absorption spectrum of the *Assay preparation* prepared as directed in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Cromolyn Sodium RS, concomitantly measured.

**Sterility** (71): meets the requirements.

**Uniformity of dosage units** (905): meets the requirements.

**pH** (791): between 4.0 and 7.0.

**Related compounds**—Apply 10-µL portions of Inhalation Solution and Standard solutions of USP Cromolyn Sodium RS in a mixture of water, stabilizer-free tetrahydrofuran, and acetone (6:4:1) containing 10 mg per mL (*Standard solution A*) and 0.1 mg per mL (*Standard solution B*) to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (9:9:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the *R<sub>f</sub>* value of the principal spot obtained from the Inhalation Solution corresponds to that obtained from *Standard solution A*. Any spot in the chromatogram obtained from the Inhalation Solution moving ahead of the principal spot is not more intense than the spot in the chromatogram obtained from *Standard solution B* (1.0%).

### Assay—

*pH 7.4 Sodium phosphate buffer* and *Standard preparation*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Assay preparation*—Dilute with water an accurately measured volume of Inhalation Solution, equivalent to about 25 mg of cromolyn sodium, to obtain a solution having a concentration of about 250 µg per mL. Pipet 10 mL of this solution into a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a 1 in 100 aqueous solution of *pH 7.4 Sodium phosphate buffer* as the blank. Calculate the quantity, in mg, of  $C_{23}H_{14}Na_2O_{11}$  in each mL of the Inhalation Solution taken by the formula:

$$(C/V)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Cromolyn Sodium RS in the *Standard preparation*; *V* is the volume, in mL, of Inhalation Solution taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cromolyn Sodium Nasal Solution

» Cromolyn Sodium Nasal Solution is an aqueous solution of Cromolyn Sodium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{23}H_{14}Na_2O_{11}$ . It may contain suitable stabilizers.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Cromolyn Sodium RS

**Identification**—It meets the requirements for *Identification* test B under *Cromolyn Sodium*.

**pH** (791): between 4.0 and 7.0.

**Related compounds**—It meets the requirements of the test for *Related compounds* under *Cromolyn Sodium Inhalation Solution*, “Nasal Solution” being read in place of “Inhalation Solution.”

**Assay**—

*pH 7.4 Sodium phosphate buffer*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Assay preparation*—Transfer 4 mL of Nasal Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer an aliquot of this solution, equivalent to 8 mg of cromolyn sodium, to a 250-mL volumetric flask. Add 2.5 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

*Standard preparation*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cromolyn Sodium Inhalation Solution*.

## Cromolyn Sodium Ophthalmic Solution

» Cromolyn Sodium Ophthalmic Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{23}H_{14}Na_2O_{11}$ . It may contain suitable antimicrobial and stabilizing agents.

**Packaging and storage**—Preserve in tight, light-resistant, single-dose or multiple-dose containers. Ophthalmic Solution that is packaged in multiple-dose containers contains a suitable antimicrobial agent.

**USP Reference standards** (11)—

USP Cromolyn Sodium RS

**Identification**—It meets the requirements for *Identification* test B under *Cromolyn Sodium*.

**Sterility** (71): meets the requirements.

**pH** (791): between 4.0 and 7.0.

**Related compounds**—It meets the requirements of the test for *Related compounds* under *Cromolyn Sodium Inhalation Solution*, “Ophthalmic Solution” being read in place of “Inhalation Solution.”

**Assay**—

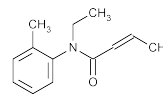
*pH 7.4 Sodium phosphate buffer*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Assay preparation*—Transfer 4 mL of Ophthalmic Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer an aliquot of this solution, equivalent to 8 mg of cromolyn sodium, to a 250-mL volumetric flask. Add 2.5 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

*Standard preparation*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cromolyn Sodium Inhalation Solution*.

## Crotamiton



$C_{13}H_{17}NO$  203.28

2-Butenamide, *N*-ethyl-*N*-(2-methylphenyl)-.

*N*-Ethyl-*o*-crotonotoluidide [483-63-6].

» Crotamiton is a mixture of *cis* and *trans* isomers containing not less than 97.0 percent and not more than 103.0 percent of  $C_{13}H_{17}NO$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Crotamiton RS

**Identification**—

**A: Infrared Absorption** (197F).

**B: Ultraviolet Absorption** (197U)—

*Solution*: 20  $\mu$ g per mL.

*Medium*: cyclohexane.

**C**: To about 10 mL of a saturated solution in water add a few drops of potassium permanganate TS: a brown color is produced, and a brown precipitate is formed on standing.

**Specific gravity** (841): between 1.008 and 1.011 at 20°.

**Refractive index** (831): between 1.540 and 1.543 at 20°.

**Residue on ignition** (281): not more than 0.1%.

**Bound halogen**—Place 4 drops in a 3-mm (ID) test tube, and add calcium oxide to a height of 1 cm. Heat the tube in a flame, starting from the top, until the reaction is complete, then ignite for a short time. Transfer the contents to a beaker containing 10 mL of water, acidify with nitric acid, and filter. To the filtrate add 0.2 mL of silver nitrate solution (1 in 60): any opalescence obtained is not more than that obtained from a blank solution treated in the same manner.

**Assay**—Transfer about 50 mg of Crotamiton, accurately weighed, to a 100-mL volumetric flask, add cyclohexane to volume, and mix. Transfer 10.0 mL of this solution to a 250-mL volumetric flask, dilute with cyclohexane to volume, and mix. Determine the absorbance of this solution and of a solution of USP Crotamiton RS in the same medium having a known concentration of about 20  $\mu$ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of  $C_{13}H_{17}NO$  in the Crotamiton taken by the formula:

$$2.5C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Crotamiton RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

## Crotamiton Cream

» Crotamiton Cream contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{13}H_{17}NO$ .

**Packaging and storage**—Preserve in collapsible tubes or tight, light-resistant containers.



**USP Reference standards** (11)—

USP Crodamiton RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay preparation*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Internal standard solution*—Dissolve butyl benzoate in methanol to obtain a solution containing about 17.5 mg per mL.

*Mobile phase*—Prepare a suitable degassed and filtered mixture of acetonitrile and water (3:2).

*Standard solution*—Dissolve a suitable quantity of USP Crodamiton RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 1 mg per mL.

*Standard preparation*—Pipet 10 mL of *Standard solution* and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Assay preparation*—Transfer an accurately weighed portion of Crodamiton Cream, equivalent to about 50 mg of crodamiton, to a tared 50-mL volumetric flask. Add about 25 mL of methanol, and shake and sonicate to disperse the cream. Dilute with methanol to volume, and mix. Filter about 20 mL through moderately retentive filter paper. Pipet 10 mL of the clear filtrate and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.

*Procedure*—Inject equal volumes of the *Standard preparation* and the *Assay preparation* into a liquid chromatograph (see *Chromatography* (621)) equipped with a 254-nm detector and a 4.6-mm × 25-cm stainless steel column that contains packing L1. In a suitable chromatogram, the resolution,  $R$ , between peaks for crodamiton and butyl benzoate is not less than 3.0; and the lowest and highest peak response ratios ( $R_s$ ) of three replicate injections of the *Standard preparation* agree within 2.0%. Calculate the quantity, in mg, of  $C_{13}H_{17}NO$  in the portion of Cream taken by the formula:

$$250C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Crodamiton RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the crodamiton peak and the butyl benzoate peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Cupric Chloride**

$CuCl_2 \cdot 2H_2O$  170.48

$CuCl_2$  134.45

Copper chloride ( $CuCl_2$ ) dihydrate;  
Copper(2+) chloride dihydrate [10125-13-0].  
Anhydrous [7447-39-4].

**DEFINITION**

Cupric Chloride contains NLT 99.0% and NMT 100.5% of  $CuCl_2$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A solution (1 in 20) meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Copper** (191): A solution (1 in 20) meets the requirements.

**ASSAY**• **PROCEDURE**

**Sample solution:** 8 mg/mL of Cupric Chloride in water

**Analysis:** To the *Sample solution* add 4 mL of acetic acid and 3 g of potassium iodide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 13.45 mg of Cupric Chloride ( $CuCl_2$ ).

**Acceptance criteria:** 99.0%–100.5% on the dried basis

**IMPURITIES**• **LIMIT OF SODIUM**

**Sample stock solution:** Transfer 10.0 g of Cupric Chloride to a 100-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.1 g/mL of cupric chloride.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* (851)), analyze the *Sample solutions* by the method of standard addition analysis according to Table 1.

**Acceptance criteria:** NMT 0.02%

Table 1

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Background Correction
Sodium	589.0	0.1	0.01/0.02	Air-acetylene	No
Potassium	766.5	0.1	0.01/0.02	Air-acetylene	No
Calcium	422.7	2.0	0.05/0.10	Air-acetylene	No
Iron	248.3	2.0	0.05/0.10	Air-acetylene	Yes
Nickel	232.0	2.0	0.10/0.20	Air-acetylene	No

• **LIMIT OF POTASSIUM**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* (851)), analyze the *Sample solutions* by the method of standard addition analysis according to Table 1.

**Acceptance criteria:** NMT 0.01%

• **LIMIT OF CALCIUM**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.005%

• **LIMIT OF IRON**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.005%

• **LIMIT OF NICKEL**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.01%

• **INSOLUBLE MATTER**

**Sample:** 10 g of Cupric Chloride

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water and 2 mL of hydrochloric acid. Cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 1 h, and pass through a tared, filtering crucible of fine-pore size. Rinse the beaker with hot water, passing the rinsings through the filter, and finally wash the filter with additional hot water. Retain the combined filtrate and washings for the test for *Limit of Sulfate*. Dry the filter at 105°.

**Acceptance criteria:** The residue weighs NMT 1.0 mg (0.01%).

• **LIMIT OF SULFATE**

**Analysis:** Heat to boiling the combined filtrate and washings retained from the test for *Insoluble Matter*. Add 10 mL of barium chloride TS, digest for 2 h on a steam bath, and allow to stand overnight. Pass the solution through a tared, porcelain filtering crucible of medium-pore size, and wash the residue with two 10-mL portions of hot water. Ignite at 800 ± 25° to constant weight.

**Acceptance criteria:** The weight of the residue, corrected for the weight obtained in a blank test, does not exceed 1.2 mg (0.005%).

**SPECIFIC TESTS**

- **LOSS ON DRYING** <731>: Dry a sample at 105° for 16 h: it loses 20.9%–21.4% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

## Cupric Chloride Injection

**DEFINITION**

Cupric Chloride Injection is a sterile solution of Cupric Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of copper (Cu).

**IDENTIFICATION**

- **A.** The *Sample solution* exhibits an absorption maximum at about 325 nm when prepared and tested as directed in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Sodium chloride solution:** 1.35 g/L of sodium chloride

**Standard stock solution:** Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. This solution contains 1000 µg/mL of copper. Store in a polyethylene bottle.

**Standard solutions:** Pipet 15 mL of *Standard stock solution* into a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0, 5.0, and 6.0 mL of this solution to separate 100-mL volumetric flasks containing 10 mL of *Sodium chloride solution*, dilute the contents of each flask with water to volume, and mix.

These *Standard solutions* contain 2.4, 3.0, and 3.6 µg of copper per mL, respectively.

**Sample stock solution:** Transfer a volume of Injection, equivalent to 2 mg of copper, into 100 mL of water.

**Sample solution:** Pipet 15 mL of the *Sample stock solution* into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium content of this flask to 13.5 mg. Dilute with water to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption

**Analytical wavelength:** 324.8 nm (copper emission line)

**Lamp:** Copper hollow-cathode

**Flame:** Air-acetylene

**Blank:** *Sodium chloride solution* and water (1 in 10)

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*  
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of copper, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Injection taken:

$$\text{Result} = [(C/V) \times F \times V_1 \times D] \times (100/L)$$

*C* = concentration of copper in the *Sample solution* (µg/mL)

*V* = volume of Injection (mL)

*F* = conversion factor from µg to mg, 1/1000

*V*<sub>1</sub> = volume of the *Sample stock solution*, 100 mL

*D* = dilution factor from the *Sample solution*, 100/15

*L* = label claim (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** <791>: 1.5–2.5
- **BACTERIAL ENDOTOXINS TEST** <85>: It contains NMT 250.0 USP Endotoxin Units/mg of copper.
- **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* <1>

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
- **LABELING:** Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid before administration.

- **USP REFERENCE STANDARDS** <11>  
USP Endotoxin RS

## Cupric Sulfate

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  249.69

$\text{CuSO}_4$  159.61

Sulfuric acid, copper(2+) salt (1:1), pentahydrate;  
Copper(2+) sulfate (1:1) pentahydrate [7758-99-8].  
Anhydrous [7758-98-7].

### DEFINITION

Cupric Sulfate, dried at 250° to constant weight, contains NLT 98.5% and NMT 100.5 % of  $\text{CuSO}_4$ .

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** <191>: A solution (1 in 10) meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Copper** <191>: A solution (1 in 10) meets the requirements.

### ASSAY

#### PROCEDURE

**Sample solution:** Place 650 mg of Cupric Sulfate in a weighed container fitted with a ground-glass stopper. Dry, allow to cool in a desiccator, and weigh again to obtain the weight of the sample. Dissolve in 50 mL of water.

**Analysis:** To the *Sample solution* add 4 mL of 6 N acetic acid and 3 g of potassium iodide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding about 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 15.96 mg of cupric sulfate ( $\text{CuSO}_4$ ).

**Acceptance criteria:** 98.5%–100.5% on the dried basis

### IMPURITIES

#### LIMIT OF SODIUM

**Sample stock solution:** Transfer 40.0 g of Cupric Sulfate to a 200-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.2 g/mL of cupric sulfate.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

**Acceptance criteria:** NMT 0.02%

Table 1

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Back-ground Cor-rec-tion
Sodium	589.0	0.05	0.005/0.01	Air-acety-lene	No
Potassi-um	766.5	0.4	0.02/0.04	Air-acety-lene	No

Table 1 (Continued)

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Back-ground Cor-rec-tion
Calcium	422.7	0.8	0.02/0.04	Nitrous oxide-acety-lene	No
Iron	248.3	4.0	0.12/0.24	Air-acety-lene	Yes
Nickel	232.0	4.0	0.10/0.20	Air-acety-lene	No

#### LIMIT OF POTASSIUM

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

**Acceptance criteria:** NMT 0.01%

#### LIMIT OF CALCIUM

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

**Acceptance criteria:** NMT 0.005%

#### LIMIT OF IRON

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

**Acceptance criteria:** NMT 0.003%

#### LIMIT OF NICKEL

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Spectrophotometry and Light-Scattering* <851>), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

**Acceptance criteria:** NMT 0.005%

### SPECIFIC TESTS

- **LOSS ON DRYING** <731>: Dry a sample at 250° to constant weight: it loses 33.0%–36.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

**Cupric Sulfate Injection**

» Cupric Sulfate Injection is a sterile solution of Cupric Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of copper (Cu).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling**—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Identification**—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 325 nm when tested as directed for *Procedure* in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 250.0 USP Endotoxin Units per mg of copper.

**pH** (791): between 2.0 and 3.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

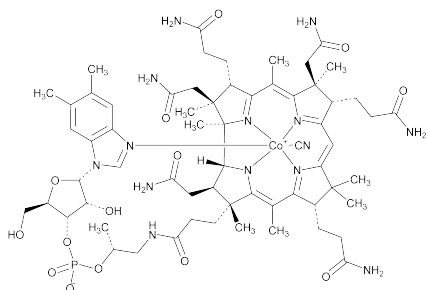
**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Sodium chloride solution*, *Copper stock solution*, and *Standard preparations*—Prepare as directed in the *Assay* under *Cupric Chloride Injection*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of copper, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 15 mL of this solution into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium content of this flask to 13.5 mg. Dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cupric Chloride Injection*.

**Cyanocobalamin**

$C_{63}H_{88}CoN_{14}O_{14}P$   
Vitamin B<sub>12</sub> [68-19-9].

1355.37

**DEFINITION**

Cyanocobalamin contains NLT 96.0% and NMT 102.0% of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION** (197U)

**Wavelength range:** 200–700 nm

**Sample solution:** Prepare as directed in the *Assay*.

**Acceptance criteria:** The absorption spectrum exhibits maxima at  $278 \pm 1$ ,  $361 \pm 1$ , and  $550 \pm 2$  nm. The absorbance ratio  $A_{361}/A_{278}$  is 1.70–1.90, and the absorbance ratio  $A_{361}/A_{550}$  is 3.15–3.40.

- **B.**

**Sample solution:** Fuse 1 mg of Cyanocobalamin with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling.

**Analysis:** Add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (100 mg/mL), dropwise, until just pink. Add 500 mg of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of nitroso R salt solution (2 mg/mL). Add 0.5 mL of hydrochloric acid, and boil for 1 min.

**Acceptance criteria:** A red or orange-red color appears immediately after the addition of nitroso R salt. The red color persists after boiling with the addition of hydrochloric acid.

- **C. HPLC**

**Mobile phase and Chromatographic system:** Proceed as directed in the test for *Related Compounds*.

**Standard solution:** 50 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in *Mobile phase*. Use within 1 h.

**Sample solution:** 50 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.

**Acceptance criteria:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**

- **PROCEDURE**

**Standard solution:** 30 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water

[NOTE—USP Cyanocobalamin RS is a solid dispersion of cyanocobalamin in mannitol. Dissolve and dilute quantitatively and stepwise NLT 30 mg of USP Cyanocobalamin RS to prepare the *Standard solution*.]

**Sample solution:** 30 µg/mL of Cyanocobalamin in water

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** 361 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ) in the portion of Cyanocobalamin taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of cyanocobalamin in the *Standard solution* (µg/mL)

$C_U$  = concentration of Cyanocobalamin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 96.0%–102.0% on the dried basis

**IMPURITIES**

- **RELATED COMPOUNDS**

**Solution A:** 10 g/L of disodium hydrogen phosphate in water

**Mobile phase:** Mixture of methanol and *Solution A* (26.5: 73.5). Adjust with phosphoric acid to a pH of 3.5.

**System suitability solution:** Dissolve 25 mg of Cyanocobalamin in 10 mL of water, warming if necessary. Allow to cool, add 5 mL of a 1.0-g/L solution of tosyl-chloramide sodium and 0.5 mL of 0.05 M hydrochloric acid, and then dilute with water to 25 mL. Shake and allow to stand for 5 min. Dilute 1.0 mL of this solution with *Mobile phase* to 10 mL, and inject immediately.

**Quantitative limit solution:** 1 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.

**Sample solution:** 1 mg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 361 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Column temperature:** 35°

**Flow rate:** 0.8 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Quantitative limit solution*

[NOTE—The chromatogram of the *System suitability solution* should exhibit two major peaks, cyanocobalamin and 7β,8β-lactocyanocobalamin. The relative retention times for the two peaks are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between cyanocobalamin and 7β, 8β-lactocyanocobalamin, *System suitability solution*

**Signal-to-noise ratio:** NLT 5.0 for the major peak, *Quantitative limit solution*

#### Analysis

**Sample:** *Sample solution*

[NOTE—Allow the run time be at least three times the retention time of cyanocobalamin peak.]

Identify the impurities listed in *Table 1*, and measure the peak responses.

Calculate the percentage of individual impurities in the portion of Cyanocobalamin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of peak responses of all the peaks from the *Sample solution*

**Acceptance criteria:** See *Table 1*.

[NOTE—Disregard any peak less than 0.1%.]

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyanocobalamin	1.0	—
7β,8β-Lactocyanocobalamin	1.2	1.0
50-Carboxycyanocobalamin	1.4	0.5
34-Methylcyanocobalamin	1.5	2.0
32-Carboxycyanocobalamin	1.6	1.0
8-Epi-cyanocobalamin	2.5	1.0
Any other unidentified impurity	—	0.5
Total impurities	—	3.0

#### SPECIFIC TESTS

##### • LOSS ON DRYING <731>

**Sample:** 25 mg

**Analysis:** Dry the *Sample* in a suitable vacuum drying apparatus at 105° and at a pressure of NMT 5 mm of mercury for 2 h.

**Acceptance criteria:** NMT 12.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>  
USP Cyanocobalamin RS

### Cyanocobalamin Injection

» Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injection, or in Water for Injection rendered isotonic by the addition of Sodium Chloride. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of anhydrous cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ).

**Packaging and storage**—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.

**USP Reference standards** <11>—

USP Cyanocobalamin RS

USP Endotoxin RS

**Identification**—The absorption spectrum, in the range of 300 nm to 550 nm, of the solution employed for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Cyanocobalamin RS, concomitantly measured, and the ratio  $A_{361}/A_{550}$  is between 3.15 and 3.40.

**Bacterial endotoxins** <85>—It contains not more than 0.4 USP Endotoxin Unit per µg of cyanocobalamin.

**pH** <791>: between 4.5 and 7.0.

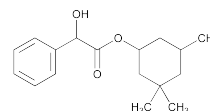
**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Dilute, if necessary, an accurately measured volume of Injection, equivalent to not less than 300 µg of cyanocobalamin, quantitatively and stepwise with water to a concentration of about 30 µg per mL. Dissolve an accurately weighed quantity of USP Cyanocobalamin RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 30 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in µg, of  $C_{63}H_{88}CoN_{14}O_{14}P$  in each mL of the Injection taken by the formula:

$$10(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Cyanocobalamin RS in the Standard solution; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Injection and the Standard solution, respectively.

### Cyclandelate



$C_{17}H_{24}O_3$  276.37

3,3,5-Trimethylcyclohexanol  $\alpha$ -phenyl- $\alpha$ -hydroxyacetate.  
1,5-*cis*-3,3,5-Trimethylcyclohexyl 2-hydroxy-2-phenyl acetate [456-59-7].

» Cyclandelate contains not less than 98.0 percent of  $C_{17}H_{24}O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store below 40°, preferably between 15° and 30°.

**USP Reference standards** (11)—

USP Cyclandelate RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 0.5 mg per mL.

*Medium:* 95% alcohol.

The solution exhibits absorption maxima between 250 and 254 nm, between 256 and 260 nm, and between 262 and 266 nm.

**B:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution*—Dissolve 10 mg of Cyclandelate in 1 mL of alcohol.

*Application volume:* 5  $\mu$ L.

*Developing solvent system:* a mixture of hexane, ethyl acetate, and glacial acetic acid (8:2:1).

**Loss on drying** (731)—Dry 1 g over silica gel for 24 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Chromatographic purity**—

*Mobile phase and Chromatographic system*—Prepare as directed in the Assay.

*Test solution*—Transfer about 100 mg of Cyclandelate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Standard solution*—Pipet 3.0 mL of the *Test solution* into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Allow the chromatogram of the *Test solution* to run for a period of time that is about 3 times the retention time of cyclandelate. The total area of all the peaks from the *Test solution*, other than the peak obtained from cyclandelate, is not greater than the peak area of cyclandelate obtained from the *Standard solution*: not more than 3.0% of total impurities is found.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Resolution solution*—Dissolve accurately weighed quantities of USP Cyclandelate RS and dicyclohexyl phthalate in *Mobile phase* to obtain a solution having known concentrations of about 0.2 mg per mL and 0.08 mg per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cyclandelate RS in *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Transfer about 100 mg of Cyclandelate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 228-nm detector and a 4.0-mm  $\times$  15-cm column that contains packing L1.

The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cyclandelate and dicyclohexyl phthalate is not less than 7. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{17}H_{24}O_3$  in the portion of Cyclandelate taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cyclandelate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclizine Hydrochloride

$C_{18}H_{22}N_2 \cdot HCl$  302.84

Piperazine, 1-(diphenylmethyl)-4-methyl-, monohydrochloride.

1-(Diphenylmethyl)-4-methylpiperazine monohydrochloride [303-25-3].

» Cyclizine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of  $C_{18}H_{22}N_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Cyclizine Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** Dissolve 500 mg in 10 mL of a mixture of 3 volumes of alcohol and 2 volumes of water, warming if necessary. Cool the solution in an ice bath, add 1 mL of 1 N sodium hydroxide and 20 mL of water, stir, and filter: the precipitate of the base so obtained, washed with water and dried in vacuum at 60° for 2 hours, melts between 106° and 109°.

**C:** It responds to the tests for *Chloride* (191).

**pH** (791): between 4.5 and 5.5, determined potentiometrically in a 1 in 50 solution, the solvent being a mixture of 2 volumes of alcohol and 3 volumes of water.

**Loss on drying** (731)—Dry it at 120° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Eluant:* a mixture of chloroform, methanol, and ammonium hydroxide (80:20:1).

*Visualization:* 2.

**Assay**—Transfer to a beaker about 400 mg of Cyclizine Hydrochloride, accurately weighed, and dissolve in 80 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.14 mg of  $C_{18}H_{22}N_2 \cdot HCl$ .

## Cyclizine Hydrochloride Tablets

» Cyclizine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{22}N_2 \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Cyclizine Hydrochloride RS

### Identification—

**A:** Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of cyclizine hydrochloride, with 25 mL of water for 5 minutes, and filter the mixture. Cool the filtrate in an ice bath, add a slight excess of 1 N sodium hydroxide, and stir well: the precipitate so obtained responds to *Identification test B* under *Cyclizine Hydrochloride*.

**B:** Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**Dissolution, Procedure for a Pooled Sample** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{18}H_{22}N_2 \cdot HCl$  dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{22}N_2 \cdot HCl$  is dissolved in 45 minutes.

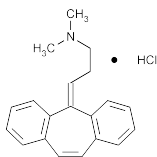
**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Proceed with Tablets as directed under *Salts of Organic Nitrogenous Bases* (501), diluting the *Standard Preparation* and the *Assay Preparation*, respectively, with an equal volume of dilute sulfuric acid (1 in 100), and determining the absorbance at the wavelength of maximum absorbance at about 264 nm. Calculate the quantity, in mg, of  $C_{18}H_{22}N_2 \cdot HCl$  in the portion of Tablets taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Cyclizine Hydrochloride RS in the *Standard Preparation*.

## Cyclobenzaprine Hydrochloride



$C_{20}H_{21}N \cdot HCl$  311.85

1-Propanamine, 3-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-, hydrochloride.

N,N-Dimethyl-5H-dibenzo[a,d]cycloheptene- $\Delta^5$ - $\gamma$ -propylamine hydrochloride [6202-23-9].

» Cyclobenzaprine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $C_{20}H_{21}N \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Cyclobenzaprine Hydrochloride RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 15  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** A solution (1 in 50) responds to the tests for *Chloride* (191).

**Melting range** (741): between 215° and 219°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

**Chromatographic purity**—Dissolve 100 mg in methanol, and dilute with methanol to 5.0 mL to obtain the *Test solution*. Dissolve a suitable quantity of USP Cyclobenzaprine Hydrochloride RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively and stepwise with methanol to obtain a *Diluted standard solution* having a concentration of about 100  $\mu$ g per mL. Apply separate 5- $\mu$ L portions of the three solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Develop the chromatogram in a suitable chamber with a freshly prepared solvent system consisting of a mixture of acetone, toluene, and ammonium hydroxide (75:25:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and view under short-wavelength UV light: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*; and any other spot obtained from the *Test solution* does not exceed, in size or intensity, the principal spot obtained from the *Diluted standard solution* (0.5%).

**Assay**—Dissolve about 400 mg of Cyclobenzaprine Hydrochloride, accurately weighed, in about 80 mL of glacial acetic acid, add 15 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a platinum ring electrode and a sleeve-type calomel electrode containing 0.1 N lithium perchlorate in glacial acetic acid (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.19 mg of  $C_{20}H_{21}N \cdot HCl$ .

## Cyclobenzaprine Hydrochloride Tablets

### DEFINITION

Cyclobenzaprine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197M)

**Sample:** Transfer an amount equivalent to 50 mg of cyclobenzaprine hydrochloride, from a quantity of finely powdered Tablets, to a small flask. Add 10 mL of methylene chloride, swirl to dissolve, and filter. Evaporate the clear filtrate to about 5 mL, transfer to a centrifuge tube, and add 1–2 mL of ether. Evaporate with the aid

of a current of air to about 1 mL, and agitate until crystallization occurs. Wash the crystals with several portions of ether, and air-dry.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

**Mobile phase:** Acetonitrile, methanol, methanesulfonic acid, and water (28: 24: 0.2: 48). Adjust with diethylamine to a pH of 3.6.

**Standard solution:** 0.05 mg/mL of USP Cyclobenzaprine Hydrochloride RS in 0.1 N hydrochloric acid

**Sample solution:** Nominally 0.05 mg/mL of cyclobenzaprine hydrochloride from finely powdered Tablets (NLT 20) in 0.1 N hydrochloric acid prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 75% of the flask volume of 0.1 N hydrochloric acid, and shake by mechanical means for 30 min. Dilute with 0.1 N hydrochloric acid to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 290 nm

**Column:** 4.6-mm × 10-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Capacity factor:** NLT 2.0

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Standard solution:** USP Cyclobenzaprine Hydrochloride RS in *Medium* having a concentration similar to the one expected in the *Sample solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary.

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 290 nm

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) dissolved.

**Tolerances:** NLT 75% (Q) of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

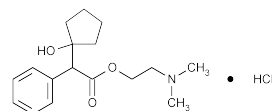
## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS <11>**

USP Cyclobenzaprine Hydrochloride RS

## Cyclopentolate Hydrochloride



$C_{17}H_{25}NO_3 \cdot HCl$  327.85

Benzeneacetic acid,  $\alpha$ -(1-hydroxycyclopentyl)-, 2-(dimethylamino)ethyl ester, hydrochloride, ( $\pm$ )-; 2-(Dimethylamino)ethyl ( $\pm$ )-1-hydroxy- $\alpha$ -phenylcyclopentaneacetate hydrochloride [5870-29-1].

» Cyclopentolate Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{17}H_{25}NO_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store in a cold place.

### USP Reference standards <11>

USP Cyclopentolate Hydrochloride RS

### Identification

**A:** *Infrared Absorption* <197K>.

**B:** A solution (1 in 500) responds to the tests for *Chloride* <191>.

**pH** <791>: between 4.5 and 5.5, in a solution (1 in 100).

**Loss on drying** <731>—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.05%.

### Chromatographic purity

*Buffer solution*, *Mobile phase*, and *Chromatographic system*—Prepare as directed under *Assay*.

*Test preparation*—Use the *Assay preparation*.

*Procedure*—Inject a volume (about 20 µL) of the *Test preparation* into the chromatograph, record the chromatogram obtained for a period of not less than twice the retention time of cyclopentolate, and measure the peak responses. Calculate the percentage of each peak, other than the solvent peak and the cyclopentolate peak, in the specimen of Cyclopentolate Hydrochloride taken by the same formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of each peak and  $r_t$  is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% individual impurity and not more than 2.0% total impurities are found.

### Assay

*Buffer solution*—Dissolve 660 mg of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and mix.

*Mobile phase*—Prepare a suitable filtered and degassed mixture of acetonitrile and *Buffer solution* (7:3). Make adjust-



ments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclopentolate Hydrochloride RS in water, dilute quantitatively, and stepwise if necessary, with water, and mix to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer about 100 mg of Cyclopentolate Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L15. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 3000 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>17</sub>H<sub>25</sub>NO<sub>3</sub> · HCl in the portion of Cyclopentolate Hydrochloride taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclopentolate Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cyclopentolate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclopentolate Hydrochloride Ophthalmic Solution

» Cyclopentolate Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Cyclopentolate Hydrochloride. It may contain suitable buffers and other additives. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>17</sub>H<sub>25</sub>NO<sub>3</sub> · HCl.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** <11>—  
USP Cyclopentolate Hydrochloride RS

**Identification**—Place in a 125-mL separator a volume of Ophthalmic Solution, equivalent to about 50 mg of cyclopentolate hydrochloride, and place in a second separator about 50 mg of USP Cyclopentolate Hydrochloride RS dissolved in 5 mL of water. Treat each solution as follows. Add 1 g of potassium carbonate, and extract with two 10-mL portions of ether. Pass the ether extracts through ether-washed filter paper, collect the filtrate in a small beaker, and evaporate to dryness: the residue so obtained responds to *Identification test A* under *Cyclopentolate Hydrochloride*.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 3.0 and 5.5.

**Assay**—

*Buffer solution*, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cyclopentolate Hydrochloride*.

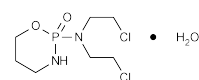
**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of cyclopentolate hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Cyclopentolate Hydrochloride*. Calculate the quantity, in mg, of cyclopentolate hydrochloride (C<sub>17</sub>H<sub>25</sub>NO<sub>3</sub> · HCl) in each mL of the Ophthalmic Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which V is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

## Cyclophosphamide



C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P · H<sub>2</sub>O 279.10

C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P 261.09

2*H*-1,3,2-Oxazaphosphorin-2-amine, *N,N*-bis(2-chloroethyl)tetrahydro-, 2-oxide, monohydrate, (±); (±)-2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate [6055-19-2]. Anhydrous [50-18-0].

### DEFINITION

Cyclophosphamide contains NLT 97.0% and NMT 103.0% of C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P, calculated on the anhydrous basis.

[**CAUTION**—Great care should be taken in handling Cyclophosphamide, as it is a potent cytotoxic agent.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (3:7)

**Ethylparaben solution:** Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.

**System suitability solution:** Transfer USP Cyclophosphamide RS, equivalent to 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Ethylparaben solution*, and dilute with water to volume.

**Standard solution:** 0.5 mg/mL of USP Cyclophosphamide RS in water

**Sample solution:** 0.5 mg/mL of Cyclophosphamide in water

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2 between cyclophosphamide and ethylparaben

**Relative standard deviation:** NMT 2% from six replicate injections, cyclophosphamide peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_7H_{15}Cl_2N_2O_2P$  in the Cyclophosphamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL). [NOTE—Concentration is calculated on the anhydrous basis.]

$C_U$  = concentration of Cyclophosphamide in the *Sample solution* (mg/mL). [NOTE—Nominal concentration is calculated on the anhydrous basis.]

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

#### IMPURITIES

##### Inorganic Impurities

• **HEAVY METALS** (231): NMT 20 ppm

*Sample solution:* 40 mg/mL, and filter if necessary

##### Organic Impurities

##### • PROCEDURE 1: LIMIT OF PROPANOLAMINE

**Diluent:** Methylene chloride and dehydrated alcohol (17:3)

**Standard solution:** 12.5 µg/mL of USP Propanolamine RS in *Diluent*. [NOTE—Propanolamine in the *Standard solution* is 0.025% of Cyclophosphamide in the *Sample solution*.]

**Sample solution:** 50 mg/mL of Cyclophosphamide in *Diluent*

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.1-mm layer of chromatographic silica gel

**Application volume:** 2 µL

**Developing solvent system A:** Toluene, methylene chloride, and methanol (5:5:1). Prepare at time of use.

**Developing solvent system B:** Methanol and glacial acetic acid (9:1)

**Solution A:** Hydrochloric acid and water (7:18)

**Solution B:** 5 g/L of potassium permanganate in water

**Reagent A:** *Solution A* and *Solution B* (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]

**Reagent B:** 100 mg of tetramethylbenzidine in 2.5 mL of methylene chloride, and diluted with cyclohexane to 100 mL

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop with *Developing solvent system A* over a path of 7 cm followed by air drying for 15 min. Develop again in *Developing solvent system B* over a path of 2 cm followed by air drying for NLT 10 min. [NOTE—Transfer *Developing solvent system B* to the chamber 15 min before development.] Dry the plate at 45° under a vacuum for 50 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for 10 min. Remove the plate and place it in a fume hood for 10 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Remove it from *Reagent B* and wait for 15 min, evaluate it with a suitable densitometer, equipped with a filter having its maximum transmittance at 375 nm, and locate and scan the

spot produced by propanolamine from the *Standard solution* and any spot from the *Sample solution* having the same  $R_f$  as that produced by propanolamine from the *Standard solution*.

##### Acceptance criteria

**Propanolamine:** The spot of propanolamine from the *Sample solution* is not more intense than the spot of propanolamine from the *Standard solution* (0.025%).

##### • PROCEDURE 2: LIMIT OF DEGRADATION PRODUCTS

**Diluent:** Methanol and water (1:1)

**Standard solution A:** 12 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*

**Standard solution B:** 12 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*

**Standard solution C:** 12 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*

**Standard solution D:** 15 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*.

[NOTE—Cyclophosphamide related compound D is free base ( $M_r = 260.66$ ) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt ( $M_r = 333.58$ ).]

**Standard solution E:** 12 µg/mL of USP Cyclophosphamide RS in *Diluent*

**Sample solution:** 20 mg/mL of Cyclophosphamide in *Diluent*

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator

**Application volume:** 20 µL

**Developing solvent system:** Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)

**Reagent A:** 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1).

[NOTE— Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]

**Reagent B:** Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol, and dilute with cyclohexane to 200 mL.

##### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*

[NOTE— Apply *Standard solution E* after the plate development in the *Developing solvent system*. Proceed as directed in the *Analysis* below.]

Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in the fresh portion of the *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate in an oven at 50° under a vacuum for 20 min or using a TLC heating plate at 50° for 20 min in a fume hood. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for at least 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B* or spraying it with *Reagent B*. Examine the plate by visual evaluation.

##### Acceptance criteria

The spot of cyclophosphamide related compound A from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A* (0.06%).

The spot of cyclophosphamide related compound B from the *Sample solution* is not more intense than the

spot of cyclophosphamide related compound B from *Standard solution B* (0.06%).

The spot of cyclophosphamide related compound C from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C* (0.06%).

The spot of cyclophosphamide related compound D from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D* (0.06%).

The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E* (0.06%).

**Individual impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D <sup>a</sup>	0.15	0.06
Cyclophosphamide related compound C <sup>b</sup>	0.20	0.06
Cyclophosphamide related compound B <sup>c</sup>	0.43	0.06
Cyclophosphamide related compound A <sup>d</sup>	0.90	0.06
Any unspecified impurity	—	0.06

<sup>a</sup> 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

<sup>b</sup> 3-Aminopropyl dihydrogen phosphate.

<sup>c</sup> 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

<sup>d</sup> Bis(2-chloroethyl)amine hydrochloride.

## SPECIFIC TESTS

### • LIMIT OF CHLORIDE

**Sample solution:** Dissolve 2.0 g of Cyclophosphamide in 30 mL of water, and add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.

**Analysis:** Titrate potentiometrically with 0.01 N silver nitrate VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* <541>). Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion. Calculate the percentage of chloride in the portion of Cyclophosphamide taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / [TN \times W \times (100 - A) / 100]$$

V = sample titrant volume (mL)

B = blank titrant volume (mL)

N = titrant normality

F = equivalence factor, 0.355 mg of chloride ion/mL of TN

TN = theoretical normality, 0.01 N

W = sample weight (mg)

A = assay correction for water

**Acceptance criteria:** NMT 0.033%

### • LIMIT OF PHOSPHATE

**Diluent:** 0.2 g/mL of hydrochloric acid in water

**Solution A:** Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

**Standard stock solution:** 0.72 g/L of monobasic potassium phosphate. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Prepare immediately before use.

**Standard solution:** *Standard stock solution* and water (1:49). Prepare immediately before use. [NOTE—This solution contains 100 µg/L of PO<sub>4</sub>.]

**Sample solution:** Dissolve 100 mg of Cyclophosphamide in water, and dilute to 100 mL.

**Analysis:** To the *Sample solution* add 4 mL of sulfomolybdic acid TS. Shake and add 0.1 mL of *Solution A*. Prepare a standard in the same manner using the *Standard solution*. After 10 min, compare the colors using 20 mL of each solution in color comparison tubes in diffused daylight, viewing vertically against a white background.

**Acceptance criteria:** Any color from the *Sample solution* is not more intense than that from the *Standard solution* (NMT 0.01%).

• **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Cyclophosphamide is sterile, it contains NMT 0.0625 USP Endotoxin Unit/mg of cyclophosphamide.

• **STERILITY TESTS** <71>: Where the label states that Cyclophosphamide is sterile, it meets the requirements.

• **PH** <791>: 3.9–7.1, in a solution (1 in 100), determined 30 min after its preparation

• **WATER DETERMINATION, Method I** <921>: 5.7%–6.8%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.

• **LABELING:** Where the label states that Cyclophosphamide is sterile, the tests for *Bacterial Endotoxins Test* <85> and *Sterility Tests* <71> should be performed.

### • USP REFERENCE STANDARDS <11>

USP Cyclophosphamide RS

USP Cyclophosphamide Related Compound A RS

Bis(2-chloroethyl)amine hydrochloride.

C<sub>4</sub>H<sub>9</sub>Cl<sub>2</sub>N · HCl 178.49

USP Cyclophosphamide Related Compound B RS

3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

C<sub>7</sub>H<sub>16</sub>ClN<sub>2</sub>O<sub>3</sub>P 242.64

USP Cyclophosphamide Related Compound C RS

3-Aminopropyl dihydrogen phosphate.

C<sub>3</sub>H<sub>10</sub>NO<sub>4</sub>P 155.09

USP Cyclophosphamide Related Compound D RS

3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride.

C<sub>7</sub>H<sub>18</sub>ClN<sub>2</sub>O<sub>4</sub>P · 2HCl 333.58

USP Endotoxin RS

USP Propanolamine RS

3-Aminopropan-1-ol.

C<sub>3</sub>H<sub>9</sub>NO 75.11

## Cyclophosphamide for Injection

» Cyclophosphamide for Injection is a sterile mixture of Cyclophosphamide with or without a suitable diluent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous cyclophosphamide (C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>. Storage at a temperature not exceeding 25° is recommended. It will withstand brief exposure to temperatures up to 30°, but is to be protected from temperatures above 30°.

### USP Reference standards <11>—

USP Cyclophosphamide RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

### Identification—

**A:** It responds to the *Thin-layer Chromatographic Identification Test* <201>, a solution of it in chloroform, equivalent to 20 mg of cyclophosphamide per mL, filtered if necessary,

being used as the test solution. Apply 5  $\mu$ L of the test solution and the Standard solution, use a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (75:20:5), and visualize the spots by placing the plate in an iodine chamber.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of cyclophosphamide.

**pH** (791): between 3.0 and 9.0, but the range does not exceed 3 pH units, in a solution containing the equivalent of 20 mg of anhydrous cyclophosphamide per mL, determined 30 minutes after its preparation.

**Other requirements**—It meets the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and *Labeling under Injections* (1).

#### Assay—

**Mobile phase**—Prepare a suitable, degassed solution of water and acetonitrile (70:30).

**Internal standard solution**—Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Transfer an accurately weighed quantity of USP Cyclophosphamide RS, equivalent to about 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add about 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.5 mg of anhydrous cyclophosphamide per mL.

**Assay preparation**—Accurately weigh a portion of Cyclophosphamide for Injection, equivalent to about 200 mg of anhydrous cyclophosphamide, to a 200-mL volumetric flask, add about 50 mL of water, and shake for about 5 minutes, dilute with water to volume, and mix. Pipet 25 mL of this solution and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatography is equipped with a 195-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2%, and the resolution factor between cyclophosphamide and ethylparaben is not less than 2.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for cyclophosphamide and 1.0 for ethylparaben. Calculate the quantity, in mg, of  $C_7H_{15}Cl_2N_2O_2P$  in the portion of Cyclophosphamide for Injection taken by the formula:

$$400C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of anhydrous cyclophosphamide in the *Standard preparation*, as determined from the concentration of USP Cyclophosphamide RS corrected for moisture by a titrimetric water determination; and  $R_U$  and  $R_S$  are the ratios of the peak responses of cyclophosphamide to those of the internal standard in the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclophosphamide Tablets

» Cyclophosphamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ).

**Packaging and storage**—Preserve in tight containers. Storage at a temperature not exceeding 25° is recommended. Tablets will withstand brief exposure to temperatures up to 30°, but are to be protected from temperatures above 30°.

**USP Reference standards** (11)—

USP Cyclophosphamide RS

#### Identification—

**A:** Extract a portion of finely powdered Tablets, equivalent to about 50 mg of cyclophosphamide, with 25 mL of chloroform, filter about 2 mL of the chloroform solution, mix the filtrate with 500 mg of potassium bromide, evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask, and use the residue to prepare a potassium bromide dispersion: the IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima, between 6.5 and 14  $\mu$ m, only at the same wavelengths as that of a similar preparation of USP Cyclophosphamide RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

**Medium:** water; 900 mL, deaerated.

**Apparatus:** 1:100 rpm.

**Time:** 45 minutes.

Determine the amount of  $C_7H_{15}Cl_2N_2O_2P$  dissolved by employing the following method.

**Mobile phase**—Prepare a suitable filtered and degassed mixture of water and acetonitrile (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Cyclophosphamide RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration corresponding to that of the solution under test.

**Test solution**—Use portions of the solution under test passed through a 0.8- $\mu$ m filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 195-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peak. Calculate the amount of cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclophosphamide RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses for cyclophosphamide obtained from the *Test solution* and *Standard solution*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amount of cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**PROCEDURE FOR CONTENT UNIFORMITY**—

**Perchloric acid solution**—Dissolve 23.5 mL of perchloric acid in water, and dilute with water to 1 L.

**4-(p-Nitrobenzyl)pyridine solution**—Dissolve 1.5 g of 4-(p-nitrobenzyl)pyridine in 200 mL of ethylene glycol.

**Sodium hydroxide solution**—Dissolve 20 g of sodium hydroxide in 1000 mL of diluted alcohol.

**Procedure**—Place 1 Tablet in a volumetric flask of suitable size so that the final concentration is about 500 µg per mL. Fill the flask about two-thirds full of water, shake until the Tablet is completely disintegrated, dilute with water to volume, and filter, discarding the first 10 mL of the filtrate. Place in separate 27-mm × 170-mm test tubes 2.0 mL of the filtrate, 2.0 mL of water to provide a blank, and 2.0 mL of the Standard solution, prepared by dissolving an accurately weighed quantity of USP Cyclophosphamide RS in water and diluting quantitatively and stepwise with water to obtain a solution having a known concentration of about 500 µg per mL. Treat each tube as follows. Add 0.7 mL of *Perchloric acid solution*, mix, and heat at 95° for 10 minutes. Cool, add 1.0 mL of sodium acetate TS, mix, add 1.6 mL of *4-(p-Nitrobenzyl)pyridine solution*, mix, and heat at 95° for 10 minutes. Cool, add 8.0 mL of *Sodium hydroxide solution*, and mix. Within 4 minutes, determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at 560 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of  $C_7H_{15}Cl_2N_2O_2P$  in the Tablet taken by the formula:

$$(T/500)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of anhydrous cyclophosphamide in the Tablet; *C* is the concentration, in µg per mL, of USP Cyclophosphamide RS, corrected for moisture by a titrimetric water determination, in the Standard solution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—

**Mobile phase**—Prepare a suitable, degassed solution of water and acetonitrile (70:30).

**Internal standard solution**—Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Transfer an accurately weighed quantity of USP Cyclophosphamide RS, equivalent to about 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add about 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.5 mg of anhydrous cyclophosphamide per mL.

**Assay preparation**—Transfer not fewer than 10 Tablets to a volumetric flask of suitable size so that the final concentration is about 1 mg of anhydrous cyclophosphamide per mL. Fill about half full with water, shake for 30 minutes, dilute with water to volume, and mix. Filter through fast, fluted filter paper, discarding the first 40 to 50 mL of the filtrate. Pipet 25 mL of the filtrate and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatography is equipped with a 195-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the rela-

tive standard deviation is not more than 2%, and the resolution factor between cyclophosphamide and ethylparaben is not less than 2.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for cyclophosphamide and 1.0 for ethylparaben. Calculate the quantity, in mg, of  $C_7H_{15}Cl_2N_2O_2P$  per Tablet taken by the formula:

$$(2CV/N)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of anhydrous cyclophosphamide in the *Standard preparation*, as determined from the concentration of USP Cyclophosphamide RS corrected for moisture by a titrimetric water determination; *V* is the volume, in mL, of the volumetric flask to which the *N* Tablets were transferred; *N* is the number of Tablets taken; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses of cyclophosphamide to those of the internal standard in the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclopropane



$C_3H_6$  42.08  
Cyclopropane.  
Cyclopropane [75-19-4].

» Cyclopropane contains not less than 99.0 percent, by volume, of  $C_3H_6$ .

**Caution**—Cyclopropane is highly flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in cylinders. [NOTE—Maintain cylinders of Cyclopropane at 25 ± 2° for not less than 6 hours prior to withdrawing specimens for the tests and assay, and correct the results to 25° and 760 mm of mercury.]

**Labeling**—The label bears a warning that cyclopropane is highly flammable and is not to be used where it may be ignited.

**Acidity or alkalinity**—Add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS to 400 mL of boiling water, and boil the solution for 5 minutes. Pour 100 mL of the boiling solution into each of three color-comparison tubes marked *A*, *B*, and *C*, respectively. To tube *B* add 0.20 mL of 0.012 N hydrochloric acid, and to tube *C* add 0.40 mL of 0.012 N hydrochloric acid. Insert the stopper in each of the tubes, and cool them to room temperature. Pass 2000 mL of Cyclopropane through the solution in tube *B* at a rate requiring about 30 minutes for the passage of the gas: the color of the solution in tube *B* is no deeper orange-red than that in tube *C* and no deeper yellow-green than that in tube *A*.

NOTE—The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.

**Carbon dioxide**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of a carbon dioxide detector tube to the container valve, and the other end to a gas flow meter. Pass 1000 mL of the Cyclopropane through the tube at a suitable rate: the indicator change corresponds to not more than 0.03%.

**Halogens**—Provide a 500-mL flask with a tightly fitting two-hole stopper. Through one opening pass a delivery tube bent at right angles and extending just beyond the lower surface of the stopper. Through the other opening insert a capillary tube bent at right angles and having a bore of  $1 \pm 0.2$  mm, in the same manner. Place in a 50-mL cylinder, having an internal diameter of  $2 \pm 0.25$  cm, 40 mL of a solution containing 850 mg of sodium carbonate in 1000 mL of water. Provide the cylinder with a two-hole stopper, and through one opening pass a right-angle delivery tube, having a bore of  $3 \pm 0.5$  mm, to within 2 mm of the bottom of the cylinder. The end of the delivery tube that extends out of the cylinder is provided with an enlargement  $8 \pm 0.5$  cm long having an internal diameter of  $2 \pm 0.25$  cm. Through the other opening in the stopper pass another right-angle delivery tube, having it extend just below the surface of the stopper. Collect 500 mL of Cyclopropane in the flask. By means of hydrostatic pressure, applied through the delivery tube, force the gas through the capillary tube, the water used being previously saturated with Cyclopropane. Ignite the gas, place the enlarged end of the delivery tube, connected with the cylinder, around the flame, extending the flame one-third of the way into the enlargement. Apply suction to the shorter delivery tube connected with the cylinder, thus drawing the spent gases through the sodium carbonate solution, the period of ignition of the 500 mL of Cyclopropane requiring approximately 30 minutes. Make any necessary correction for the amount of halogen in the volume of air used for the ignition of the gas. Transfer the sodium carbonate solution to a 500-mL volumetric flask, and rinse the cylinder thoroughly, collecting the rinsings in the flask. Dilute the solution with water to volume, and mix. To a 50-mL aliquot add sufficient nitric acid to make it acid to litmus paper, and then add 1 mL of acid in excess. Prepare a blank containing 0.50 mL of 0.0012 N hydrochloric acid and 4 mL of the sodium carbonate solution in 46 mL of water, acidify to litmus with nitric acid, then add 1 mL of acid in excess and 1 mL of silver nitrate TS to each solution: after 5 minutes any opalescence in the solution representing the Cyclopropane does not exceed that in the blank (0.02% as chloride).

**Propylene, allene, and other unsaturated hydrocarbons**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of an olefin detector tube to the container valve, and the other end to a gas flow meter. Pass the Cyclopropane through the detector tube at a suitable rate: the color of the indicating layer of the tube contents matches the color standard after the passage of not less than 400 mL of Cyclopropane (0.9% as propylene).

**Assay**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Withdraw 100 mL of Cyclopropane, accurately measured in a gas buret previously filled with mercury and equipped with a leveling bulb at the lower end. Connect one arm of the buret stopcock to a pipet that previously has been filled with sulfuric acid. By appropriate manipulation of the stopcock and the leveling bulb, transfer the gas between the pipet and the buret, bringing about sufficient contact of the gas with the acid to reduce the volume of unabsorbed gas to a minimum as measured in the buret. Not more than 1.0 mL of gas remains.

(+)-4-Amino-3-isoxazolidinone [68-41-7].

» Cycloserine has a potency of not less than 900  $\mu\text{g}$  of  $\text{C}_3\text{H}_6\text{N}_2\text{O}_2$  per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cycloserine RS

**Identification**—Dissolve about 1 mg in 10 mL of 0.1 N sodium hydroxide. To 1 mL of the resulting solution add 3 mL of 1 N acetic acid and 1 mL of a mixture, prepared 1 hour before use, of equal parts of sodium nitroprusside solution (1 in 25) and 4 N sodium hydroxide: a blue color gradually develops.

**Condensation products**—Its absorptivity (see *Spectrophotometry and Light-Scattering* (851)) at 285 nm, determined in a 0.1 N sodium hydroxide solution containing 0.40 mg per mL is not more than 0.80.

**Specific rotation** (781S): between  $108^\circ$  and  $114^\circ$ .

*Test solution*: 50 mg per mL, in 2 N sodium hydroxide.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 5.5 and 6.5, in a solution (1 in 10).

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at  $60^\circ$  for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay**—

*pH 6.8 Phosphate buffer*—Prepare as directed in *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*.

*Mobile phase*—Dissolve 0.5 g of sodium 1-decanesulfonate in 800 mL of water, add 50 mL of acetonitrile and 5 mL of glacial acetic acid, and mix. Adjust with 1 N sodium hydroxide to a pH of 4.4. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in *pH 6.8 Phosphate buffer* to obtain a solution having a known concentration of about 0.4 mg per mL.

*Assay preparation*—Transfer about 20 mg of Cycloserine, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *pH 6.8 Phosphate buffer* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 219-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about  $30^\circ$ . Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in  $\mu\text{g}$ , of  $\text{C}_3\text{H}_6\text{N}_2\text{O}_2$  in each mg of Cycloserine taken by the formula:

$$50,000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard preparation*; W is the quantity, in mg, of Cycloserine taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for cycloserine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cycloserine



$\text{C}_3\text{H}_6\text{N}_2\text{O}_2$  102.09  
3-Isloxazolidinone, 4-amino-, (R)-.

## Cycloserine Capsules

» Cycloserine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cycloserine ( $C_3H_6N_2O_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cycloserine RS

**Identification**—Shake a quantity of the contents of Capsules, equivalent to about 10 mg of cycloserine, with 100 mL of 0.1 N sodium hydroxide, and filter: 1 mL of the filtrate so obtained responds to the *Identification* test under *Cycloserine*.

**Dissolution** (711)—

*Medium:* pH 6.8 Phosphate buffer (see *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 30 minutes.

Determine the amount of  $C_3H_6N_2O_2$  dissolved by employing the following method.

pH 6.8 Phosphate buffer, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in pH 6.8 Phosphate buffer to obtain a solution having a known concentration of about 0.25 mg per mL.

*Test solution*—Use a filtered portion of the solution under test.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in mg, of cycloserine ( $C_3H_6N_2O_2$ ) dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses for cycloserine obtained from the *Test solution* and the *Standard solution*, respectively.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_3H_6N_2O_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of the contents of Capsules in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Assay**—

pH 6.8 Phosphate buffer, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cycloserine*.

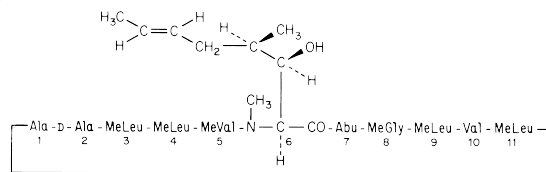
*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cycloserine, to a 250-mL volumetric flask, dilute with pH 6.8 Phosphate buffer to volume, mix, and filter.

*Procedure*—Proceed as directed in the *Assay* under *Cycloserine*. Calculate the quantity, in mg, of cycloserine ( $C_3H_6N_2O_2$ ) in the portion of Capsules taken by the formula:

$$250C(r_U / r_S)$$

in which the terms are as defined therein.

## Cyclosporine



$C_{62}H_{111}N_{11}O_{12}$  1202.61

Cyclo[[*(E)*-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl].

[*R*-[*R*\*,*R*\*-*(E)*]-Cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L- $\alpha$ -aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl)] [59865-13-3].

» Cyclosporine contains not less than 98.5 percent and not more than 101.5 percent of cyclosporine A ( $C_{62}H_{111}N_{11}O_{12}$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Cyclosporine RS

USP Cyclosporine Resolution Mixture RS

This material is a 100:1 mixture of cyclosporine and cyclosporine U.

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cyclosporine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Heavy metals**, *Method II* (231): 0.002%.

**Related compounds**—Using the chromatograms obtained from *Standard preparation 2* and the *Assay preparation* in the *Assay*, calculate the percentage of each impurity by the formula:

$$2000(C / W)(r_i / r_{S2})$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in *Standard preparation 2*; W is the weight, in mg, of Cyclosporine taken to prepare the *Assay preparation*;  $r_i$  is the response of an individual impurity observed in the chromatogram of the *Assay preparation*; and  $r_{S2}$  is the response of the main cyclosporine peak in the chromatogram obtained from *Standard preparation 2*: not more than 0.7% of any individual impurity is found, and the sum of all such impurities is not more than 1.5%, any impurities corresponding to less than 0.05% being disregarded.

**Assay**—

*Mobile phase*—Prepare a mixture of water, acetonitrile, *tert*-butyl methyl ether, and phosphoric acid (520:430:50:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of acetonitrile and water (1:1).

*Standard preparation 1*—Dissolve an accurately weighed quantity of USP Cyclosporine RS in *Diluent* to obtain a solution having a known concentration of about 1.25 mg per mL.

**Standard preparation 2**—Transfer 2.0 mL of *Standard preparation 1* to a 250-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.01 mg of USP Cyclosporine RS per mL.

**Assay preparation**—Dissolve about 25 mg of Cyclosporine, accurately weighed, in *Diluent*, dilute with *Diluent* to 20.0 mL, and mix.

**Resolution solution**—Prepare a solution of USP Cyclosporine Resolution Mixture RS in *Diluent* having a concentration of about 1.25 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector, a 0.25-mm × 1-m stainless steel tube connected to a 4-mm × 25-cm column that contains 3- to 5-μm packing L1. The tube and column are maintained at 80°. The flow rate is about 1.2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the cyclosporine U peak and the main cyclosporine peak are resolved from each other. Chromatograph *Standard preparation 1*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph *Standard preparation 2*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μL) of *Standard preparation 1*, *Standard preparation 2*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of cyclosporine A (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>) in the Cyclosporine taken by the formula:

$$(CP/10U)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in *Standard preparation 1*; P is the specified purity, in μg per mg, of USP Cyclosporine RS; U is the concentration, in mg per mL, of specimen in the *Assay preparation*; and  $r_U$  and  $r_S$  are the main cyclosporine peak responses obtained from the *Assay preparation* and *Standard preparation 1*, respectively.

## Cyclosporine Capsules

» Cyclosporine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

WHERE CAPSULES CONTAIN LIQUID—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than

30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

WHERE CAPSULES CONTAIN POWDER—

**Medium:** 0.1 N hydrochloric acid containing 0.5% of sodium lauryl sulfate; 1000 mL.

**Apparatus 1:** 150 rpm.

**Time:** 90 minutes.

Determine the amount of C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> dissolved by employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (900:450:50:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Cyclosporine RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.001 L mg per mL, L being the labeled quantity, in mg, of cyclosporine in each Capsule. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. This solution contains about 0.0005 L mg of USP Cyclosporine RS per mL.

**Test solution**—Filter a portion of the solution under test. Transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L1 and is maintained at a constant temperature of about 80°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 700 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the solution estimated to contain 0.1 mg of cyclosporine per mL, or 40 μL of the solution estimated to contain 0.025 mg of cyclosporine per mL of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> dissolved by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the cyclosporine peak areas obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> is dissolved in 90 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921)—For Capsules that contain powder, not more than 3.5% is found, using finely ground Capsule contents.

**Assay**—

WHERE CAPSULES CONTAIN LIQUID—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Cyclosporine Injection*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclosporine RS in dehydrated alcohol to obtain a solution having a known concentration of about 1 mg per mL. Use this solution promptly after preparation.

**Assay preparation**—Using a sharp blade, carefully cut open not fewer than 20 Capsules, and with the aid of dehydrated alcohol transfer the contents of the Capsules to a suitable volumetric flask. Wash the blade with dehydrated alcohol, and transfer the washings to the volumetric flask. Dilute the contents of the volumetric flask with dehydrated alcohol to volume, and mix. Quantitatively dilute an accu-



rately measured volume of this solution with dehydrated alcohol to obtain a solution having a concentration of about 1 mg of cyclosporine per mL.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in each Capsule taken by the formula:

$$(L/D)(CP/1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of cyclosporine in each Capsule taken; *D* is the concentration, in mg per mL, of the *Assay preparation*, based on the labeled quantity of cyclosporine in the Capsules taken and the extent of dilution; *C* is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*; *P* is the purity, in  $\mu$ g per mg, of USP Cyclosporine RS; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

WHERE CAPSULES CONTAIN POWDER—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (605:400:50:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluting solvent**—Prepare a mixture of acetonitrile, tetrahydrofuran, and dehydrated alcohol (9:5:4).

**Standard preparation**—Transfer about 25 mg of USP Cyclosporine RS, accurately weighed, to a 25-mL volumetric flask. Add 2.5 mL of water, and sonicate for 10 minutes. Add about 10 mL of *Diluting solvent*, sonicate for 5 minutes, dilute with *Diluting solvent* to volume, and mix.

**Assay stock preparation**—Transfer the contents of 20 Capsules to a volumetric flask of such capacity, *V*, in mL, to make a final concentration of 10 mg of cyclosporine per mL. Add 0.1*V* mL of water to the flask, and sonicate for 10 minutes. Add 0.4*V* mL of *Diluting solvent* to the flask, and sonicate for 5 minutes. Dilute with *Diluting solvent* to volume, and mix.

**Assay preparation**—Transfer 5.0 mL of *Assay stock preparation* to a 50-mL volumetric flask, add 5 mL of water, dilute with *Diluting solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L13 and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in each Capsule taken by the formula:

$$10CV(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*; *V* is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cyclosporine peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclosporine Injection

» Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers.

**Labeling**—Label it to indicate that it is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

**USP Reference standards** (11)—

USP Cyclosporine RS

USP Endotoxin RS

**Identification**—

**A:** Prepare a solution of it in methanol containing about 0.5 mg of cyclosporine per mL (test solution). Prepare a Standard solution containing 0.5 mg per mL of USP Cyclosporine RS in methanol. Separately apply 10- $\mu$ L portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using ethyl ether as the developing solvent, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with a freshly prepared mixture of 5 mL of *Solution A* (340 mg of bismuth subnitrate dissolved in 20 mL of 20% acetic acid), 5 mL of *Solution B* (8 g of potassium iodide dissolved in 20 mL of water), 20 mL of glacial acetic acid, and water to make 100 mL. Immediately again spray the plate with hydrogen peroxide TS. Cyclosporine appears as a brown spot having an *R<sub>F</sub>* value of about 0.45 on the chromatograms: the *R<sub>F</sub>* value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution. [NOTE—Disregard any spots at the origin.]

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cyclosporine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—Prepare the test specimen as follows, using USP Endotoxin RS. Make a 1:10 dilution of the Injection with Water for Injection. Add 0.1 mL of the resulting suspension and 0.1 mL of appropriately constituted LAL reagent to a suitable pyrogen-free test tube, and mix on a vortex mixer for about 5 seconds: the article under test contains not more than 0.84 USP Endotoxin Unit per mg of cyclosporine.

**Sterility** (71): meets the requirements.

**Alcohol content** (where present)—

**Internal standard solution**—Mix 3 mL of *n*-propyl alcohol and 50 mL of butyl alcohol.

**Standard stock solution**—Transfer about 1.6 g of dehydrated alcohol, accurately weighed, to a 25-mL volumetric flask, dilute with butyl alcohol to volume, and mix.

**Standard preparation**—Transfer 5.0 mL of *Standard stock solution* and 6.0 mL of *Internal standard solution* to a 25-mL

volumetric flask, dilute with butyl alcohol to volume, and mix.

**Test preparation**—Transfer an accurately weighed portion of Injection, equivalent to about 320 mg of  $C_2H_5OH$ , to a 25-mL volumetric flask, add 6.0 mL of *Internal standard solution*, dilute with butyl alcohol to volume, and mix.

**Chromatographic system**—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm  $\times$  2-m glass column packed with support  $S_3$ . The injection port is maintained at a temperature of about 280°, the detector is maintained at about 290°, and the column is maintained at 145° for 8 minutes and is programmed thereafter to rise to 270° at a rate of 32° per minute. Nitrogen is used as the carrier gas, flowing at a rate of about 35 mL per minute. [NOTE—Make adjustments if necessary to obtain satisfactory chromatography.]

**System suitability**—Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the relative standard deviation for replicate injections is not greater than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Inject separate suitable portions (about 1  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol. Calculate the quantity, in mg, of  $C_2H_5OH$  in the portion of Injection taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of  $C_2H_5OH$  in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the alcohol peak to the *n*-propyl alcohol internal standard peak obtained from the *Test preparation* and the *Standard preparation*, respectively: it contains between 80.0% and 120.0% of the labeled amount of  $C_2H_5OH$ .

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (550:400:50:0.5), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclosporine RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Use this solution promptly after preparation.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Using a suitable hypodermic needle and syringe, withdraw all of the withdrawable contents from 1 container of Injection, and dilute quantitatively with methanol to obtain a solution containing about 0.5 mg of cyclosporine per mL. Use this solution promptly after preparation.

**Assay preparation 2** (where the label states the quantity of cyclosporine in a given volume)—Dilute an accurately measured volume of Injection quantitatively with methanol to obtain a solution containing about 0.5 mg of cyclosporine per mL. Use this solution promptly after preparation.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm analytical column that contains packing L16. The column is maintained at 70°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $K'$ , is not less than 3 and not more than 10; the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{26}H_{111}N_{11}O_{12}$ ) withdrawn from the container or in each mL of the Injection taken by the same formula:

$$(L / D)(CP / 1000)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of cyclosporine in the container or in each mL of Injection;  $D$  is the concentration, in mg of cyclosporine per mL, of *Assay preparation 1* or *Assay preparation 2* based on the labeled quantity in the container or in the volume of Injection taken and the extent of dilution, respectively;  $C$  is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*;  $P$  is the purity, in  $\mu$ g per mg, of USP Cyclosporine RS; and  $r_U$  and  $r_S$  are the peak responses obtained from *Assay preparation 1* or *Assay preparation 2* and the *Standard preparation*, respectively.

## Cyclosporine Oral Solution

» Cyclosporine Oral Solution is a solution of Cyclosporine in a suitable vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Cyclosporine RS

#### Identification—

**A:** Using a solution of it in a mixture of methanol and chloroform (4:1) containing about 1 mg of cyclosporine per mL (test solution) and a Standard solution containing 1 mg of USP Cyclosporine RS in the same solvent mixture, proceed as directed in *Identification* test A under *Cyclosporine Injection*, beginning with "Separately apply 10- $\mu$ L portions of the test solution": the Oral Solution meets the requirements of the test.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

#### Alcohol content (where present)—

**Internal standard solution, Chromatographic system, and System suitability**—Proceed as directed in the test for *Alcohol content* under *Cyclosporine Injection*.

**Standard stock solution**—Transfer about 2.5 g of dehydrated alcohol, accurately weighed, to a 50-mL volumetric flask, dilute with butyl alcohol to volume, and mix.

**Standard preparation**—Transfer 5.0 mL of *Standard stock solution* and 6.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with butyl alcohol to volume, and mix.

**Test preparation**—Transfer an accurately weighed portion of Oral Solution, equivalent to about 250 mg of  $C_2H_5OH$ , to a 25-mL volumetric flask, add 6.0 mL of *Internal standard solution*, dilute with butyl alcohol to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the test for *Alcohol content* under *Cyclosporine Injection*. Calculate the quantity, in mg, of  $C_2H_5OH$  in the portion of Oral Solution taken by the formula:

$$25C(R_U / R_S)$$

in which the terms are as defined therein: between 80.0% and 120.0% of the labeled amount of  $C_2H_5OH$  is found.

#### Assay—

**Mobile phase**—Prepare as directed in the Assay under *Cyclosporine Injection*.

**Solvent mixture**—Prepare a mixture of methanol and chloroform (4:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclosporine RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL. Use this solution promptly after preparation.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Oral Solution with *Solvent mixture* to obtain a solution containing about 1 mg of cyclosporine per mL. Use this solution promptly after preparation.

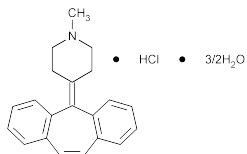
**Chromatographic system**—Proceed as directed for *Chromatographic system* in the Assay under *Cyclosporine Injection*, except to maintain the column at 50° instead of 70°.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in each mL of the Oral Solution taken by the formula:

$$(L/D)(CP/1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of cyclosporine in each mL of Oral Solution taken; *D* is the concentration, in mg per mL, of the *Assay preparation*, based on the labeled quantity of cyclosporine in the volume of Oral Solution taken and the extent of dilution; *C* is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*; *P* is the purity, in  $\mu$ g per mg, of USP Cyclosporine RS; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyproheptadine Hydrochloride



$C_{21}H_{21}N \cdot HCl \cdot 1\frac{1}{2}H_2O$  350.88

Piperidine, 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1-methyl-, hydrochloride, sesquihydrate.

4-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate [41354-29-4].

Anhydrous 323.87 [969-33-5].

» Cyproheptadine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of  $C_{21}H_{21}N \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Cyproheptadine Hydrochloride RS

#### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 16  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 286 nm do not differ by more than 3.0%.

**C:** Dissolve 100 mg of Cyproheptadine Hydrochloride in 10 mL of methanol. Place 1 drop of the solution on a filter paper, dry, and view under short-wavelength UV light: a bright blue fluorescence is observed.

**Acidity**—Dissolve 1.0 g of Cyproheptadine Hydrochloride in 25 mL of methanol, add methyl red TS, and titrate with 0.10 N sodium hydroxide: not more than 0.15 mL is required (0.05% as HCl).

**Water, Method I** (921): between 7.0% and 9.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.003%.

**Assay**—Dissolve about 650 mg of Cyproheptadine Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, heating to dissolve. Cool, add 10 mL of mercuric acetate TS, 0.5 mL of acetic anhydride, and 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.39 mg of  $C_{21}H_{21}N \cdot HCl$ .

## Cyproheptadine Hydrochloride Oral Solution

» Cyproheptadine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyproheptadine hydrochloride ( $C_{21}H_{21}N \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cyproheptadine Hydrochloride RS

**Identification**—Place about 50 mL of Oral Solution in a separator, add 25 mL of sodium bicarbonate solution (2 in 100), and extract with three 15-mL portions of isoctane. Wash the combined isoctane extracts with 15 mL of sodium bicarbonate solution (2 in 100), and discard the washing. Evaporate the isoctane solution on a steam bath to dryness, and dissolve the residue in 1 mL of carbon disulfide, filtering if necessary. Determine the IR absorption spectrum as directed under *Identification—Organic Nitrogenous Bases* (181), obtaining the spectrum of USP Cyproheptadine Hydrochloride RS as directed: the Oral Solution meets the requirements of the test.

**pH** (791): between 3.5 and 4.5.

#### Assay—

**Methanesulfonic acid solution, Mobile phase, and Chromatographic system**—Proceed as directed in the Assay under *Cyproheptadine Hydrochloride Tablets*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyproheptadine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 2 mg of cyproheptadine hydrochloride, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a 0.45- $\mu$ m or finer porosity.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cyproheptadine hydrochloride ( $C_{21}H_{21}N \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyproheptadine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cyproheptadine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyproheptadine Hydrochloride Tablets

» Cyproheptadine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{21}N \cdot HCl$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Cyproheptadine Hydrochloride RS

**Identification**—Tablets meet the requirements under *Identification*—Organic Nitrogenous Bases (181).

**Dissolution** (711)—

*Medium*: 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{21}H_{21}N \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 285 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cyproheptadine Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{21}H_{21}N \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Methanesulfonic acid solution**—Prepare a solution of methanesulfonic acid in water (3:1000).

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, isopropyl alcohol, and *Methanesulfonic acid solution* (20:15:65); while mixing adjust with triethylamine to a pH of  $4.0 \pm 0.05$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyproheptadine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg per mL.

**Assay preparation**—Transfer a number of Tablets, accurately weighed, equivalent to 80 mg of cyproheptadine hydrochloride, to a 1-liter volumetric flask, dissolve by sonication in 500 mL of *Mobile phase* for 15 minutes, and agitate for 30 minutes. Dilute with *Mobile phase* to volume, and mix. Pass through a filter having a 0.45- $\mu$ m or finer porosity.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 285-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as di-

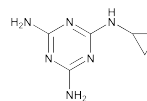
rected for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{21}N \cdot HCl$  in each of the Tablets taken by the formula:

$$1000(C / N)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyproheptadine Hydrochloride RS in the *Standard preparation*; N is the number of Tablets taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the cyproheptadine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyromazine



$C_6H_{10}N_6$  166.18

N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine.

2-Cyclopropylamino-4,6-diamino-s-triazine [66215-27-8].

» Cyromazine contains not less than 98.0 percent and not more than 102.0 percent of  $C_6H_{10}N_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cyromazine RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 219° and 226°.

**Loss on drying** (731)—Dry it at 105° to a constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Assay**—

**Mobile phase**—Mix 930 mL of water, 3.72 g of dibasic potassium phosphate, and 6.48 g of monobasic potassium phosphate. Add 50 mL of methanol and 20 mL of acetonitrile, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyromazine RS in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

**Assay preparation**—Dissolve an accurately weighed quantity of Cyromazine in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

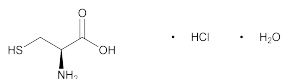
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the content, in percentage, of C<sub>6</sub>H<sub>10</sub>N<sub>6</sub> in the portion of Cyromazine taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which C<sub>s</sub> is the concentration, in μg per mL, of USP Cyromazine RS in the *Standard preparation*; C<sub>u</sub> is the concentration, in μg per mL, of Cyromazine in the *Assay preparation*; and r<sub>u</sub> and r<sub>s</sub> are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cysteine Hydrochloride



C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S · HCl · H<sub>2</sub>O 175.63

C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S · HCl 157.62  
L-Cysteine hydrochloride monohydrate [7048-04-6].  
Anhydrous [52-89-1].

### DEFINITION

Cysteine Hydrochloride is L-cysteine hydrochloride monohydrate and contains NLT 98.5% and NMT 101.5% of L-cysteine hydrochloride (C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S · HCl), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>

### ASSAY

#### Change to read:

#### PROCEDURE

**Sample:** 250 mg of Cysteine Hydrochloride

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to an iodine flask. Add 20.0 mL of water and 4 g of potassium iodide, and mix. Cool the solution in an ice bath, and add 5 mL of 3 N hydrochloric acid and 25.0 mL of 0.1 N iodine VS. • Insert the stopper, and allow to stand in the dark for 20 min, while remaining in the ice bath. • (ERR 1-Jul-2012) Titrate the excess iodine with the *Back titrant*. • Titrate the starch TS as the endpoint is approached. Perform the *Blank* determination. Calculate the percentage of cysteine hydrochloride (C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S · HCl) in the *Sample* taken:

teine hydrochloride (C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S · HCl) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F] / W\} \times 100$$

V<sub>B</sub> = Back titrant volume consumed by the *Blank* (mL)

V<sub>S</sub> = Back titrant volume consumed by the *Sample* (mL)

N = actual normality of the *Back titrant* (mEq/mL)

F = equivalency factor, 157.6 mg/mEq

W = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.4%

- **CHLORIDE AND SULFATE**, *Sulfate* <221>

**Standard solution:** 0.10 mL of 0.020 N sulfuric acid

**Sample:** 0.33 g of Cysteine Hydrochloride

**Acceptance criteria:** NMT 0.03%.

- **IRON** <241>: NMT 30 ppm

- **HEAVY METALS**, *Method I* <231>: NMT 15 ppm

### RELATED COMPOUNDS

**N-Ethylmaleimide solution:** 40 mg/mL of N-ethylmaleimide in alcohol

**Standard stock solution:** Dissolve 20 mg of USP L-Cysteine Hydrochloride RS in 10.0 mL of water. Add 10.0 mL of *N-Ethylmaleimide solution*, and mix. Allow the solution to stand for 5 min before using.

**Standard solution:** 0.05 mg/mL from *Standard stock solution* in water. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

**System suitability solution:** Transfer 10 mg of USP L-Tyrosine RS and 10 mL of the *Standard stock solution* to a 25-mL volumetric flask. Dilute with water to volume.

**Sample stock solution:** Transfer 0.2 g of Cysteine Hydrochloride to a 10-mL volumetric flask, dissolve, and dilute with water to volume.

**Sample solution:** To 5.0 mL of the *Sample stock solution* add 5.0 mL of *N-Ethylmaleimide solution*, and mix. Allow the solution to stand for 5 min before using.

### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5 μL

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

### System suitability

**Suitability requirements:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

### Analysis

**Samples:** *Standard solution*, *System suitability solution*, and *Sample solution*.

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

**Acceptance criteria:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

**Individual impurities:** NMT 0.5%

**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* <781S>

**Sample solution:** 80 mg/mL in 6 N hydrochloric acid

**Acceptance criteria:** +5.7° to +6.8°

- **LOSS ON DRYING** <731>: Dry a sample at room temperature at a pressure not exceeding 5 mm of mercury for 24 h: it loses 8.0%–12.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP L-Cysteine Hydrochloride RS  
USP L-Tyrosine RS

**Cysteine Hydrochloride Injection**

» Cysteine Hydrochloride Injection is a sterile solution of Cysteine Hydrochloride in Water for Injection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of  $C_3H_7NO_2S \cdot HCl \cdot H_2O$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP L-Cysteine Hydrochloride RS  
USP Endotoxin RS

**Identification**—

**A:** To 2 mL of Injection add 3 mL of water, and mix. Add 10 mL of cupric sulfate TS: a bluish-gray precipitate is formed.

**B:** To 2 mL of Injection add 3 mL of water, and mix. Add 2 mL of 3 N sodium hydroxide and 2 drops of sodium nitroferricyanide solution (1 in 20): a red-purple color is produced, and it rapidly changes to yellow.

**Bacterial endotoxins** (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of cysteine hydrochloride.

**pH** (791): between 1.0 and 2.5.

**Heavy metals, Method II** (231): 2 ppm.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Standard stock preparation**—Dissolve an accurately weighed quantity of USP L-Cysteine Hydrochloride RS in nitrogen-saturated water to obtain a solution having a known concentration of about 1 mg per mL.

**Standard preparation**—Transfer 20.0 mL of *Standard stock preparation* to a 200-mL volumetric flask, dilute with nitrogen-saturated 1.0 N sodium hydroxide to volume, and mix.

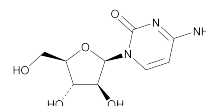
**Assay preparation**—Dilute an accurately measured volume of Injection, equivalent to about 250 mg of cysteine hydrochloride, quantitatively and stepwise with nitrogen-saturated 1.0 N sodium hydroxide, to obtain a solution having a concentration of about 0.1 mg per mL.

**Procedure**—Transfer a suitable amount of *Standard preparation* to a polarographic cell. With mercury dropping from the electrode, lower the dropping mercury electrode of a polarograph so that the end is submerged in the liquid. Bubble oxygen-free, water-saturated nitrogen through the liquid for 15 minutes. Record the polarogram from  $-0.2$  volt to  $-1.10$  volts, using a saturated calomel electrode as the reference electrode. In a similar manner, record the polarograms obtained using portions of the *Assay preparation* and of the nitrogen-saturated 1.0 N sodium hydroxide. Determine the height of the diffusion current wave at  $-0.4$  volt. Calculate the quantity, in mg, of  $C_3H_7NO_2S \cdot HCl \cdot H_2O$  in each mL of the Injection taken by the formula:

$$2500(C/V)[(i_a)_U / (i_a)_S]$$

in which C is the concentration, in mg per mL, of USP L-Cysteine Hydrochloride RS. In the *Standard preparation*, V is the volume, in mL, of Injection taken; and  $(i_a)_U$  and  $(i_a)_S$  are the observed diffusion currents, corrected for the diffu-

sion current of the 0.1 N sodium hydroxide, of the *Assay preparation* and the *Standard preparation*, respectively.

**Cytarabine**

$C_9H_{13}N_3O_5$  243.22

2(1H)-Pyrimidinone, 4-amino-1- $\beta$ -D-arabinofuranosyl-1- $\beta$ -D-Arabinofuranosylcytosine [147-94-4].

» Cytarabine contains not less than 98.0 percent and not more than 102.0 percent of  $C_9H_{13}N_3O_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cytarabine RS  
USP Endotoxin RS  
USP Uracil Arabinoside RS

**Identification**—

**A:** *Infrared Absorption* (197M): previously dried at a pressure of not more than 5 mm of mercury at  $60^\circ$  for 3 hours.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between  $+154^\circ$  and  $+160^\circ$ .

*Test solution:* 10 mg per mL, in water.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** (231): 0.001%.

**Chromatographic purity**—

**Phosphate buffer**—Prepare a solution containing 0.01 M monobasic sodium phosphate and 0.01 M dibasic sodium phosphate in a suitable container. Adjust with 0.1 M sodium hydroxide or 0.1 M phosphoric acid to a pH of 7.0.

**Solution A**—Prepare a filtered and degassed mixture of *Phosphate buffer* and methanol (49:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Prepare this solution fresh daily.

**Solution B**—Prepare a filtered and degassed mixture of *Phosphate buffer* and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Prepare this solution fresh daily.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system*.

**System suitability solution**—Dissolve suitable quantities of uridine, USP Uracil Arabinoside RS, and USP Cytarabine RS in water to obtain a solution containing about 0.02, 0.02, and 5.0 mg per mL, respectively.

**Standard solution**—Dissolve an accurately weighed quantity of USP Cytarabine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 4  $\mu$ g per mL.

**Test solution**—Transfer about 25 mg of Cytarabine, accurately weighed, to a 5.0-mL volumetric flask, dissolve in and

dilute with water to volume, and mix. [NOTE—Prepare this solution fresh daily.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B*, the percentage of *Solution B* being 0% at the time of injection. This composition is held for 10 minutes. *Solution B* is then linearly increased to 100% over a period of 10 minutes. After maintaining this composition for 5 minutes, the percentage of *Solution B* is then linearly decreased to 0% over a period of 5 minutes. This composition is maintained for 20 minutes to equilibrate the system. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.55 for uracil, 1.14 for uridine, 1.62 for uracil arabinoside, and 1.0 for cytarabine; and the resolution, *R*, between cytarabine and uridine is not less than 1.25. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of uracil arabinoside in the portion of Cytarabine taken by the formula:

$$500(C / W)(r_i / 1.34r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cytarabine RS in the *Standard solution*; *W* is the weight, in mg, of the specimen, 1.34 is the relative response factor for uracil arabinoside; *r<sub>i</sub>* is the peak response of uracil arabinoside in the *Test solution*; and *r<sub>s</sub>* is the peak response of USP Cytarabine RS in the *Standard solution*: not more than 0.30% is found.

Calculate the percentage of all other impurities in the portion of Cytarabine taken by the formula:

$$500(C / W)(r_i / F_r)$$

in which *C* is the concentration, in mg per mL, of USP Cytarabine RS in the *Standard solution*; *W* is the weight, in mg, of the specimen; *r<sub>i</sub>* is the peak response of each impurity in the *Test solution*; *r<sub>s</sub>* is the peak response of USP Cytarabine RS in the *Standard solution*; and *F<sub>r</sub>*, the relative response factor, equals 2.5 for the uracil peak, with a relative retention time of 0.55, 1.5 for peaks with relative retention times of 0.38, 0.43, and 1.14, and 1.0 for all other peaks. Not more than 0.10% of any individual impurity is found, and not more than 0.30% of total impurities is found (including uracil arabinoside).

**Other requirements**—Where the label states that Cytarabine is sterile, it meets the requirements for *Sterility Tests* <71> and for *Bacterial endotoxins* under *Cytarabine for Injection*. Where the label states that Cytarabine must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cytarabine for Injection*.

#### Assay—

**Phosphate buffer**—Dissolve 0.73 g of monobasic sodium phosphate and 1.4 g of dibasic sodium phosphate in 1 L of water, mix, and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Phosphate buffer* and methanol (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cytarabine RS in water, and dilute quantita-

tively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

**Resolution solution**—Dissolve an accurately weighed quantity of USP Uracil Arabinoside RS in *Standard preparation*, and dilute quantitatively, and stepwise if necessary, with *Standard preparation* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer about 10 mg of Cytarabine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for cytarabine and 1.3 for uracil arabinoside; and the resolution, *R*, between cytarabine and uracil arabinoside is not less than 2.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—After chromatography has been completed, flush the column with a mixture of water and methanol (7:3).]

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub> in the portion of Cytarabine taken by the formula:

$$100C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cytarabine RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cytarabine for Injection

» Cytarabine for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cytarabine (C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub>).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

#### USP Reference standards <11>—

USP Cytarabine RS

USP Uracil Arabinoside RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 0.07 USP Endotoxin Unit per mg of cytarabine.

**pH** <791>: between 4.0 and 6.0, in a solution containing the equivalent of 10 mg of cytarabine per mL.

**Water, Method I** <921>: not more than 3.0%.

**Other requirements**—It meets the requirements for *Sterility Tests* <71>, *Uniformity of Dosage Units* <905>, and *Labeling* under *Injections* <1>. The drug substance in the vial meets the requirements for *Cytarabine*.

**Assay—**

*Phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under Cytarabine.

*Assay preparation*—Separately constitute 5 vials of Cytarabine for Injection in a volume of water, accurately measured, corresponding to the volume specified in the labeling. Pool and mix the constituted solutions in a suitable container. Transfer an accurately measured volume of the constituted solution, equivalent to about 100 mg of cytarabine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

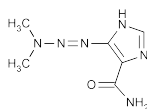
*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_9H_{13}N_3O_5$  in the portion of constituted solution taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cytarabine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Dacarbazine



$C_6H_{10}N_6O$  182.18

1H-Imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazenyl)-.  
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide  
[4342-03-4].

» Dacarbazine contains not less than 97.0 percent and not more than 102.0 percent of  $C_6H_{10}N_6O$ .

**Caution**—Great care should be taken in handling Dacarbazine, as it is a potent cytotoxic agent.

**Packaging and storage**—Preserve in tight, light-resistant containers, in a refrigerator.

### USP Reference standards (11)—

USP Dacarbazine RS

USP Dacarbazine Related Compound A RS

5-Aminoimidazole-4-carboxamide hydrochloride.

USP Dacarbazine Related Compound B RS

2-Azahypoxanthine.

$C_4H_3N_5O$  137.10

**Identification**—The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dacarbazine RS.

**Residue on ignition** (281): not more than 0.1%.

**Related compounds**—Dissolve an accurately weighed quantity of Dacarbazine in 0.1 N hydrochloric acid to obtain a solution having a concentration of 40 mg per mL, and apply 5  $\mu$ L of the solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Apply, separately, 5  $\mu$ L of a methanolic solution containing 0.40 mg of USP Dacarbazine Related Compound A RS per mL, and 5  $\mu$ L of an aqueous solution containing 0.40 mg of USP Dacarbazine Related Compound B RS per mL. Develop the chromatogram in a mixture of butanol, water, and acetic acid (5:2:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: any spots obtained from the test solution are not greater in size or intensity than the spots, occurring at the respective  $R_f$  values, produced by the Standard solutions, corresponding to not more than 1.0% of dacarbazine related compound A and not more than 1.0% of dacarbazine related compound B.

**Assay**—[NOTE—Throughout this procedure, avoid exposing Dacarbazine and its solutions to light.]

**Standard preparations**—Transfer about 30 mg of USP Dacarbazine RS, accurately weighed, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix (*Standard stock solution*). Dilute a portion of *Standard stock solution* quantitatively and stepwise with 0.1 N hydrochloric acid to obtain an *Acidic standard preparation* having a known concentration of about 6  $\mu$ g per mL. Dilute a portion of *Standard stock solution* quantitatively and stepwise with pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to obtain a *Neutral standard preparation* having a known concentration of about 6  $\mu$ g per mL.

**Assay preparations**—Prepare as directed under *Standard preparations*, except to use about 30 mg of Dacarbazine, accurately weighed.

**Procedure**—Concomitantly determine the absorbances of the *Acidic standard preparation* and the *Acidic assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 323 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Concomitantly determine the absorbances of the *Neutral standard preparation* and the *Neutral assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 329 nm, using pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) as the blank. Calculate the quantity, in mg, of  $C_6H_{10}N_6O$  in the portion of Dacarbazine taken by the formula:

$$5C[(A_{323} + A_{329})_U / (A_{323} + A_{329})_S]$$

in which C is the concentration, in  $\mu$ g per mL, of USP Dacarbazine RS in the *Standard preparations*, and the parenthetical expressions are the sums of the absorbances of the *Assay preparations* (U) and the *Standard preparations* (S), respectively, measured at the wavelengths indicated by the subscripts.

## Dacarbazine for Injection

» Dacarbazine for Injection is a sterile, freeze-dried mixture of Dacarbazine and suitable buffers or diluents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_{10}N_6O$ .

**Caution**—Great care should be taken to prevent inhaling particles of Dacarbazine for Injection and exposing the skin to it.

**Packaging and storage**—Preserve in single-dose or multiple-dose *Containers for Sterile Solids* as described under *Injections* (1), preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Dacarbazine RS

USP Dacarbazine Related Compound B RS

2-Azahypoxanthine.

$C_4H_3N_5O$  137.10

USP Endotoxin RS

**Completeness of solution**—When dissolved as directed in the labeling, it yields a clear, pale yellow to yellow solution.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

### Identification—

**A:** Dissolve a suitable quantity of Dacarbazine for Injection in water to obtain a solution having a concentration of 10 mg of dacarbazine per mL. Apply separately 1  $\mu$ L of the freshly prepared solution and 1  $\mu$ L of an aqueous solution, containing 10 mg each of USP Dacarbazine RS and citric acid per mL, to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol and 1 N ammonium hydroxide (3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate evenly with a freshly prepared solution containing 1% of ferric chloride and 1% of potassium ferricyanide (prepared by mixing 5 mL of a 10% aqueous solution of ferric chloride with 5 mL of a 10% aqueous solution of potassium ferricyanide and diluting with water to 50 mL). Dacarbazine

appears as an intense blue spot on a light yellow background: the  $R_f$  value of the spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B:** To 1 mL of a solution (1 in 100) in a test tube add a few crystals of periodic acid and 4 drops of methanol. Shake, and after 1 minute add 5 mL of a 0.2% acetylacetone reagent solution (prepared by mixing 15.0 g of ammonium acetate, 0.30 mL of glacial acetic acid, and 0.20 mL of acetylacetone in a 100-mL volumetric flask, adding water to volume, and mixing). Shake, and place in a water bath maintained at a temperature of 60°: an intense yellow color develops in a few minutes (*presence of mannitol*).

**C:** To 2 drops of an aqueous solution (1 in 100) in a 15-mL test tube add 10 mL of a solution prepared by mixing 10 mL of acetic anhydride with 30 mL of pyridine: an intense yellow color is produced immediately and after a few minutes becomes red-violet (*presence of citric acid*).

**Bacterial endotoxins** (85)—It contains not more than 0.52 USP Endotoxin Unit per mg of dacarbazine.

**pH** (791): between 3.0 and 4.0, in a solution containing an amount of Dacarbazine for Injection equivalent to about 1 g of dacarbazine in 100 mL of water.

**Water, Method I** (921): not more than 1.5%.

**Limit of 2-azahypoxanthine**—[NOTE—The *Mobile phase* employed in this procedure is corrosive. The system should be rinsed well with methanol following completion of analysis.]

*Mobile phase*—Transfer 2.2 g of docusate sodium to a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 15 mL of glacial acetic acid, and dilute with water to volume. Filter the solution through a 0.5- $\mu$ m porosity filter. Prepare this solution fresh daily.

*Standard solution*—Prepare a solution of USP Dacarbazine Related Compound B RS to contain 0.04 mg per mL.

*Test solution*—Constitute the contents of 1 vial of Dacarbazine for Injection. Using the contents of the constituted vial, dilute quantitatively with water to obtain a solution containing 4 mg of dacarbazine per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph five replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the peak responses at corresponding retention times obtained from the *Standard solution* and the *Test solution*, and calculate the quantity, in mg, of 2-azahypoxanthine monohydrate in the dacarbazine taken by the formula:

$$(CV)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dacarbazine Related Compound B RS in the *Standard solution*; V is the final volume, in mL, of the *Test solution*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is found.

**Other requirements**—It meets the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905) and *Labeling under Injections* (1).

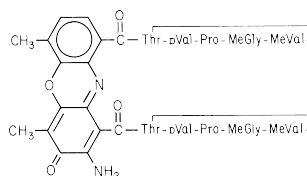
**Assay**—Dissolve the contents of not fewer than 10 containers of Dacarbazine for Injection in 0.1 N hydrochloric acid. Transfer and combine the solutions quantitatively rinsing as necessary with 0.1 N hydrochloric acid. Dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric

acid to obtain a solution containing about 0.4 mg per mL. Transfer 2.0 mL of this solution to a 250-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Dacarbazine RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 3.2  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 323 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the average quantity, in mg, of  $C_6H_{10}N_6O$  in each container of Dacarbazine for Injection taken by the formula:

$$125(VC / N)(A_U / A_S)$$

in which V is the volume, in mL, of the solution produced by dilution of the combined container contents to a concentration of 0.4 mg per mL taking account of dilution factors in the case of stepwise dilution; C is the concentration, in mg per mL, of USP Dacarbazine RS in the Standard solution; N is the number of vials taken; and  $A_U$  and  $A_S$  are the absorbances of the solution of Dacarbazine for Injection and the Standard solution, respectively.

## Dactinomycin



$C_{62}H_{86}N_{12}O_{16}$  1255.42

Actinomycin D.

Actinomycin D [50-76-0].

» Dactinomycin contains not less than 950  $\mu$ g and not more than 1030  $\mu$ g of  $C_{62}H_{86}N_{12}O_{16}$  per mg, calculated on the dried basis.

**Caution**—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers, protected from light and excessive heat.

**USP Reference standards** (11)—

USP Dactinomycin RS

USP Endotoxin RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 25  $\mu$ g per mL.

*Medium:* methanol.

Absorptivity at 445 nm, calculated on the dried basis, is not less than 95.0% and not more than 103.0% of that of USP Dactinomycin RS, the potency of the Reference Standard being taken into account.

*Ratio:*  $A_{240}/A_{445}$  between 1.30 and 1.50.

**B:** The chromatogram obtained from the *Assay preparation* in the Assay exhibits a major peak for dactinomycin, the retention time of which corresponds to that exhibited by the *Standard preparation*, and the chromatogram compares qualitatively to that obtained from the *Standard preparation*.

**Specific rotation** (781S): between  $-293^\circ$  and  $-329^\circ$  measured at 20°.

*Test solution:* 1 mg per mL, in methanol.

**Crystallinity** (695): meets the requirements.

**Bacterial endotoxins** (85)—It contains not more than 100 USP Endotoxin Units per mg.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Assay**—[NOTE—In this procedure, use a freshly prepared *Standard preparation* and *Assay preparation*, protected from light.]

*Mobile phase*—Prepare a suitable mixture of acetonitrile, 0.04 M sodium acetate, and 0.07 M acetic acid (approximately 46:25:25), filter through a membrane filter (1-μm or finer porosity), and degas. [NOTE—The acetonitrile concentration may be varied to provide appropriate *Chromatographic system* performance and to provide a suitable elution time.]

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dactinomycin RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 1200 μg of dactinomycin per mL.

*Assay preparation*—Dissolve about 30 mg of Dactinomycin, accurately weighed, in *Mobile phase* to make 25.0 mL, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 25 minutes for Dactinomycin. Calculate the potency, in μg, of C<sub>62</sub>H<sub>86</sub>N<sub>12</sub>O<sub>16</sub> per mg taken by the formula:

$$25(C/W)(r_U/r_S)$$

in which C is the concentration, in μg, of dactinomycin in each mL of the *Standard preparation*; W is the weight, in mg, of Dactinomycin taken; and  $r_U$  and  $r_S$  are the peak responses of the *Assay preparation* and the *Standard preparation*, respectively.

## Dactinomycin for Injection

### DEFINITION

Dactinomycin for Injection is a sterile mixture of Dactinomycin and Mannitol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of C<sub>62</sub>H<sub>86</sub>N<sub>12</sub>O<sub>16</sub>.

[**CAUTION**—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

### IDENTIFICATION

#### A. PROCEDURE

*Standard solution:* 25 μg/mL of USP Dactinomycin RS in methanol

*Sample solution:* 25 μg/mL of dactinomycin in methanol

**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as the *Standard solution*, concomitantly measured.

**Ratio:** A<sub>240</sub>/A<sub>445</sub>, 1.30–1.50

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Use freshly prepared *Standard solution* and *Sample solution*, protected from light.]

*Mobile phase:* Acetonitrile and water (3:2)

*Standard solution:* 250 μg/mL of USP Dactinomycin RS in *Mobile phase*

*Sample solution:* 250 μg/mL of dactinomycin from Dactinomycin for Injection diluted with *Mobile phase*. Filter, if necessary, to obtain a clear solution. [NOTE—Prepare the solution by adding a suitable aliquot of *Mobile phase* to one container of Dactinomycin for Injection.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.5 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for dactinomycin is 6 min.]

#### Suitability requirements

**Column efficiency:** NLT 1200 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>62</sub>H<sub>86</sub>N<sub>12</sub>O<sub>16</sub> in the portion of Dactinomycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dactinomycin RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of dactinomycin in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–120.0%

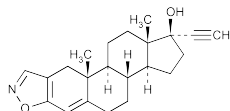
### SPECIFIC TESTS

- PH (791):** 5.5–7.5, in the solution constituted as directed in the labeling
- LOSS ON DRYING (731):** Dry a portion in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 4.0% of its weight.
- OTHER REQUIREMENTS:** It meets the requirements under *Injections* (1).
- BACTERIAL ENDOTOXINS TEST (85):** NMT 100.0 USP Endotoxin Units/mg of dactinomycin.
- STERILITY TESTS (71):** Meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined*, *Membrane Filtration*, each container being constituted aseptically by injecting Sterile Water for Injection through the stopper, and the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.
- CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* (1), *Constituted Solutions*.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in light-resistant Containers for Sterile Solids as described under *Injections* (1).
- LABELING:** Label it to include the statement "Protect from light."
- USP REFERENCE STANDARDS (11)**
  - USP Dactinomycin RS
  - USP Endotoxin RS

## Danazol



$C_{22}H_{27}NO_2$  337.46

Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol, (17 $\alpha$ )-  
17 $\alpha$ -Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol  
[17230-88-5].

» Danazol contains not less than 97.0 percent and not more than 102.0 percent of  $C_{22}H_{27}NO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Danazol RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* prepared as directed in the Assay.

**Specific rotation** (781S): between +21° and +27°.

*Test solution:* 10 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at 60° to constant weight: it loses not more than 2.0% of its weight.

**Chromatographic purity**—

*Solvent*—Prepare a mixture of chloroform and methanol (9:1).

*Standard solutions*—Dissolve an accurately weighed quantity of USP Danazol RS in *Solvent* to obtain a solution having a known concentration of 1 mg per mL. Dilute quantitatively with *Solvent* to obtain *Standard solutions* having the following compositions:

Standard solution	Dilution	Concentration ( $\mu$ g RS per mL)	Percentage (% for comparison with test specimen)
A	(1 in 2)	500	1.0
B	(1 in 4)	250	0.5
C	(1 in 10)	100	0.2
D	(1 in 20)	50	0.1

*Test solution*—Dissolve an accurately weighed quantity of Danazol in *Solvent* to obtain a solution containing 50 mg per mL.

*Procedure*—Apply separately 5  $\mu$ L of the *Test solution* and 5  $\mu$ L of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber and develop the chromatograms in a solvent system consisting of a mixture of cyclohexane and ethyl acetate (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light. Expose the plate to iodine vapors for 5 minutes. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*: the sum of the intensities of secondary spots obtained from the *Test solution* corresponds to not more than

1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

**Assay**—Dissolve about 100 mg of Danazol, accurately weighed and previously dried, in about 50 mL of alcohol in a 100-mL volumetric flask, swirl until dissolved, dilute with alcohol to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Similarly, dissolve an accurately weighed quantity of USP Danazol RS in alcohol to obtain a Standard solution having a known concentration of about 20  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, using alcohol as the blank. Calculate the quantity, in mg, of  $C_{22}H_{27}NO_2$  in the portion of Danazol taken by the formula:

$$5C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Danazol RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Danazol and the Standard solution, respectively.

## Danazol Capsules

» Danazol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{27}NO_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Danazol RS

**Identification**—Shake the contents of a sufficient number of Capsules, equivalent to about 50 mg of Danazol, with 50 mL of chloroform, and filter. Evaporate the filtrate on a steam bath with the aid of a stream of nitrogen to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue, previously dried, exhibits maxima at the same wavelengths as that of a similar preparation of USP Danazol RS.

**Dissolution** (711)—

*Medium:* 0.75% sodium lauryl sulfate solution; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_{22}H_{27}NO_2$  dissolved as follows. Remove an aliquot from the solution under test at a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Measure the amount in solution in filtered portions of the *Dissolution Medium*, suitably diluted with the *Dissolution Medium*, at the wavelength of maximum absorbance at about 286 nm, with a suitable spectrophotometer, in comparison with a solution of known concentration of USP Danazol RS prepared as follows. Transfer 10 mg of USP Danazol RS, accurately weighed, to a 10-mL volumetric flask, and dissolve in isopropyl alcohol. Transfer 2.0 mL to a 100-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{22}H_{27}NO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (4:3:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Danazol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Accurately weigh the contents of not less than 20 Capsules. Mix the contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of danazol, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 5 mL of the filtrate. Pipet 5 mL of the filtrate into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.45-μm porosity filter, discarding the first 5 mL of the filtrate.

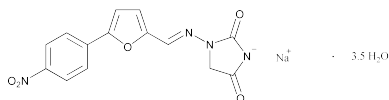
**Chromatographic system** (see *System Suitability* under *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a 3.9-mm × 15-cm column that contains 4-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of danazol in the portion of Capsules taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Danazol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dantrolene Sodium



$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  399.29  
2,4-Imidazolidinedione, 1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-, sodium salt, hydrate (2:7).  
1-[[5-(p-Nitrophenyl)furfurylidene]amino]hydantoin sodium salt hydrate [24868-20-0].

» Dantrolene Sodium contains not less than 90.0 percent and not more than 96.0 percent of  $C_{14}H_{10}N_4O_5$ , the free acid form of Dantrolene Sodium, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

### USP Reference standards (11)—

USP Dantrolene RS

USP Dantrolene Related Compound A RS  
5-(4-Nitrophenyl)-2-furaldehyde azine.  
 $C_{22}H_{14}N_4O_6$

USP Dantrolene Related Compound B RS  
5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.  
 $C_{14}H_{12}N_4O_6$

USP Dantrolene Related Compound C RS  
5-(4-Nitrophenyl)-2-furancarboxyaldehyde.  
 $C_{11}H_7NO_4$

USP Dantrolene Sodium RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** Ignite about 200 mg; the residue meets the requirements of the flame test for *Sodium* (191).

**Water**, *Method Ia* (921): between 14.5% and 17.0%.

**Heavy metals**, *Method II* (231): 0.002%.

### Limit of dantrolene related compound A—

**Test stock solution**—Prepare as directed for the *Assay stock preparation* as directed in the *Assay*.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Dantrolene Related Compound A RS and USP Dantrolene Sodium RS in dimethylformamide to obtain a solution having known concentrations of 17.5 μg per mL of dantrolene related compound A and 50 μg per mL of dantrolene sodium. Dilute with acetonitrile to obtain a solution having concentrations of about 0.35 μg per mL of dantrolene related compound A and 1 μg per mL of dantrolene sodium.

**Test solution**—Dilute the *Test stock solution* with acetonitrile to obtain a solution having a concentration of about 0.175 mg per mL of dantrolene sodium.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor for dantrolene related compound A is not more than 1.5; and the relative standard deviation for replicate injections is not more than 5% for dantrolene related compound A. [NOTE—The dantrolene peak elutes at void volume at approximately 1.5 minutes.]

**Procedure**—Inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the response for the dantrolene related compound A peak. Calculate the percentage of dantrolene related compound A in the portion of Dantrolene Sodium taken by the formula:

$$100(r_U / r_S)(C_S / C_T)$$

in which  $r_U$  and  $r_S$  are the peak responses of dantrolene related compound A obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene related compound A in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of Dantrolene Sodium in the *Test solution*: not more than 0.15% of dantrolene related compound A is found.

### Related compounds—

**Mobile phase**, **System suitability stock solution B**, **Diluent**, and **Chromatographic system**—Prepare as directed in the *Assay*.

**Standard solution**—Dilute quantitatively the *System suitability stock solution B* with *Diluent* to obtain a solution having a known concentration of 0.25 μg per mL each of dantrolene related compound B and dantrolene related compound C.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Inject equal volumes (about 20 μL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each of the relevant

dantrolene related compounds in the portion of Dantrolene Sodium taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of dantrolene related compound B or dantrolene related compound C in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Dantrolene Sodium in the *Test solution*;  $r_U$  is the individual peak response for dantrolene related compound B or dantrolene related compound C obtained from the *Test solution*; and  $r_S$  is the response of the corresponding peak obtained from the *Standard solution*. Not more than 0.50% of dantrolene related compound B is found; and not more than 0.30% of dantrolene related compound C is found.

#### Assay—

**Buffer**—Dissolve 3.85 g of ammonium acetate in 1.0 L of water, and adjust with glacial acetic acid to a pH of  $4.5 \pm 0.1$ .

**Diluent**—Prepare a mixture of water and acetonitrile (50:50).

**Solution A**—Prepare a filtered and degassed mixture of water, *Buffer*, and acetonitrile (70:20:10).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and *Buffer* (80:20).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability stock solution A**—Transfer 62.5 mg of USP Dantrolene Sodium RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 1.25 mg per mL of dantrolene sodium.

**System suitability stock solution B**—Transfer 6.3 mg each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having known concentrations of about 0.125 mg per mL each of dantrolene related compound B and dantrolene related compound C.

**System suitability solution**—Quantitatively dilute suitable volumes of *System suitability stock preparation A* and *System suitability stock preparation B* with *Diluent* to obtain a solution having concentrations of about 0.125 mg per mL of dantrolene sodium and 2.5 µg per mL each of dantrolene related compound B and dantrolene related compound C.

**Standard stock preparation**—Transfer 50 mg of USP Dantrolene RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 1.0 mg per mL of dantrolene.

**Standard preparation**—Dilute the *Standard stock preparation* with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL of dantrolene.

**Assay stock preparation**—Transfer an accurately weighed quantity of dantrolene sodium into a 100-mL volumetric flask, and dissolve in 5 mL of dimethylformamide. Add 5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a concentration of about 1.25 mg per mL of dantrolene sodium.

**Assay preparation**—Dilute the *Assay stock preparation* with *Diluent* to obtain a solution having a concentration of about 0.125 mg per mL of dantrolene sodium.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector

and a 4.6-mm × 15-cm column that contains 5-µm L1 packing. The flow rate is about 2.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solvent A (%)	Solvent B (%)	Elution
0–10	90→60	10→40	linear gradient
10–20	60→10	40→90	linear gradient
20–25	10	90	isocratic
25–25.1	10→90	90→10	linear gradient
25.1–35	90	10	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between dantrolene and dantrolene related compound C is not less than 8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%. [NOTE—For the purpose of identification, the approximate relative retention times are 0.68 for dantrolene related compound B, 1.24 for dantrolene related compound C, and 1.0 for dantrolene.]

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dantrolene peaks. Calculate the percentage of dantrolene ( $C_{14}H_9N_4O_5$ ) in the portion of Dantrolene Sodium taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Dantrolene Sodium in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dantrolene Sodium Capsules

» Dantrolene Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dantrolene sodium ( $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Dantrolene RS

USP Dantrolene Related Compound B RS

5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$C_{14}H_{12}N_4O_6$

USP Dantrolene Sodium RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

**Medium:** 0.5% methylbenzethonium chloride in water, pH 6.8 (adjusted with 0.1 N potassium hydroxide or 0.1 N hydrochloric acid); 900 mL, deaerated.

**Apparatus 1:** 100 rpm.

**Time:** 40 minutes.

Determine the amount of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  dissolved by employing the following method.

**Standard solution 1** (for Capsules labeled to contain 100 mg)—Transfer 25 mg of USP Dantrolene RS, accurately weighed, to a 250-mL volumetric flask. Dissolve in 5.0 mL of dimethylformamide. Add 200 mL of *Medium* and 10.0 mL of 0.1 N potassium hydroxide. Mix, dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu$ m polytetrafluoroethylene (PTFE) filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Standard solution 2** (for Capsules labeled to contain 50 mg)—Transfer 25.0 mL of *Standard solution 1* to a 50-mL volumetric flask containing 0.5 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu$ m PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Standard solution 3** (for Capsules labeled to contain 25 mg)—Transfer 25.0 mL of *Standard solution 1* to a 100-mL volumetric flask containing 1.0 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu$ m PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Test solution**—Withdraw 10 mL of the solution under test. Pass through a 0.45- $\mu$ m PTFE filter, previously wetted with a few drops of isopropyl alcohol. Discard the first 5 mL. Collect the filtered solution in a tube that contains 1 drop of 1 N potassium hydroxide, and mix.

**System suitability**—[NOTE—All absorbance values should be obtained on solutions within 2 hours of their preparation.] Using a 0.1-cm cell, measure the absorbance of the *Medium*, using water as the blank, and measure the absorbance of each of the three *Standard solutions* using *Medium* as the blank, at the wavelength of maximum absorbance at about 395 nm. The system is considered suitable for use if the following criteria are met: the absorbance of the *Medium* is less than 10% of the absorbance of *Standard solution 1*; the absorbance of *Standard solution 2* is between 0.3 and 0.5; and the ratio of the absorbance of *Standard solution 1* to that of *Standard solution 3* is  $4.00 \pm 0.10$ .

Determine the amount of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  dissolved by measuring the absorbance of the *Test solution* at the wavelength of maximum absorbance at about 395 nm in comparison with the appropriate *Standard solution*, using a 0.1-cm cell and *Medium* as the blank. All absorbance values are obtained on solutions within 2 hours of their preparation. Calculate the percentage of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times 0.79186 \times LC}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; 0.79186 is the correction for water of hydration and sodium contained in the dantrolene sodium monohydrate form of the drug, assuming that the bulk drug contains 15% of water and 6.84% of sodium; and  $LC$  is the Capsule label claim, in mg.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  is dissolved in 40 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Related compounds—

*Diluent*, *Solution A*, *Solution B*, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the Assay.

**Standard solution**—Transfer 5 mg, accurately weighed, of USP Dantrolene Related Compound B RS into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.1 mg per mL. Quantitatively dilute this solution with *Diluent* to obtain a

solution having a known concentration of about 0.0005 mg per mL of dantrolene related compound B.

**Test solution**—Use the Assay preparation.

**Procedure**—Inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Capsules taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which  $r_U$  is the individual peak response for dantrolene related compound B obtained from the *Test solution*;  $r_S$  is the response of the corresponding peak in the *Standard solution*;  $C_S$  is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of dantrolene sodium in the *Test solution*: not more than 2% of dantrolene related compound B is found.

#### Assay—

**Diluent**—Prepare a solution of acetonitrile and water (70:30).

**Buffer solution**—Dissolve 3.3 g of ammonium acetate in 1 L of water.

**Solution A**—Prepare a filtered and degassed mixture of *Buffer solution*, acetonitrile, and glacial acetic acid (120:76:7).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Use the *Standard solution*, prepared as directed in the test for *Related compounds*.

**Standard preparation**—Transfer 40 mg, accurately weighed, of USP Dantrolene RS to a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.8 mg per mL. Quantitatively dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

**Assay preparation**—Mix the combined contents of not fewer than 20 Capsules, and transfer an accurately weighed portion, equivalent to the average weight of one Capsule, to a 50-mL volumetric flask. Add 10 mL of dimethylformamide, and sonicate for 15 minutes to dissolve. Add 5 mL of glacial acetic acid, and dilute with acetone to volume. Quantitatively dilute this solution with *Diluent* to obtain a solution having 0.1 mg per mL of dantrolene sodium, and pass through a 0.45- $\mu$ m nylon filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–8	100	0	isocratic
8–8.1	100→0	0→100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0→100	100→0	linear gradient
13.1–20	100	0	re-equilibration

Separately inject the *System suitability solution* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the

relative standard deviation for replicate injections for dantrolene is not more than 1.0%. [NOTE—For the purpose of peak identification, the approximate relative retention times are 0.68 for dantrolene related compound B and 1.0 for dantrolene.]

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dantrolene peaks. Calculate the percentage of dantrolene sodium ( $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ ) in the portion of Capsules taken by the formula:

$$100(399.29/314.25)(r_U / r_S)(C_S / C_U)$$

in which 399.29 is the molecular weight of dantrolene sodium; 314.25 is the molecular weight of dantrolene;  $r_U$  and  $r_S$  are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard preparation*; and  $C_U$  is the concentration, in mg per mL, of dantrolene sodium ( $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ ) in the *Assay preparation*.

## Dantrolene Sodium for Injection

» Dantrolene Sodium for Injection is a sterile, non-pyrogenic, lyophilized formulation containing Dantrolene Sodium, and one or more suitable buffering or sequestering agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ .

**Packaging and storage**—Preserve in tight containers. Store at controlled room temperature, protected from light.

### USP Reference standards (11)—

USP Dantrolene RS

USP Dantrolene Related Compound B RS

5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$C_{14}H_{12}N_4O_6$

USP Endotoxin RS

### Identification—

**A:** *Infrared Absorption* (197K)—

**Test specimen**—To 0.5 g of Dantrolene Sodium for Injection, add 10 mL of 0.1 N hydrochloric acid and 10 mL of ethyl acetate, and mix. Allow the phases to separate, and transfer the upper ethyl acetate phase to a suitable glass container. Evaporate the solvent, dry the residue at 105° for 10 minutes, and use the residue.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of dantrolene sodium.

**Sterility** (71): meets the requirements.

**Uniformity of dosage units** (905): meets the requirements.

**pH** (791)—Dissolve the contents of 1 vial in 60 mL of USP Water for Injection: the pH is between 8.8 and 11.0.

**Water, Method Ia** (921): not more than 3.0%.

### Related compounds—

**Mobile phase and Diluent**—Proceed as directed in the *Assay*.

**Standard solution**—Transfer 10 mg of USP Dantrolene Related Compound B RS, accurately weighed, into a 50-mL

volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.2 mg per mL. Dilute with *Diluent* to obtain a solution having a known concentration of about 0.002 mg per mL of dantrolene related compound B.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system**—Proceed as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections for dantrolene related compound B is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Dantrolene Sodium for Injection taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which  $r_U$  is the peak response for dantrolene related compound B obtained from the *Test solution*;  $r_S$  is the corresponding peak response in the *Standard solution*;  $C_S$  is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Test solution*. Not more than 8% of dantrolene related compound B is found.

**Other requirements:** meets the requirements under *Injections* (1).

### Assay—

**Buffer**—Dissolve 3.3 g of ammonium acetate in 1 L of water, and adjust with acetic acid to a pH of 4.5 ± 0.1.

**Solution A**—Prepare a filtered and degassed mixture of *Buffer*, acetonitrile, and glacial acetic acid (120:80:7).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of acetonitrile and water (60:40).

**Standard preparation**—Transfer 40 mg of USP Dantrolene RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.8 mg per mL. Dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

**Assay preparation**—Using 70 mL of water for each vial, transfer the entire contents of the required number of vials to a suitable flask necessary to obtain a solution having a known concentration of about 0.1 mg of dantrolene sodium hydrate per mL. Sonicate for 2 to 5 minutes to dissolve the sample. Dilute with *Diluent* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–8	100	0	isocratic
8–8.1	100→0	0→100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0→100	100→0	linear gradient
13.1–20	100	0	re-equilibration



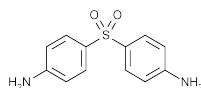
Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections for dantrolene is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for dantrolene. Calculate the percentage of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  in the portion of Dantrolene Sodium for Injection taken by the formula:

$$(399.29/314.25)(r_U/r_S)(C_S/C_U)$$

in which 399.29 and 314.25 are the molecular weights of dantrolene sodium hydrate and dantrolene, respectively;  $r_U$  and  $r_S$  are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard preparation*; and  $C_U$  is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Assay preparation*.

## Dapsone



$C_{12}H_{12}N_2O_2S$  248.30  
Benzenamine, 4,4'-sulfonylbis-  
4,4'-Sulfonyldianiline [80-08-0].

» Dapsone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{12}H_{12}N_2O_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Dapsone RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 5  $\mu$ g per mL.

*Medium:* methanol.

**Melting range** (741): between 175° and 181°.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Selenium** (291): 0.003%, a 100-mg test specimen, mixed with 100 mg of magnesium oxide, being used.

**Chromatographic purity**—

**Standard solutions**—Dissolve USP Dapsone RS in methanol and mix to obtain *Standard solution A* having a known concentration of 12.5 mg per mL. Dilute quantitatively with methanol to obtain *Standard solution B*, containing 125  $\mu$ g of the USP Reference Standard per mL, and *Standard solution C*, containing 62.5  $\mu$ g of the USP Reference Standard per mL.

**Test solution**—Dissolve an accurately weighed quantity of Dapsone in methanol to obtain a solution containing 12.5 mg per mL.

**Procedure**—[NOTE—Prepare the solvent system fresh daily. Equilibrate the chromatographic chamber with the solvent

system for 30 minutes prior to development of the chromatographic plate.] Separately apply 4  $\mu$ L of the *Test solution* and each of the *Standard solutions* to a suitable high-performance thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 150- to 200- $\mu$ m layer of chromatographic silica gel. Dry the applications with the aid of a stream of nitrogen. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of chloroform, acetone, *n*-butyl alcohol, and formic acid (60:15:15:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber and air-dry. Spray the plate lightly with a 0.1% (w/v) solution of 4-dimethylaminocinnamaldehyde in a mixture of equal volumes of glacial acetic acid and water. Examine the spots that are developed immediately, and compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatogram of the *Standard solutions*: no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution C* (0.5%), and the sum of the intensities of all the secondary spots obtained from the *Test solution* corresponds to not more than 1.0%.

**Assay**—

**Mobile phase**—Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dapsone RS in *Mobile phase* to obtain a solution having a known concentration of about 250  $\mu$ g per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 25  $\mu$ g per mL.

**Assay preparation**—Transfer about 50 mg of Dapsone, accurately weighed, to a 200-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains 10- $\mu$ m diameter packing L3. Chromatograph a sufficient number of injections of the *Standard preparation* as directed for *Procedure*: the relative standard deviation is not more than 2%.

**Procedure**—Separately introduce equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters to obtain satisfactory chromatograms. Measure the responses for the major peaks obtained at corresponding retention times with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of  $C_{12}H_{12}N_2O_2S$  in the portion of Dapsone taken by the formula:

$$2C(P_U / P_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Dapsone RS in the *Standard preparation*; and  $P_U$  and  $P_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Add the following:****▲Dapsone Oral Suspension****DEFINITION**

Dapsone Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dapsone ( $C_{12}H_{12}N_2O_2S$ ). Prepare Dapsone Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Dapsone tablets <sup>a</sup> equivalent to	200 mg
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Dapsone 25-mg tablets, Jacobus Pharmaceutical Company, Princeton, NJ.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a dapsone liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Solution A:** 50 mM ammonium phosphate adjusted to a pH of 4.6

**Mobile phase:** Acetonitrile and *Solution A* (12:88). Filter and degas.

**Internal standard solution:** 1.0 mg/mL of diazoxide in methanol

**Standard stock solution:** 2.0 mg/mL of USP Dapsone RS in methanol

**Standard solution:** Pipet 2.5 mL of *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of dapsone and 50 µg/mL of diazoxide. Centrifuge.

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 2.5 mL of Oral Suspension into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of dapsone and 50 µg/mL of diazoxide. Centrifuge.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 295 nm

**Column:** 3.0-mm × 15-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 0.7 mL/min

**Injection volume:** 10 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention times for dapsone and diazoxide are about 8.9 and 12.9 min, respectively.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.3% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dapsone ( $C_{12}H_{12}N_2O_2S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of dapsone to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of dapsone to the internal standard from the *Standard solution*

$C_S$  = concentration of dapsone in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of dapsone in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH (791):** 3.8–4.8

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature or at controlled room temperature.

**• LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled cold temperature or controlled room temperature

**• USP REFERENCE STANDARDS (11)**

USP Dapsone RS<sup>▲</sup><sub>USP36</sub>

**Dapsone Tablets****DEFINITION**

Dapsone Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dapsone ( $C_{12}H_{12}N_2O_2S$ ).

**IDENTIFICATION**

**• A. INFRARED ABSORPTION (197K)**

**Sample solution:** Transfer a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, to a suitable container, add 5 mL of acetone, shake for 5 min, filter, and evaporate the filtrate to dryness. Dry this residue at 105° for 1 h.

**Acceptance criteria:** Meets the requirements

**• B. ULTRAVIOLET ABSORPTION (197U)**

**Sample solution:** Nominally 0.01 µg/mL prepared as follows. Triturate a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, with 50 mL of methanol, and filter. Dilute a portion of the filtrate with methanol to make approximately a 1 in 200,000 solution.

**Acceptance criteria:** Meets the requirements

**ASSAY****• PROCEDURE**

**Mobile phase:** Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.

**Standard solution:** 25 µg/mL of USP Dapsone RS in *Mobile phase*

**Sample solution:** Nominally 25 µg/mL of dapsone prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 mg of dapsone, to a 200-mL volumetric flask. Add 150 mL of methanol, and place the flask in an ultrasonic bath at a temperature of 35° for 15 min, with occasional shaking. Allow to cool to room temperature, and add methanol to volume. Centrifuge a portion of the mixture until clear. Transfer 5.0 mL of the

clear supernatant to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; 10-μm diameter, packing L3

Injection volume: 10 μL

#### System suitability

Sample: *Standard solution* (chromatograph a sufficient number)

#### Suitability requirements

Relative standard deviation: NMT 2%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dapsone RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of dapsone in the *Sample solution* (μg/mL)

Acceptance criteria: 92.5%–107.5%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

Medium: Dilute hydrochloric acid (2 in 100); 1000 mL

Apparatus 1: 100 rpm

Time: 60 min

Standard solution: USP Dapsone RS of a known concentration in *Medium*

Sample solution: Withdraw and filter a portion of the *Sample solution*. Transfer a portion of the filtrate estimated to contain 0.2 mg of dapsone to a 25-mL volumetric flask, add 5 mL of 1 N sodium hydroxide, and dilute with water to volume.

#### Instrumental conditions

Mode: UV

Analytical wavelength: 290 nm

Tolerances: NLT 75% (Q) of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS <905>

Procedure for content uniformity

Standard solution: 8 μg/mL of USP Dapsone RS in methanol

Sample solution: Nominally 8 μg/mL of dapsone prepared as follows. To 1 Tablet in a 100-mL volumetric flask add 2.0 mL of water, and allow to stand for 30 min, swirling occasionally. Add 70 mL of methanol, and place the flask in an ultrasonic bath until the specimen is completely dispersed. Add methanol to volume, and centrifuge a portion of the mixture. Quantitatively dilute a measured volume of the clear supernatant with methanol.

#### Instrumental conditions

Mode: UV

Analytical wavelength: 296 nm

Cell: 1 cm

Blank: Methanol

#### Analysis

Samples: *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Dapsone RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of dapsone in the *Sample solution* (μg/mL)

Acceptance criteria: Meet the requirements

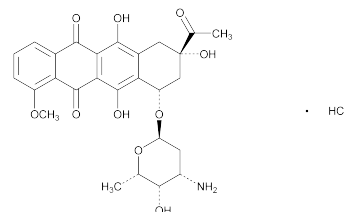
### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS <11>**

USP Dapsone RS

## Daunorubicin Hydrochloride



C<sub>27</sub>H<sub>29</sub>NO<sub>10</sub> · HCl 563.98

5,12-Naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-, (8*S*-*cis*-), hydrochloride.

(1*S*,3*S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside hydrochloride [23541-50-6].

» Daunorubicin Hydrochloride has a potency equivalent to not less than 842 μg and not more than 1030 μg of C<sub>27</sub>H<sub>29</sub>NO<sub>10</sub> per mg.

*Caution—Great care should be taken to prevent inhaling particles of Daunorubicin Hydrochloride and exposing the skin to it.*

**Packaging and storage**—Preserve in tight containers, protected from light and excessive heat.

**USP Reference standards <11>**—USP Daunorubicin Hydrochloride RS

#### Identification—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Daunorubicin Hydrochloride RS.

**B:** The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

**Crystallinity <695>**: meets the requirements.

**pH <791>**: between 4.5 and 6.5, in a solution containing 5 mg per mL.

**Water, Method I <921>**: not more than 3.0%.

#### Assay—

*Mobile phase*—Mix 62 volumes of water and 38 volumes of acetonitrile, and adjust with phosphoric acid to a pH of 2.2 ± 0.2. The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for daunorubicin. Filter the solution through a membrane filter (1 μm or finer porosity), and degas.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Daunorubicin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 250 μg of daunorubicin per mL.

**Resolution solution**—Prepare a solution of doxorubicin hydrochloride in the *Standard preparation* containing about 250 µg per mL.

**Assay preparation**—Transfer about 25 mg of Daunorubicin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for doxorubicin and 1.0 for daunorubicin; and the resolution,  $R$ , between the doxorubicin peak and the daunorubicin peak is not less than 3. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in µg of  $C_{27}H_{29}NO_{10}$  per mg, taken by the formula:

$$100(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in µg per mL, of daunorubicin in the *Standard preparation*;  $W$  is the weight, in mg, of Daunorubicin Hydrochloride taken; and  $r_U$  and  $r_S$  are the daunorubicin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Daunorubicin Hydrochloride for Injection

» Daunorubicin Hydrochloride for Injection is a sterile mixture of Daunorubicin Hydrochloride and Mannitol. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{27}H_{29}NO_{10}$ .

**Packaging and storage**—Preserve in light-resistant Containers for Sterile Solids as described under *Injections* <1>.

**USP Reference standards** <11>—  
USP Daunorubicin Hydrochloride RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Identification**—The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 4.3 USP Endotoxin Units per mg of daunorubicin.

**pH** <791>: between 4.5 and 6.5, in the solution constituted as directed in the labeling.

**Water, Method I** <921>: not more than 3.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Daunorubicin Hydrochloride*.

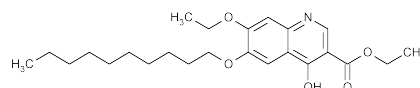
**Assay preparation**—Transfer the contents of 1 vial of Daunorubicin Hydrochloride for Injection with the aid of *Mobile phase* to an appropriate volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing about 0.25 mg of daunorubicin per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Daunorubicin Hydrochloride*. Calculate the quantity, in mg, of  $C_{27}H_{29}NO_{10}$  in the vial of Daunorubicin Hydrochloride for Injection taken by the formula:

$$(CV/1000)(r_U/r_S)$$

in which  $V$  is the volume, in mL, of the *Assay preparation*, and the other terms are as defined therein.

## Decoquinatate



$C_{24}H_{35}NO_5$  417.54

3-Quinolinecarboxylic acid, 6-(decyloxy)-7-ethoxy-4-hydroxy-, ethyl ester.

Ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate [18507-89-6].

» Decoquinatate contains not less than 99.0 percent and not more than 101.0 percent of  $C_{24}H_{35}NO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** <11>—

USP Decoquinatate RS

### Identification—

**A: Infrared Absorption** <197K>.

**B:** Transfer about 40 mg of it, accurately weighed, to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm add about 60 mL of dehydrated alcohol. Allow to cool, dilute with dehydrated alcohol to volume, and mix. Promptly transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Decoquinatate RS, concurrently measured, and the respective absorptivities, calculated on the dried basis, at the wavelength of maximum absorption at about 265 nm do not differ by more than 2.5%.

**Loss on drying** <731>—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

### Ordinary impurities <466>—

**Test solution:** chloroform, prepared with the aid of heat.

**Standard solution:** chloroform, using dilutions of the *Test solution*.

**Eluant:** a mixture of chloroform, dehydrated alcohol, and anhydrous formic acid (85:10:5).

**Visualization:** 1.

**Tolerances:** no impurity exceeds 1%, and the total does not exceed 2%.

**Assay**—Dissolve about 1000 mg of Decoquinatate, accurately weighed, in 100 mL of glacial acetic acid, with the aid of gentle heat. Allow to cool, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.76 mg of  $C_{24}H_{35}NO_5$ .

## Decoquinatate Premix

» Decoquinatate Premix contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{35}NO_5$ , the labeled amount being between 1 g and 10 g per 100 g of Premix.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Decoquinatate RS

**Identification**—To an accurately weighed quantity of it, equivalent to about 100 mg of decoquinatate, add 40 mL of chloroform, and heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Use the filtrate as the test solution. Apply 10- $\mu$ L portions of the test solution and of a Standard solution in chloroform containing 2.5 mg of USP Decoquinatate RS per mL to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and alcohol (70:30) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to dry in a current of air. Locate the spots under short-wave-length UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

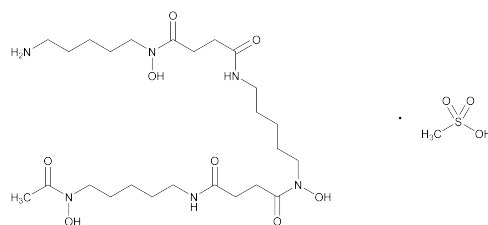
**Assay**—Extract an accurately weighed quantity of Premix, equivalent to about 200 mg of decoquinatate, with 50 mL of chloroform in a small continuous extraction apparatus for 8 reflux cycles. Transfer the extract to a 100-mL volumetric flask with the aid of chloroform, cool, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 5.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix (*Assay preparation*). Transfer 50 mg of USP Decoquinatate RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm slowly add 70 mL of dehydrated alcohol. Allow to cool, dilute with dehydrated alcohol to volume, and mix. Immediately transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix. This *Standard preparation* contains about 0.005 mg of USP Decoquinatate RS per mL. Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 265 nm, with a spectrophotometer, using a mixture

of dehydrated alcohol, 0.1 N hydrochloric acid, and chloroform (90:10:0.25) as the blank. Calculate the percentage of  $C_{24}H_{35}NO_5$  in the portion of Premix taken by the formula:

$$4000(C/W)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Decoquinatate RS in the *Standard preparation*; W is the quantity, in g, of Premix taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Deferoxamine Mesylate



$C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$  656.79  
Butanediamide, *N'*-[5-[[4-[[5-(acetylhydroxyamino)pentyl]-amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxy-, monomethanesulfonate;  
*N*-[5-[3-[(5-Aminopentyl)hydroxycarbonyl]propionamido]pentyl]-3-[[5-(*N*-hydroxyacetamido)pentyl]carbonyl]propionohydroxamic acid monomethanesulfonate (salt) [138-14-7].

### DEFINITION

Deferoxamine Mesylate contains NLT 93.0% and NMT 102.0% of  $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Solution A:** 1.32 g/L of dibasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile and *Solution A* (1:1)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	80	20
35	57.5	42.5
35.1	88	12
40	88	12

**Diluent:** Acetonitrile and water (6:94)

**Standard solution:** 1.0 mg/mL of USP Deferoxamine Mesylate RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Deferoxamine Mesylate in *Diluent*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 7.5-cm; 3.5-μm packing L1**Column temperature:** 32°**Autosampler temperature:** 5°**Flow rate:** 1.5 mL/min**Injection size:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of deferoxamine mesylate

(C<sub>25</sub>H<sub>48</sub>N<sub>6</sub>O<sub>8</sub> · CH<sub>3</sub>O<sub>3</sub>S) in the portion of Deferoxamine

Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of deferoxamine from the *Sample solution* $r_S$  = peak response of deferoxamine from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 93.0%–102.0% on the anhydrous basis**IMPURITIES**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%, 2.0 g being used
- **CHLORIDE AND SULFATE, Chloride** <221>: A 1.2-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (NMT 0.012%).
- **CHLORIDE AND SULFATE, Sulfate** <221>: A 0.5-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (NMT 0.04%).
- **HEAVY METALS, Method II** <231>: NMT 10 ppm
- **ORGANIC IMPURITIES**

**Solution A, Solution B, Diluent, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.**Standard stock solution:** Use the *Standard solution*, prepared as directed in the *Assay*. [NOTE—USP Deferoxamine Mesylate RS contains impurity A as a minor component.]**Standard solution:** 0.01 mg/mL of USP Deferoxamine Mesylate RS in *Diluent* from the *Standard stock solution***System suitability****Samples:** *Standard stock solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between the impurity A and deferoxamine peaks, *Standard stock solution***Relative standard deviation:** NMT 5.0% for the deferoxamine peak, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Deferoxamine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of deferoxamine from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 2*.

[NOTE—The reporting level for impurities is 0.04%.]

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity A*	0.85	3.0
Deferoxamine	1.0	—
Any unspecified impurity	—	1.0
Total impurities eluting before deferoxamine	—	5.0
Total impurities eluting after deferoxamine	—	2.0

\* Des-methylene impurity (desferrioxamine A).

**SPECIFIC TESTS**

- **PH** <791>: 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION, Method I** <921>: NMT 2.0%
- **STERILITY TESTS** <71>: Where the label states that Deferoxamine Mesylate is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Deferoxamine Mesylate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a cold place.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** <11>  
USP Deferoxamine Mesylate RS  
USP Endotoxin RS

**Deferoxamine Mesylate for Injection**

» Deferoxamine Mesylate for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>25</sub>H<sub>48</sub>N<sub>6</sub>O<sub>8</sub> · CH<sub>3</sub>O<sub>3</sub>S.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.**USP Reference standards** <11>—

USP Deferoxamine Mesylate RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.**Identification**—Dissolve 5 mg in 5 mL of water, add 2 mL of tribasic sodium phosphate solution (1 in 200), mix, then add 10 drops of β-naphthoquinone-4-sodium sulfonate solution (1 in 40); a blackish brown color is produced.**Bacterial endotoxins** <85>—It contains not more than 0.33 USP Endotoxin Unit per mg of deferoxamine mesylate.**PH** <791>: between 4.0 and 6.0, in a solution (1 in 100).**Water, Method I** <921>: not more than 1.5%.**Other requirements**—It meets the requirements under *Injections* <1> and *Uniformity of Dosage Units* <905>.**Assay**—*Ferric chloride solution*—Dissolve 6.7 g of ferric chloride in dilute hydrochloric acid (1 in 100) in a 100-mL volumetric

flask. Add dilute hydrochloric acid (1 in 100) to volume, mix, and filter.

**Standard preparation**—Dissolve a suitable quantity of USP Deferoxamine Mesylate RS, accurately weighed, in water to obtain a solution having a known concentration of about 1000 µg per mL.

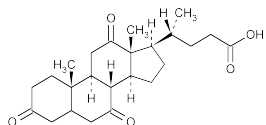
**Assay preparation**—Constitute the contents of 1 vial in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a concentration of about 1 mg per mL.

**Procedure**—Pipet 2 mL each of the *Standard preparation*, *Assay preparation*, and water to provide a blank, into separate 25-mL volumetric flasks. To each flask add 3 mL of *Ferric chloride solution*, dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparation* and the *Assay preparation* against the blank, in 1-cm cells, at the wavelength of maximum absorbance at about 485 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of deferoxamine mesylate ( $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ ) in the vial of Deferoxamine Mesylate for Injection taken by the formula:

$$CV(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Deferoxamine Mesylate RS in the *Standard preparation*; V is the volume, in mL, of water used to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dehydrocholic Acid



$C_{24}H_{34}O_5$  402.52  
Cholan-24-oic acid, 3,7,12-trioxo-, (5β)-;  
3,7,12-Trioxo-5β-cholan-24-oic acid [81-23-2].

### DEFINITION

Dehydrocholic Acid contains NLT 98.5% and NMT 101.0% of  $C_{24}H_{34}O_5$ , calculated on the dried basis. Dehydrocholic Acid for parenteral use melts between 237° and 242°.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Sample solution:** Transfer 500 mg of Dehydrocholic Acid to a 300-mL conical flask, add 60 mL of neutralized alcohol, and warm on a steam bath until dissolved. Allow to cool.

**Analysis:** Add phenolphthalein TS and 20 mL of water to the *Sample solution*. Titrate with 0.1 N sodium hydroxide VS, adding 100 mL of water shortly before the endpoint is reached. Each mL of 0.1 N sodium hydroxide is equivalent to 40.25 mg of  $C_{24}H_{34}O_5$ .

**Acceptance criteria:** 98.5%–101.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

#### • BARIUM

**Sample:** 2 g

**Analysis:** Boil the *Sample* with 100 mL of water for 2 min. Add 2 mL of hydrochloric acid, and again boil for

2 min. Cool, filter, and wash the filter with water until the filtrate measures 100 mL. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid.

**Acceptance criteria:** No turbidity is produced.

- **HEAVY METALS**, *Method II* (231): 20 ppm

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 231°–242°, but the range between beginning and end of melting does not exceed 3°.
- **OPTICAL ROTATION**, *Specific Rotation* (781S): +29.0° to +32.5°  
**Sample solution:** 20 mg/mL in dioxane
- **LOSS ON DRYING** (731): Dry at 105° for 2 h: it loses NMT 1.0% of its weight.
- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the test for absence of *Salmonella* species.
- **ODOR ON BOILING:** Boil 2 g with 100 mL of water for 2 min: the mixture is odorless.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Dehydrocholic Acid RS

## Dehydrocholic Acid Tablets

### DEFINITION

Dehydrocholic Acid Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of  $C_{24}H_{34}O_5$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Mix a quantity of finely powdered Tablets equivalent to 500 mg of dehydrocholic acid with 15 mL of water, and add slowly, with stirring, 2 mL of sodium carbonate TS. Filter, and add to the filtrate 3 N hydrochloric acid (about 2 mL) dropwise until no more precipitate is formed. Filter the precipitate, wash with small portions of cold water until free from chloride, and dry at 105° for 2 h. [NOTE—Reserve a portion of the material obtained for use in *Identification* test B.]

- **B. MELTING RANGE OR TEMPERATURE** (741): 231°–242°, but the range between beginning and end of melting does not exceed 3°.

**Sample:** Use the material reserved from *Identification* test A.

### ASSAY

#### • PROCEDURE

**Sample:** Finely powder NLT 20 Tablets. Transfer a portion of the powder equivalent to 500 mg of dehydrocholic acid to a 300-mL conical flask. Add 60 mL of neutralized alcohol, and warm on a steam bath for 10 min. Allow to cool.

**Analysis:** Add phenolphthalein TS and 20 mL of water. Titrate with 0.1 N sodium hydroxide VS, adding 100 mL of water shortly before the endpoint is reached. Each mL of 0.1 N sodium hydroxide is equivalent to 40.25 mg of  $C_{24}H_{34}O_5$ .

**Acceptance criteria:** 94.0%–106.0%

### PERFORMANCE TESTS

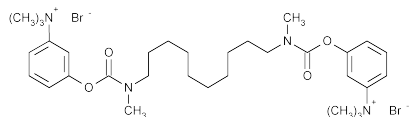
- **DISINTEGRATION** (701)  
Time: NMT 30 min
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the test for absence of *Salmonella* species

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Dehydrocholic Acid RS

**Demecarium Bromide**

$C_{32}H_{52}Br_2N_4O_4$  716.59

Benzenaminium, 3,3'-[1,10-decanediylbis[(methylimino)carbonyloxy]]bis[*N,N,N*-trimethyl]-, dibromide.

(*m*-Hydroxyphenyl)trimethylammonium bromide decamethylenebis[methylcarbamate] (2:1) [56-94-0].

» Demecarium Bromide contains not less than 95.0 percent and not more than 100.5 percent of  $C_{32}H_{52}Br_2N_4O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Demecarium Bromide RS

**Identification—**

**A:** *Infrared Absorption* <197K>.

**B:** Dissolve about 100 mg in 50 mL of 1 N sodium hydroxide, and reflux for 15 minutes. Cool, and add 3 mL of the refluxed solution to 25 mL of saturated sodium bicarbonate solution. Add, with mixing, 4 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000) and 2 mL of sodium hypochlorite solution (1.5 in 20,000): a violet-blue color is produced.

**C:** Dissolve about 50 mg in 20 mL of water, add 10 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling: a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.

**D:** A solution of it responds to the tests for *Bromide* <191>.

**pH** <791>: between 5.0 and 7.0, in a solution (1 in 100).

**Water, Method I** <921>: not more than 2.0%.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method I** <231>: 0.002%.

**Limit of *m*-trimethylammonio-phenol bromide—**

**Control solution**—Dissolve 100 mg of *m*-dimethylaminophenol in 10 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 1 mL of this solution into a 500-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Transfer 100 mg of Demecarium Bromide to a 100-mL volumetric flask, add water to volume, and mix.

**Procedure**—Pipet 25 mL of the *Test solution* into a glass-stoppered, 50-mL centrifuge tube, and pipet 25 mL of the

*Control solution* into a second, similar tube. To each tube add 3 mL of pH 7.0 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000), 5 mL of isobutyl alcohol, and 1 mL of sodium hypochlorite solution (1.5 in 20,000). Insert the stoppers in the tubes, shake the mixtures for 5 minutes, and centrifuge: any blue color produced in the upper layer obtained from the *Test solution* is not more intense than that obtained from the *Control solution*.

**Assay**—Dissolve about 0.8 g of Demecarium Bromide, accurately weighed, in a mixture of 75 mL of glacial acetic acid and 15 mL of mercuric acetate TS, warming slightly, if necessary, to effect solution. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 35.83 mg of  $C_{32}H_{52}Br_2N_4O_4$ .

**Demecarium Bromide Ophthalmic Solution**

» Demecarium Bromide Ophthalmic Solution is a sterile, aqueous solution of Demecarium Bromide. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{32}H_{52}Br_2N_4O_4$ . It contains a suitable antimicrobial agent.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Demecarium Bromide RS

**Identification—**

**A:** Mix about 10 mL with 12.5 mL of 1 N sodium hydroxide, and proceed as directed in *Identification test B* under *Demecarium Bromide*, beginning with "reflux for 15 minutes": a violet-blue color is produced.

**B:** To 10 mL add 5 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling: a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.

**C:** It responds to the tests for *Bromide* <191>.

**Sterility** <71>: meets the requirements.

**Assay**—Into each of two 50-mL volumetric flasks marked 1 and 2 pipet a volume of Ophthalmic Solution, equivalent to about 2.5 mg of demecarium bromide. Into each of two additional 50-mL volumetric flasks, marked 3 and 4, pipet 5.0 mL of a freshly prepared Standard solution made by dissolving about 50 mg of USP Demecarium Bromide RS, accurately weighed, in 100.0 mL of water. To flasks 1 and 3, add 1 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of each of these solutions to separate glass-stoppered tubes, insert the stoppers loosely, heat the tubes in a water bath for 15 minutes, then cool to room temperature. The concentration of USP Demecarium Bromide RS in the Standard solution is about 50 µg per mL. To flasks 2 and 4, add pH 10.0 alkaline borate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*) to volume, and mix. Concomitantly determine the absorbances of the two sodium hydroxide solutions at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using the corresponding borate buffer solutions as the respective solvent blanks. Calculate the quantity, in mg,

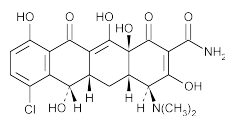


of  $C_{32}H_{52}Br_2N_4O_4$  in each mL of the Ophthalmic Solution taken by the formula:

$$(50C / V)(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Demecarium Bromide RS in the Standard solution;  $V$  is the volume, in mL, of Ophthalmic Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the sodium hydroxide solution from the Ophthalmic Solution and the Standard solution, respectively.

## Demeclocycline



$C_{21}H_{21}ClN_2O_8$  464.85

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, [4S-(4 $\alpha$ ,4a $\alpha$ ,5a $\alpha$ ,6 $\beta$ ,12a $\alpha$ )]-. 7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide [127-33-3].

Sesquihydrate 491.88 [13215-10-6].

» Demeclocycline has a potency equivalent to not less than 970  $\mu$ g of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Demeclocycline Hydrochloride RS

### Identification—

**A:** Transfer about 40 mg, accurately weighed, to a 250-mL volumetric flask, add 2 mL of 0.1 N hydrochloric acid to dissolve, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add about 75 mL of water and 5.0 mL of 5 N sodium hydroxide, dilute with water to volume, and mix: the UV absorption spectrum of this solution, measured at 6 minutes, accurately timed, after the addition of the sodium hydroxide, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Demeclocycline Hydrochloride RS, concomitantly measured, and the absorptivity, calculated on the anhydrous basis, at the wavelength of maximum absorbance at about 380 nm is between 103.5% and 111.3% of that of the USP Demeclocycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**B:** Transfer 40 mg, accurately weighed, to a 200-mL volumetric flask, add 100 mL of 0.1 N hydrochloric acid, shake to dissolve, dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution into each of two 50-mL volumetric flasks (*Solutions 1* and *2*). Prepare similar solutions of USP Demeclocycline Hydrochloride RS (*Solutions 3* and *4*). To *Solutions 1* and *3*, add 10 mL of 6 N hydrochloric acid, and to *Solutions 2* and *4*, add 10 mL of 3 N hydrochloric acid. Heat the four flasks in a water bath for 20 minutes, cool, dilute the contents with water to volume, and mix. Determine the absorbances of *Solutions 1* and *3* at the wavelength of maximum absorbance at about 430 nm, with a suitable spectrophotometer, using *Solutions 2* and *4*, respectively, as the blanks. Determine the absorbances of

ance at about 368 nm, using *Solutions 1* and *3*, respectively, as the blanks. Calculate the ratio:

$$(W_S P / 1000)(A_{368} + A_{430})_U / W_U(A_{368} + A_{430})_S$$

in which  $W_S$  is the weight, in mg, of USP Demeclocycline Hydrochloride RS taken, calculated on the dried basis;  $P$  is the potency, in  $\mu$ g per mg, of the USP Demeclocycline Hydrochloride RS;  $W_U$  is the weight, in mg, of specimen taken, calculated on the anhydrous basis; and the final two parenthetic expressions are the absorbances of the four solutions at the wavelengths indicated by the subscripts for the specimen ( $U$ ) and the Standard ( $S$ ): the ratio is between 0.97 and 1.17.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.0 and 5.5, in a solution containing 10 mg per mL.

**Water, Method I** (921): between 4.3% and 6.7%.

### Assay—

**Mobile phase**—Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water, add 100 mL of 0.2 M pH 9.0 phosphate buffer (prepared by mixing appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate), 150 mL of 0.02 M tetrabutylammonium hydrogen sulfate (adjusted with sodium hydroxide TS to a pH of 9.0), and 100 mL of 0.01 M edetate disodium (adjusted with sodium hydroxide TS to a pH of 9.0). Dilute with water to volume, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Decreasing the amount of tertiary butyl alcohol increases the resolution.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer about 45 mg of Demeclocycline, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Resolution solution**—Prepare a solution of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid, and allow to stand for 3 hours.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column containing packing L21 and maintained at  $60 \pm 0.5^\circ$ . The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for epidemethylchlortetracycline and 1.0 for demeclocycline; and the resolution between the epidemethylchlortetracycline peak and the demeclocycline peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) equivalent in each mg of the Demeclocycline taken by the formula:

$$50(CE / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Demeclocycline Hydrochloride RS in the *Standard preparation*;  $E$  is the demeclocycline hydrochloride equivalent, in  $\mu$ g per mg, of USP Demeclocycline Hydrochloride RS;  $W$  is the quantity, in mg, of the Demeclocycline taken; and  $r_U$  and  $r_S$  are the demeclocycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Demeclocycline Oral Suspension

» Demeclocycline Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ). It may contain one or more suitable buffers, preservatives, stabilizers, and suspending agents.

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** (11)—

USP Demeclocycline Hydrochloride RS

**Identification**—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of demeclocycline hydrochloride, add 50 mL of methanol, shake, and allow the mixture to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 5.8.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Demeclocycline*.

*Assay preparation*—Transfer an accurately weighed quantity of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ), to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Sonicate for 5 minutes, and centrifuge for 5 minutes. Pass a portion of the supernatant through a suitable filter having a 1.5- $\mu$ m or finer porosity, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Demeclocycline*. Calculate the quantity, in mg, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) equivalent in each mL of Oral Suspension taken by the formula:

$$0.05(CE / V)(r_U / r_S)$$

in which *V* is the volume, in mL, of Oral Suspension taken; and the other terms are as defined therein.

## Demeclocycline Hydrochloride

$C_{21}H_{21}ClN_2O_8 \cdot HCl$  501.31

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, monohydrochloride, 4S-(4 $\alpha$ , 4a $\alpha$ , 5a $\alpha$ , 6 $\beta$ , 12a $\alpha$ )-.

7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-73-3].

» Demeclocycline Hydrochloride has a potency of not less than 900  $\mu$ g of  $C_{21}H_{21}ClN_2O_8 \cdot HCl$  per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Demeclocycline Hydrochloride RS

**Identification**—

**A:** It responds to *Identification* test *A* under *Demeclocycline*, except that its absorptivity, calculated on the dried basis, is between 95.8% and 104.2% of that of the USP Demeclocycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**B:** It responds to *Identification* test *B* under *Demeclocycline*, except that the ratio is 0.9 to 1.1.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 2.0 and 3.0, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Demeclocycline*.

*Assay preparation*—Transfer about 50 mg of Demeclocycline Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Demeclocycline*. Calculate the quantity, in  $\mu$ g, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in each mg of the Demeclocycline Hydrochloride taken by the formula:

$$50(CE / W)(r_U / r_S)$$

in which *W* is the quantity, in mg, of the Demeclocycline Hydrochloride taken, and the other terms are as defined therein.

## Demeclocycline Hydrochloride Capsules

» Demeclocycline Hydrochloride Capsules contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Demeclocycline Hydrochloride RS

**Identification**—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing about 1 mg of demeclocycline hydrochloride per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{21}ClN_2O_8$  dissolved from UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Demeclocycline Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of demeclocycline ( $C_{21}H_{21}ClN_2O_8$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: the material loses not more than 2.0% of its weight, except that if the Capsules contain starch the material loses not more than 8.0% of its weight.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the Assay under Demeclocycline.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 10 Capsules, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ), to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Sonicate for 5 minutes, and centrifuge for 5 minutes. Pass a portion of the supernatant through a suitable filter having a 1.5- $\mu$ m or finer porosity, and use the clear filtrate as the Assay preparation.

*Procedure*—Proceed as directed for Procedure in the Assay under Demeclocycline. Calculate the quantity, in mg, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in the portion of Capsule contents taken by the formula:

$$0.05CE(r_U / r_S)$$

in which the terms are as defined therein.

## Demeclocycline Hydrochloride Tablets

» Demeclocycline Hydrochloride Tablets contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Demeclocycline Hydrochloride RS

**Identification**—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing about 1 mg of demeclocycline hydrochloride per mL, and filter. Using the filtrate as the Test Solution, proceed as directed for Method II under Identification—Tetracyclines (193).

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 75 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{21}ClN_2O_8$  dissolved from UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with Medium, if necessary, in comparison with a Standard solution having a known concentration of USP Demeclocycline Hydrochloride RS in the same Medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of demeclocycline ( $C_{21}H_{21}ClN_2O_8$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of finely ground Tablet powder, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 2% of its weight.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the Assay under Demeclocycline.

*Assay preparation*—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ), to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Sonicate for 5 minutes, and centrifuge for 5 minutes. Pass a portion of the supernatant through a suitable filter of 1.5- $\mu$ m or finer porosity, and use the clear filtrate as the Assay preparation.

*Procedure*—Proceed as directed for Procedure in the Assay under Demeclocycline. Calculate the quantity, in mg, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$0.05CE(r_U / r_S)$$

in which the terms are as defined therein.

## Cryopreserved Human Fibroblast-Derived Dermal Substitute

» Cryopreserved Human Fibroblast-Derived Dermal Substitute is a living monolayer skin substitute derived from neonatal foreskins. It is composed of fibroblasts, an extracellular matrix, and a bioabsorbable scaffold. Human fibroblasts are seeded onto a bioabsorbable, nonantigenic and nonpyrogenic mesh scaffold composed of polyglactin, a copolymer of glycolide and lactide. The fibroblasts proliferate to fill the interstices of this scaffold to create a three-dimensional human dermal substitute that secretes human dermal collagen, matrix proteins, growth factors, and cytokines. Cryopreserved Human Fibroblast-Derived Dermal Substitute does not contain macrophages, lymphocytes, blood vessels, hair follicles, muscle fibers, or keratin. The fibroblast-cell banks, from which Cryopreserved Human Fibroblast-Derived Dermal Substitute is derived, test negative for human and animal viruses and retroviruses and are also tested for normal cell morphology, human karyology, and isoenzymes. Maternal blood sera are tested for evidence of infection with human immunodeficiency virus types 1 and 2, hepatitis B and C viruses, syphilis, and human T-lymphotropic virus type 1 and are found negative for the purpose of donor selection. Cryopreserved Human Fibroblast-Derived Dermal Substitute is manufactured with sterile components under aseptic conditions within the final package. Reagents used in the manufacture of Cryopreserved Human Fibroblast-Derived Dermal Substitute are tested and found free of viruses, retroviruses, endotoxins, and mycoplasma before use. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy. During subsequent screening of the fibroblast cell strain at various

stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus and human T-lymphotropic virus type 2, is carried out and found to be negative. The final product tests negative for the presence of mycoplasma.

**Packaging and storage**—Cryopreserved Human Fibroblast-Derived Dermal Substitute is aseptically packaged and supplied frozen in a clear ethyl vinyl acetate bag containing one piece of approximately 5 cm × 7.5 cm for a single application. The solution within the bag is a saline-based cryoprotectant supplemented with 10% dimethyl sulfoxide and bovine serum to facilitate long-term storage. An aluminum-plastic foil envelope surrounds the bag for its protection. Cryopreserved Human Fibroblast-Derived Dermal Substitute should be stored at  $-75 \pm 10^\circ$  for no longer than 6 months.

**Labeling**—The label indicates the dimensions of the Cryopreserved Human Fibroblast-Derived Dermal Substitute material enclosed. It contains the expiry date, the required storage conditions, and the lot number. The label cautions that Cryopreserved Human Fibroblast-Derived Dermal Substitute is not to be used if the package shows signs of damage. Additional labeling requirements include instructions on the proper thawing and handling of Cryopreserved Human Fibroblast-Derived Dermal Substitute, the timeframe for use after package opening, and a statement that cytotoxic agents are not to be used.

**USP Reference standards** (11)—  
USP Endotoxin RS

**USP Authentic Visual References** (11)—USP Cryopreserved Human Fibroblast-Derived Dermal Substitute Reference Photomicrographs. [NOTE—These three photomicrographs represent examples of passing units. They are specified to assist in ascertaining histological quality.]

**Sterility** (71): meets the requirements.

**Test solution**—Thaw Cryopreserved Human Fibroblast-Derived Dermal Substitute by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of  $34^\circ$  to  $37^\circ$  for 2 to 3 minutes. The minimum amount of water in the water bath is 2 L per Cryopreserved Human Fibroblast-Derived Dermal Substitute unit. Perform the test on 20 mL of the cryopreservative.

**Bacterial endotoxins** (85)—Thaw Cryopreserved Human Fibroblast-Derived Dermal Substitute by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of  $34^\circ$  to  $37^\circ$  for 2 to 3 minutes. The minimum amount of water in the water bath is 2 L per Cryopreserved Human Fibroblast-Derived Dermal Substitute unit. Remove the unit from the ethyl vinyl acetate bag, and immerse in 25 mL of LAL Reagent Water. Extract for 60 minutes at  $37^\circ$  with shaking on an orbital shaker set at 125 revolutions per minute. Remove a 4-mL aliquot of the extract for testing: it contains not more than 0.5 USP Endotoxin Unit per mL.

#### **Histological characterization—**

**BUFFERED FORMALIN**—Prepare a solution containing 10% (w/v) formaldehyde solution and 1.0% to 1.5% methanol in a suitable buffer, adjusted to a pH of 6.8 to 7.2.<sup>1</sup>

**PREPARATION OF TISSUE FOR STAINING**—Cut Cryopreserved Human Fibroblast-Derived Dermal Substitute into 3-mm × 3-mm sections. Place three sections into suitable histological tissue cassettes,<sup>2</sup> and insert the cassettes into suitable histological cassette basket(s).<sup>3</sup> At a temperature of  $40^\circ$ , sequentially immerse the histological cassette basket(s) in separate

solutions of *Buffered formalin* (2 hours), two changes of 80% alcohol (30 minutes per step), alcohol (30 minutes), three changes of dehydrated alcohol (30 minutes per step), suitable histological xylene substitute (30 minutes),<sup>4</sup> and two changes of suitable xylene substitute (30 minutes per step). Immerse the histological cassette basket(s) into molten paraffin<sup>5</sup> that is at a temperature of  $60^\circ$  for 30 minutes. Remove the cassette basket(s), and immerse in a fresh container of molten paraffin ( $60^\circ$ ) for 60 minutes. Remove the histological tissue cassette from the container and basket, and disassemble. Fill preheated embedding molds with molten paraffin heated to  $56^\circ$  to  $60^\circ$ , and place on top of a preheated warming platform that is designed for histology work. Transfer Cryopreserved Human Fibroblast-Derived Dermal Substitute specimens from the cassettes using forceps, and place specimens into individual molds. Orient the specimens in molds so as to cut cross-sections. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at  $4^\circ$  for 15 to 30 minutes. Clamp the tissue block into the block holder of the microtome. Fill a histological tissue flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,<sup>6</sup> and heat to  $5^\circ$  less than the melting point of the paraffin. Properly mount and adjust the tissue and paraffin block into a microtome. Set the microtome to make cuts 5 microns thick with a blade angle of  $5 \pm 2^\circ$ . Insert a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife into the knife holder. Cut a ribbon that contains 6 to 10 sections of Cryopreserved Human Fibroblast-Derived Dermal Substitute. Pick up the ribbon with forceps, and stretch it across the tissue flotation water bath. Separate 2 to 3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. For each staining procedure, prepare three slides from each of the three starting Cryopreserved Human Fibroblast-Derived Dermal Substitute 3-mm × 3-mm sections. Allow the mounted sections to air-dry completely, or dry the slide in a  $60^\circ$  oven for 1 hour.

#### **HEMATOXYLIN-EOSIN STAINING—**

**Hematoxylin-alcohol solution**—Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol with heating.

**Potassium alum solution**—Dissolve 50.0 g of potassium alum in 500 mL of water with heating.

**Hematoxylin staining solution**—Mix *Hematoxylin-alcohol solution* and *Potassium alum solution*. Bring to a boil as rapidly as possible with constant stirring. Do not heat for more than 1 minute. Slowly add 0.185 g of sodium iodate. Reheat to a simmer until the solution becomes a deep purple. Remove from heat, and quickly cool. Filter daily before use.

**10% Acid alcohol**—Add 5.0 mL of hydrochloric acid to 495 mL of 70% alcohol.

**Eosin solution**—Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

**Procedure**—The microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 minutes per step), three changes of dehydrated alcohol (1 minute per step), alcohol

<sup>1</sup>A suitable *Buffered formalin* can be obtained from VWR International, 1310 Goshen Pkwy., West Chester, PA 19380.

<sup>2</sup>A suitable histological tissue cassette can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>3</sup>A suitable histological tissue cassette basket can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>4</sup>A suitable histological xylene substitute is Citrosolve® Clearing Agent, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>5</sup>A suitable paraffin for use is Tissue Prep® 2 Embedding Media, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>6</sup>A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific Research Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.

(20 seconds), running tap water rinse (1 minute), *Hematoxylin staining solution* (4 to 5 minutes), running tap water rinse (1 minute), 10% *Acid alcohol* (15 seconds), running tap water rinse (1 minute), a suitable bluing reagent<sup>7</sup> (20 to 30 seconds), running tap water rinse (1 minute), alcohol (20 seconds), *Eosin solution* (10 to 20 seconds, until a red-dish-brown color is obtained in the tissue), three changes of dehydrated alcohol (10 seconds per step), and three changes of a suitable histological xylene substitute (10 seconds per step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue using a coverslip mountant. Using USP Cryopreserved Human Fibroblast-Derived Dermal Substitute Reference Photomicrograph 1 for comparison, the test article shows a polyglactin scaffold mesh and a secreted collagen-based matrix; the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary dermis. Fibroblasts appear elongated and spindle-shaped. The tissue, which is about 100 to 300  $\mu\text{m}$  thick, contains about 106 cells per  $\text{cm}^2$ .

#### COLLAGEN STAINING—

*Bouin's solution*—Mix 75.0 mL of 1.22% picric acid solution, 25.0 L of dimethoxymethane, and 5.0 L of acetic acid.

*Weigert's iron hematoxylin solution A*—Dissolve 1.0 g of hematoxylin in 100.0 mL of alcohol.

*Weigert's iron hematoxylin solution B*—Mix 4.0 mL of 29% ferric chloride, 95.0 mL of water, and 1.0 mL of hydrochloric acid.

*Weigert's iron hematoxylin working solution*—Mix equal volumes of *Weigert's iron hematoxylin solution A* and *Weigert's iron hematoxylin solution B*. Pass the solution through a filter having a 0.45- $\mu\text{m}$  porosity. Prepare fresh as needed.

*Gomori's trichrome solution*—Mix 1.0 mL of acetic acid and 100 mL of water. Dissolve 0.6 g of chromotrope 2R, 0.3 g of Fast Green FCF, and 0.6 g of phosphotungstic acid.

1% *Acetic acid*—Mix 1.0 mL of acetic acid and 100 mL of water.

*Procedure*—The microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 minutes per step), three changes of dehydrated alcohol (1 minute per step), alcohol (20 seconds), and running tap water rinse (1 minute). Immerse the slide in *Bouin's solution*, and place in a 42° water bath for 1 hour. Rinse the slide in water for 10 seconds. Sequentially immerse the slide in *Weigert's iron hematoxylin working solution* (10 minutes) and running tap water rinse (10 minutes). Rinse the slide in water for 10 seconds, and immerse in *Gomori's trichrome solution* (3 to 5 minutes). Rinse the slide in 1% *Acetic acid* for 20 seconds. Sequentially immerse the slide in three changes of alcohol (10 seconds per step) and three changes of a suitable histological, aliphatic xylene substitute (10 seconds per step). Affix a coverslip over the tissue using a suitable coverslip mountant. Nuclei will stain black; cytoplasm, keratin, and muscle fibers will stain red; and collagen and mucin will stain blue. Using USP Cryopreserved Human Fibroblast-Derived Dermal Substitute Reference Photomicrograph 2 for comparison, the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary; dermis, muscle fibers, and keratin are absent.

#### DISTRIBUTION OF FIBRONECTIN—

*Tris-saline buffer*—Prepare a solution containing 0.1 M tris(hydroxymethyl)aminomethane hydrochloride and 0.15 M sodium chloride, adjusted to a pH of 7.8.

3% *Hydrogen peroxide*—Mix 30 mL of hydrogen peroxide in water or methanol.

*Diaminobenzidine solution*—Use a suitable solution.<sup>8</sup>

*Hematoxylin staining solution*—Prepare as directed for *Hematoxylin-eosin staining*.

*Procedure*—The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is dried either overnight at 37° or for 1 hour at 60°. The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 minutes per step), three changes of dehydrated alcohol (1 minute per step), alcohol (20 seconds), and running tap water rinse (1 minute). Sequentially immerse the slide in *Tris-saline buffer* (10 minutes), 3% *Hydrogen peroxide* (30 minutes), three changes of *Tris-saline buffer* (1 minute per step), a suitable normal rabbit serum<sup>9</sup> (30 minutes), water (5 minutes), and three changes of *Tris-saline buffer* (1 minute per step). Incubate the slide with a suitable solution of rabbit anti-human fibronectin antibody,<sup>10</sup> diluted using a suitable antibody diluent<sup>11</sup> to an antibody titer of  $21.0 \pm 2.1$  mg per L for 1 hour. Sequentially immerse the slide in water (5 minutes) and three changes of *Tris-saline buffer* (1 minute per step). Place enough drops of a biotinylated goat anti-rabbit antibody solution<sup>12</sup> to cover the tissue section, and incubate for 30 minutes. Sequentially immerse the slide in water (5 minutes) and three changes of *Tris-saline buffer* (1 minute per step). Place enough drops of a streptavidin conjugated horseradish peroxidase solution<sup>13</sup> to cover the tissue section, and incubate for 30 minutes. Sequentially immerse the slide in water (5 minutes) and three changes of *Tris-saline buffer* (1 minute per step). Incubate the slide with *Diaminobenzidine solution* for 1 to 5 minutes, until a suitable difference in staining is seen by comparison with a control in which the fibronectin (primary) antibody is omitted. Sequentially immerse the slide in water (1 minute), *Hematoxylin staining solution* (4 to 5 minutes), and water (1 minute). Do not allow the tissue to dry. Affix a coverslip over the tissue using a low-viscosity, aqueous, synthetic-resin coverslip mountant. Using USP Cryopreserved Human Fibroblast-Derived Dermal Substitute Reference Photomicrograph 3 for comparison, fibronectin is found colocalizing with the collagen fibers. The intensity of staining may vary from region to region of the slide.

#### Metabolic activity assessment—

*DPBS solution A*—Dissolve 1.32 g of calcium chloride and 1.21 g of magnesium sulfate heptahydrate in 2 L of water.

*DPBS solution B*—Dissolve 80.0 g of sodium chloride; 2.0 g of potassium chloride; 11.5 g of dibasic sodium phosphate; 2.0 g of monobasic potassium phosphate; 10.0 g of glucose; 0.36 g of sodium phosphate; 0.5 g of streptomycin sulfate; and 1,000,000 USP Units of penicillin G sodium in 8 L water.

*DPBS working solution*—Mix *DPBS solution B* with *DPBS solution A* (8:2). Pass the solution through a filter having a 0.22- $\mu\text{m}$  porosity.

*Dulbecco's modified Eagle's tissue culture medium*—Prepare a solution that contains the following components:

Component	mg per L
Calcium chloride	264.9
Ferric nitrate nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate heptahydrate	200.0

<sup>8</sup>A suitable *Diaminobenzidine solution* can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178; catalog number D-6815.

<sup>9</sup>A suitable normal rabbit serum can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>10</sup>Suitable rabbit anti-human fibronectin antibodies can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>11</sup>Suitable antibody diluent can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>12</sup>Suitable biotinylated goat anti-rabbit antibody solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

<sup>13</sup>A suitable streptavidin conjugated horseradish peroxidase solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

<sup>7</sup>A suitable bluing reagent can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

Component	mg per L
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

**L-Glutamine solution**—Prepare a 100-mL solution containing 2.92 g of L-glutamine.

**Sodium pyruvate solution**—Prepare 100 mL of a solution containing 1.10 g of sodium pyruvate.

**Antibiotic-antimycotic solution**—Prepare 100 mL of a solution containing 0.85 g of sodium chloride, 10,000 USP Units of penicillin G sodium, 10,000 µg of streptomycin (base), and 25 µg of amphotericin B.

**Assay stock medium**—Mix 1000 mL of *Dulbecco's modified Eagle's tissue culture medium*, 10 mL of *L-Glutamine solution*, 10 mL of *Sodium pyruvate solution*, 10 mL of *Antibiotic-antimycotic solution*, and 20 mL of fetal bovine serum.<sup>14</sup>

**MTT-assay solution**—Dissolve 0.50 g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Assay stock medium*, using constant stirring. Sterilize the solution by passing it through a suitable filter having a 0.2-µm porosity.

**MTT formazan stock solution**—Prepare a solution containing 100 µg of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan per mL of isopropyl alcohol.

**MTT formazan calibration solutions**—Prepare solutions containing the following five MTT formazan concentrations: 15 µg per mL, 30 µg per mL, 45 µg per mL, 60 µg per mL, and 75 µg per mL of MTT formazan, using *MTT formazan stock solution* and diluting with isopropyl alcohol.

**Procedure**—Thaw Cryopreserved Human Fibroblast-Derived Dermal Substitute by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2 to 3 minutes. The minimum amount of water in the water bath is 2 L per Cryopreserved Human Fibroblast-Derived Dermal Substitute unit. Cut three 11-mm × 11-mm sections of Cryopreserved Human Fibroblast-Derived Dermal Substitute, and immerse the sections into separate, 3.0-mL portions of *MTT-assay solution*. Incubate for 2 hours at 37 ± 2° in a 3% to 7% CO<sub>2</sub>-air environment with shaking on an orbital shaker at 150 to 200 rpm. After incu-

bation remove from the 37°, 3% to 7% CO<sub>2</sub>-air environment. Remove the *MTT-assay solution*, and rinse twice with *DPBS working solution*. Immerse the Cryopreserved Human Fibroblast-Derived Dermal Substitute in 2 mL of isopropyl alcohol, and incubate at ambient temperature for 1 hour with shaking on an orbital shaker at approximately 125 rpm. Transfer 200-µL aliquots of the five *MTT formazan calibration solutions*, in triplicate, and 200-µL aliquots of the three isopropyl alcohol extracts of Cryopreserved Human Fibroblast-Derived Dermal Substitute to a suitable 96-well flat-bottom plate. Read the absorbance of each aliquot at 540 nm, using 200 µL of isopropyl alcohol as the blank. Plot the responses of the *MTT formazan calibration solutions* versus concentration, in µg of MTT formazan per mL, and calculate the regression line using the least-squares method; the test is considered valid if the regression line has a square of the correlation coefficient not less than 0.95: the absorbance value of individual, thawed, Cryopreserved Human Fibroblast-Derived Dermal Substitute sections is between 0.30 and 0.86.

#### DNA content—

**Cell culture water**—Use sterile water containing not more than 0.005 USP Endotoxin Unit per mL.

**DNA extraction buffer**—Transfer about 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Dissolve 12.110 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, 23.380 g of sodium chloride, and 0.080 g of sodium dodecyl sulfate, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**Proteinase K solution**—Prepare a solution of *Tritirachium album* proteinase K in 10 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to a pH of 7.5, having an activity of 600 units per mL.<sup>15</sup>

**Working DNA extraction buffer**—Add 1.22 mL of *Proteinase K solution* to 38.78 mL of *DNA extraction buffer*, and mix.

**Dilution buffer**—Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Add 1.211 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, and 5.844 g of sodium chloride, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution**—Prepare a solution containing 8.00 g of sodium chloride, 1.15 g of dibasic sodium phosphate (anhydrous), 0.20 g of potassium chloride, and 0.20 g of monobasic potassium phosphate per L.

**Calf thymus DNA solution**—Prepare a solution containing 1 mg of calf thymus DNA per mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*, mixing thoroughly for 12 to 24 hours at ambient temperature. Dilute the resulting solution with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* to prepare a solution containing 50 µg of calf thymus DNA per mL, mixing thoroughly on a vortex mixer for 10 minutes.

**Calf thymus DNA calibration solutions**—Prepare four calibration solutions containing 5 µg per mL, 10 µg per mL, 15 µg per mL, and 20 µg per mL of calf thymus DNA, using *Calf thymus DNA solution* and diluting with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

**DNA staining solution**—Prepare a solution containing 0.5 µg of 2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole trihydrochloride pentahydrate per mL of *Dilution buffer*. Store in low-actinic glassware.

**Procedure**—Thaw Cryopreserved Human Fibroblast-Derived Dermal Substitute by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2 to 3 minutes. The minimum amount of water in the water bath is 2 L per Cryopreserved Human Fibroblast-Derived Dermal Substitute unit. Cut three 11-mm × 11-mm sections of Cryopreserved Human Fibroblast-De-

<sup>14</sup>A suitable fetal bovine serum can be obtained from HyClone, 925 West 1800 South, Logan, UT 84321; catalog number SH30070.03.

<sup>15</sup>A suitable *Proteinase K solution* can be obtained from Roche Diagnostics Corp., Roche Applied Sciences, P.O. Box 50414, 9115 Hague Rd., Indianapolis, IN 46250-0414.

rived Dermal Substitute. To each of three microcentrifuge tubes add 1 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Immerse a single Cryopreserved Human Fibroblast-Derived Dermal Substitute 11-mm × 11-mm section into each microcentrifuge tube to remove the cryopreservative. Aspirate the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* from each tube, and replace with 1 mL of *Working DNA extraction buffer*, making sure that each Cryopreserved Human Fibroblast-Derived Dermal Substitute is completely submerged in the extraction buffer. Incubate the samples in a 56° to 60° water bath for 4 to 18 hours. Sonicate for 10 to 15 seconds using an ultrasonic cell disrupter to achieve complete cellular disruption of the tissue and to mix the contents of the tube. Centrifuge the microcentrifuge tubes at 12,000 to 15,000 × *g* to pellet non-DNA material. Transfer three 50-μL aliquots of each sample supernatant to individual wells of a 96-well black plate suitable for performing fluorescent analysis. Transfer triplicate 50-μL aliquots of each of the *Calf thymus DNA calibration solutions* to the 96-well plate, as well as a 50-μL aliquot of *DPBS working solution* for the blank. Add 150 μL of *DNA staining solution* to all wells containing the tissue samples, *Calf thymus DNA calibration solutions*, and the blank. Cover with aluminum foil, and place in a dark cabinet for 30 to 45 minutes at 15° to 30°. Read the fluorescence of each well, using an excitation wavelength of 355 nm and an emission wavelength of 460 nm, blanking against the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* well. Plot the responses of the *Calf thymus DNA calibration solutions* versus concentration, in μg of calf thymus DNA per mL, and calculate the regression line using the least-squares method. The test is considered valid if the %CV of the replicate values is less than 15%, the slope is between 4.48 and 6.27, the *y*-intercept is between -2.04 and 3.65, and the square of the correlation coefficient is not less than 0.990. From the regression line so obtained, determine the amount of DNA, in μg per 11-mm × 11-mm sample: the amount of DNA of individual Cryopreserved Human Fibroblast-Derived Dermal Substitute 11-mm × 11-mm section is between 6 and 15 μg.

#### Total collagen content—

*DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*—Proceed as directed for DNA content.

*DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*—Prepare a solution containing 8.00 g of sodium chloride, 1.15 g of dibasic sodium phosphate (anhydrous), 0.20 g of potassium chloride, 0.20 g of monobasic potassium phosphate, 0.10 g of magnesium chloride hexahydrate, and 0.10 g of calcium chloride (anhydrous) per L.

*Collagenase extraction solution*—Prepare a solution of *Clostridium histolyticum* collagenase, type 2, in *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution* having an activity of at least 250 units per mL.

*2% Acetic acid solution*—Mix 10 mL of acetic acid with 490 mL of water.

*Collagen standard stock solution*—Prepare a solution having a concentration of 2 mg of collagen, type I, per mL of *2% Acetic acid solution*.

*Collagen calibration standards*—Cut polyglactin mesh<sup>16</sup> into seventeen 11-mm × 11-mm squares, and place one square into 17 individual wells of a 24-well plate. Each well of the 24-well plate has a surface area of about 220 mm<sup>2</sup> and a volume of about 3.5 mL. In quadruplicate, prepare wells containing 0.050 mg, 0.100 mg, 0.200 mg, and 0.400 mg of collagen by adding 25 mL, 50 mL, 100 mL, and 200 mL, respectively, of the *Collagen standard stock solution*. The remaining well to which no *Collagen standard stock solution* has been added is used as the blank. Allow the wells to air dry.

*Sirius red solution*—Dissolve 0.5 g of Direct Red 80 in 500 mL of saturated picric acid.

<sup>16</sup>A suitable polyglactin mesh can be obtained from Ethicon Co., Johnson & Johnson Corp., 425 Hoes Ln., P.O. Box 6800, Piscataway, NJ 08855.

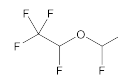
*1% (p-tert-Octylphenoxy)polyethoxyethanol solution*—Mix 10 mL of (p-tert-octylphenoxy)polyethoxyethanol in 990 mL of *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

*Procedure*—Thaw Cryopreserved Human Fibroblast-Derived Dermal Substitute by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2 to 3 minutes. The minimum amount of water in the water bath is 2 L per Cryopreserved Human Fibroblast-Derived Dermal Substitute unit. Cut three 11-mm × 11-mm sections of Cryopreserved Human Fibroblast-Derived Dermal Substitute. Place each test section into separate wells of a 24-well plate. Add 200 μL of *1% (p-tert-Octylphenoxy)polyethoxyethanol solution* to each sample. Shake on a rotating platform shaker at 100 to 150 rpm for 60 to 70 minutes at room temperature. Aspirate off the *1% (p-tert-Octylphenoxy)polyethoxyethanol solution*, and rinse three times with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Transfer each of the collagen standards to individual wells of the 24-well plate. Add 0.5 mL of *Sirius red solution* to each test sample and collagen standards. Shake on a rotating platform shaker at 100 to 150 rpm for 60 minutes at room temperature. Aspirate off the *Sirius red solution* from each well. Rinse each well twice with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Add an additional 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* to each well, and allow to stand for 2 minutes. Aspirate off the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*, and rinse twice more with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Aspirate off all traces of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Add 0.5 mL of *Collagenase extraction solution* to each well containing the *Collagen calibration standards*. Add 2.0 mL of *Collagenase extraction solution* to each well containing test samples. Rotate the plate on an orbital rotator at about 150 rpm for 90 minutes at 37°. Transfer 200 μL from each well, and transfer to a suitable 96-well flat-bottom plate. Read the absorbance of each aliquot at 540 nm. Dilute the Cryopreserved Human Fibroblast-Derived Dermal Substitute sample preparation further with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* if the absorbance is greater than the absorbance of the highest of the *Collagen calibration standards*. Plot the responses of the *Collagen calibration standards* versus the amount, in mg of collagen, and calculate the regression line using the least-squares method. The test is considered valid if the slope is between 3.00 and 5.00 and the square of the correlation coefficient is greater than or equal to 0.950. Determine the mg of collagen per Cryopreserved Human Fibroblast-Derived Dermal Substitute section from the regression line and using the following equation:

$$CC_{SR} = Df \times A \times SC_{SR}$$

in which  $CC_{SR}$  is the collagen content of the piece, in mg;  $Df$  is the dilution factor (normally 4, unless the sample had to be further diluted);  $A$  is the absorbance at 540 nm; and  $SC_{SR}$  is the slope of the regression line of the standards calculated above. The test is considered valid if the slope of the regression line is between 3.00 and 5.00 and has a square of the correlation coefficient greater than 0.950: the amount of collagen in individual Cryopreserved Human Fibroblast-Derived Dermal Substitute 11-mm × 11-mm samples is between 0.40 and 2.0 mg.

## Desflurane



C<sub>3</sub>H<sub>2</sub>F<sub>6</sub>O  
Ethane, 2-(difluoromethoxy)-1,1,1,2-tetrafluoro-, (±)-;  
(±)-2-Difluoromethyl 1,2,2,2-tetrafluoroethyl ether  
[57041-67-5].

168.04

**DEFINITION**

Desflurane contains NLT 99.7% and NMT 100.0% of  $C_3H_2F_6O$ .

**IDENTIFICATION**

- The IR absorption spectrum of Desflurane obtained using a gas cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Desflurane RS.

**ASSAY****PROCEDURE**

Using the results from the *Organic Impurities* procedure, calculate the percentage of  $C_3H_2F_6O$  in the sample of Desflurane taken by subtracting the sum of all impurities found from 100.0%.

**Acceptance criteria:** 99.7%–100.0%

**IMPURITIES****Inorganic Impurities**

- LIMIT OF NONVOLATILE RESIDUE:** Transfer 20.0 mL of Desflurane to an evaporating dish, and evaporate with a stream of nitrogen to dryness: the weight of the residue does not exceed 2.0 mg (0.01%).

**LIMIT OF ANTIMONY**

**Diluent A:** Nitric acid and water (1:1)

**Diluent B:** Nitric acid and hydrochloric acid (9:1)

**Standard solutions:** Transfer 0.1 mL (234 mg) of antimony pentachloride to a 50-mL volumetric flask, dilute with *Diluent B* to volume, and mix. This stock solution contains about 1906 µg of antimony/mL. Dilute a portion of this solution quantitatively and stepwise with *Diluent B* to obtain *Standard solutions* containing 2.5, 5.0, and 10.0 µg of antimony/mL.

**Sample solution:** Weigh a stoppered stock bottle containing a quantity of Desflurane at ambient temperature, and then cool it in powdered dry ice. Using a cold syringe, transfer 5–7 mL of Desflurane from the cold bottle to a separator containing 20 mL of *Diluent A*. Allow the stock bottle containing the remaining Desflurane to come to ambient temperature, weigh it, and calculate the quantity, in g, of Desflurane taken for the test. Allow the Desflurane in the separator to evaporate, and with the aid of a few mL of *Diluent A*, transfer the acid solution to a clean, dry beaker. Add 1 mL of hydrochloric acid to the solution in the beaker, and reduce the volume to 8 mL by evaporating on a hot plate. Transfer this solution to a 10-mL volumetric flask, and dilute with *Diluent B* to volume.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometer

**Analytical wavelength:** Antimony emission line at 217.6 nm

**Lamp:** Antimony hollow-cathode

**Flame:** Air–acetylene

**Blank:** *Diluent B*

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*

**Calculation:** Plot the absorbances of the *Standard solutions* versus the concentration (µg/mL) of antimony, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of antimony in the *Sample solution*. Calculate the quantity, in µg/g, of antimony in the portion of Desflurane taken:

$$\text{Result} = (C/W) \times V$$

- C = concentration of antimony in the *Sample solution* (µg/mL)  
 W = weight of Desflurane taken to prepare the *Sample solution* (g)  
 V = volume of *Sample solution*

**Acceptance criteria:** NMT 3 µg/g

**LIMIT OF FLUORIDE**

[NOTE— Store all solutions except *Solution A* in plastic containers.]

**Solution A:** 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo)tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of  $5.25 \pm 0.25$ , and dilute with water to 1000 mL. An equivalent commercial preparation may be used.

**Standard stock solution:** 2210 µg/mL of USP Sodium Fluoride RS in water. Each mL of this solution contains 1000 µg of fluoride/mL.

**Standard solutions:** Dilute volumes of *Standard stock solution* with *Solution A* to obtain solutions with concentrations of 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0 µg of fluoride/mL.

**Sample solution:** Transfer 20.0 mL of Desflurane to a 60-mL separator, add 20.0 mL of water, shake for 1 min, and allow the layers to separate. Drain the lower organic layer and a small portion of the aqueous layer into a beaker, and discard. Transfer 10.0 mL of the aqueous phase remaining in the separator to a plastic cup, and add 10.0 mL of *Solution A*.

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*

Concomitantly measure the potentials (see pH (791)), in mV, of the *Samples* with a pH meter capable of a minimum reproducibility of  $\pm 0.2$  mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode.

[NOTE—When taking measurements, immerse the electrodes in the solution, stir with a polytetrafluoroethylene-coated stirring bar and a magnetic stirrer having an insulated top until equilibrium is attained (about 1–2 min), and record the potential. Rinse the electrodes with *Solution A*, and dry, taking care to avoid damaging the crystal of the specific-ion electrode.]

Plot the logarithms of the fluoride concentrations (µg/mL) of the *Standard solutions* versus the potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, C (µg/mL), of fluoride in the *Sample solution*. Multiply C by 0.0002 to obtain the percentage of fluoride in the portion of Desflurane taken.

**Acceptance criteria:** NMT 0.001%

**Organic Impurities****PROCEDURE**

**Standard stock solution:** To a suitable tared vial, fitted with a septum, add 20 mL (29.4 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial sequentially add 20 µL of USP Desflurane Related Compound A RS, 23 µL of dichloromethane, 20 µL of chloroform, 38 µL of acetone, and 21 µL of USP Isoflurane RS. Record the weight after the addition of each impurity and determine the total weight.

Calculate the percentage of each impurity in the *Standard stock solution*:

$$\text{Result} = W_i/W_T \times P_i$$

- $W_i$  = weight of each impurity added (g)  
 $W_T$  = total weight of the *Standard stock solution* (g)  
 $P_i$  = purity of each impurity added (%)

**Standard solution:** To a suitable tared vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial add 250 µL of the *Standard stock solution*, and record the weight to determine the weight of the *Standard stock solution* added and the final weight of the *Standard solution*.



Calculate the spiked concentration ( $C_s$ ) of each impurity in the *Standard solution*:

$$\text{Result} = W_i/W_T \times C_i$$

$W_i$  = weight of *Standard stock solution* added (g)  
 $W_T$  = total weight of the *Standard solution* (g)  
 $C_i$  = concentration of each impurity in the *Standard stock solution* (%)

**System suitability solution:** To a suitable vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal the vial. To this vial add 100  $\mu$ L of the *Standard stock solution*.

**Sample:** Desflurane

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm  $\times$  105-m capillary column coated with 1.5- $\mu$ m film of G6

**Carrier gas:** Helium

**Autosampler/Syringe temperature:** 2°–5°

**Flow rate:** 3 mL/min

**Split flow:** 25 mL/min

**Temperature**

**Injection port:** 150°

**Detector:** 200°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
30	—	30	11
30	20	50	13

**Injection size:** 3  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between isoflurane and dichloromethane, *Standard solution*

**Tailing factor:** NMT 1.5 for isoflurane, *Standard solution*

**Relative standard deviation:** NMT 5% for each impurity, *Standard solution*

**Signal-to-noise ratio:** NLT 40 for isoflurane, *System suitability solution*

#### Analysis

[NOTE—Injections of Desflurane used to prepare the *Standard solution* must be made to estimate the amount of known impurities that may be present in the solvent. The final concentration of each impurity is equal to the concentration of the impurity added plus the concentration inherent in the matrix.]

**Samples:** *Standard solution* and *Sample solution*

Calculate the final concentration of each impurity in the *Standard solution*:

$$\text{Result} = r_U/(r_S - r_U) \times C_S + C_S$$

$r_U$  = peak response of each impurity from the Desflurane used as the solvent

$r_S$  = peak response of each impurity from the *Standard solution*

$C_S$  = spiked concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of each impurity observed in the *Sample solution* that is also present in the *Standard solution*:

$$\text{Result} = (r_U/r_S) \times C_F$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of each impurity from the *Standard solution*

$C_F$  = final concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of all other impurities:

$$\text{Result} = (r_U/r_S) \times C_S \times 1/F$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of Isoflurane from the *Standard solution*

$C_S$  = concentration of USP Isoflurane RS in the *Standard solution* (%)

$F$  = relative response factor relative to isoflurane (see *Impurity Table 1*)

**Acceptance criteria:** See *Impurity Table 1*.

**Total impurities:** NMT 0.3%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desflurane <sup>a</sup>	1.0	—	—
Dichlorofluoromethane	1.04	0.43	0.01
Trichlorofluoromethane	1.07	0.15	0.001
Desflurane related compound A <sup>b,a</sup>	1.12	—	0.10
Trichlorotrifluoroethane	1.35	1.3	0.001
Dichloromethane <sup>a</sup>	1.44	—	0.001
Isoflurane <sup>a</sup>	1.55	1.0	0.20
Chloroform <sup>a</sup>	1.88	—	0.006
Acetone <sup>a</sup>	2.12	—	0.010

<sup>a</sup> These impurities are present in the *Standard solution* and are quantified by external standards.

<sup>b</sup> Bis-(1,2,2,2-tetrafluoroethyl)ether.

#### SPECIFIC TESTS

##### • ACIDITY OR ALKALINITY

**Bromocresol purple solution:** 0.5 mg/mL of bromocresol purple. Prepared by dissolving 50 mg of bromocresol purple in 0.92 mL of 0.1 M sodium hydroxide and 20 mL of ethanol, and then diluting with water to 100 mL.

**Sample solution:** Transfer 20 mL of Desflurane to a separatory funnel, and add 20 mL of carbon dioxide-free water. Shake for 3 min, allow the layers to separate, and discard the lower organic layer. Collect the upper layer, and add 0.2 mL of *Bromocresol purple solution*.

**Acceptance criteria:** NMT 0.1 mL of 0.01 M sodium hydroxide or 0.6 mL of 0.01 M hydrochloric acid is required to change the color of the indicator.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Replace the cap securely after each use.

##### • USP REFERENCE STANDARDS <11>

USP Desflurane RS

USP Desflurane Related Compound A RS

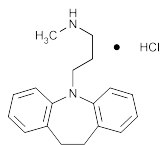
Bis-(1,2,2,2-tetrafluoroethyl)ether.

C<sub>4</sub>H<sub>2</sub>F<sub>8</sub>O 218.05

USP Isoflurane RS

USP Sodium Fluoride RS

## Desipramine Hydrochloride



$C_{18}H_{22}N_2 \cdot HCl$  302.84

5*H*-Dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N*-methyl-, monohydrochloride.

10,11-Dihydro-5-[3-(methylamino)propyl]-5*H*-dibenz[*b,f*]azepine monohydrochloride [58-28-6].

» Desipramine Hydrochloride, dried in vacuum at 105° for 2 hours, contains not less than 98.0 percent and not more than 100.5 percent of  $C_{18}H_{22}N_2 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Desipramine Hydrochloride RS

USP Iminodibenzyl RS  
 $C_{14}H_{13}N$  195.28

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 30 µg per mL.

*Medium:* 0.1 N hydrochloric acid.

Absorptivities at 251 nm, calculated on the dried basis, do not differ by more than 2.0%.

**C:** Add about 5 mg to 2 mL of nitric acid on a spot plate; an intense blue color is produced.

**D:** A 1 in 20 solution in alcohol responds to the tests for Chloride (191).

**Loss on drying** (731)—Dry it in vacuum at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

**Limit of iminodibenzyl**—

*Standard solution*—Dissolve an accurately weighed quantity of USP Iminodibenzyl RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution having a concentration of 50 µg per mL. Transfer 1.0 mL of this solution to a low-actinic, 25-mL volumetric flask, add 10 mL of a mixture of equal volumes of hydrochloric acid and alcohol, and mix.

*Test solution*—Transfer 50.0 mg of Desipramine Hydrochloride to a low-actinic, 25-mL volumetric flask, add 10 mL of a mixture of equal volumes of hydrochloric acid and alcohol, and mix.

*Procedure*—To the two flasks containing the *Standard solution* and the *Test solution*, and to a third flask containing 10 mL of a mixture of equal volumes of hydrochloric acid and alcohol to provide the blank, add slowly 5 mL of a 0.4% (v/v) solution of furfural in alcohol, mix, then add 5 mL of hydrochloric acid, and allow the flasks to stand in a constant-temperature bath at 25° for 3 hours. Dilute each flask with a mixture of equal volumes of hydrochloric acid and alcohol to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 565 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance of the solution obtained from the *Test solution* is not greater than that obtained from the *Standard solution* (0.1%).

**Assay**—Dissolve about 0.6 g of Desipramine Hydrochloride, previously dried and accurately weighed, in 100 mL of gla-

cial acetic acid, add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.29 mg of  $C_{18}H_{22}N_2 \cdot HCl$ .

## Desipramine Hydrochloride Tablets

» Desipramine Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{18}H_{22}N_2 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Desipramine Hydrochloride RS

**Identification**—Finely powder a number of Tablets, equivalent to about 350 mg of desipramine hydrochloride, and triturate the powder with 15 mL of chloroform. Filter the chloroform extract through paper into a wide-mouth test tube, and evaporate the filtrate to about 3 mL. Carefully add ether until the liquid becomes turbid, heat cautiously to produce a clear solution, then cool, and allow to stand. Collect the crystals, wash with ether, and dry in vacuum at 80° for 30 minutes: the desipramine hydrochloride so obtained responds to *Identification test A* under *Desipramine Hydrochloride*.

**Dissolution** (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 60 minutes.

*Procedure*—Determine the amount of  $C_{18}H_{22}N_2 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 251 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Desipramine Hydrochloride RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{22}N_2 \cdot HCl$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask, add 50 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 15 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise with 0.1 N hydrochloric acid to provide a solution containing approximately 25 µg of desipramine hydrochloride per mL. Concomitantly determine the absorbances of this solution and a Standard solution of USP Desipramine Hydrochloride RS in the same medium having a known concentration of about 25 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 251 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{18}H_{22}N_2 \cdot HCl$  in the tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which *T* is the labeled quantity, in mg, of desipramine hydrochloride in the Tablet, *C* is the concentration, in µg per mL, of USP Desipramine Hydrochloride RS in the Standard solution, *D* is the concentration, in µg per mL, of desipramine hydrochloride in the test solution, based upon the labeled quantity per Tablet and the extent of dilution,

and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

#### Assay—

**Standard preparation**—Dissolve a suitable quantity of USP Desipramine Hydrochloride RS, accurately weighed, in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 25 µg per mL.

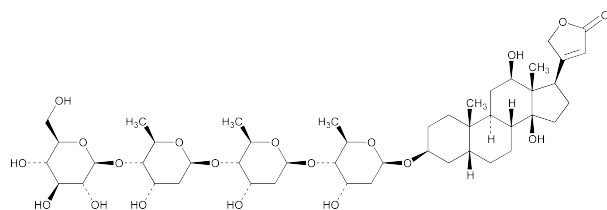
**Assay preparation**—Finely powder a number of Tablets, equivalent to about 0.5 g of desipramine hydrochloride, and transfer the powder to a 500-mL volumetric flask with the aid of 100 mL of 0.1 N hydrochloric acid. Add 150 mL of the 0.1 N acid to the mixture, insert the stopper, and shake by mechanical means for 30 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the subsequent filtrate to a 200-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Transfer 15.0 mL each of the *Standard preparation* and the *Assay preparation*, respectively, to separate glass-stoppered, 50-mL centrifuge tubes, and to each tube add 2 mL of 2.5 N sodium hydroxide and 15.0 mL of cyclohexane suitable for use in UV spectrophotometry. Insert the stoppers, shake the tubes by mechanical means for 30 minutes, and centrifuge at about 1500 rpm until the clear phases separate (5 to 10 minutes). Concomitantly determine the absorbances of the cyclohexane extracts in 1-cm cells at the wavelength of maximum absorbance at about 255 nm, with a suitable spectrophotometer, using cyclohexane suitable for use in UV spectrophotometry as the blank. Calculate the quantity, in mg, of  $C_{18}H_{22}N_2 \cdot HCl$  in the portion of Tablets taken by the formula:

$$20C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Desipramine Hydrochloride RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Deslanoside



$C_{47}H_{74}O_{19}$  943.08

Card-20(22)-enolide, 3-[(O-β-D-glucopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-, (3β,5β,12β)-. Deacetyl lanatoside C [17598-65-1].

» Deslanoside contains not less than 95.0 percent and not more than 103.0 percent of  $C_{47}H_{74}O_{19}$ , calculated on the dried basis.

**Caution**—Handle Deslanoside with exceptional care, since it is highly potent.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Deslanoside RS

**Identification**—Prepare a solution of it in methanol containing about 4 mg per mL. Apply 5 µL of this solution and 5 µL of a methanol solution of USP Deslanoside RS containing 4 mg per mL to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methanol, and water (130:36:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute perchloric acid (1 in 20) and heating at about 100° for 3 minutes. Cool, and examine under UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Specific rotation** (781S): between +7.0° and +8.5°.

**Test solution:** 20 mg per mL, in anhydrous pyridine.

**Loss on drying** (731)—Dry 0.5 g in vacuum at 100° to constant weight: it loses not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

#### Assay—

**Standard preparation**—Dissolve a suitable quantity of USP Deslanoside RS in alcohol, and quantitatively dilute with alcohol to obtain a solution having a known concentration of about 200 µg per mL.

**Assay preparation**—Transfer about 20 mg of Deslanoside, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix.

**Procedure**—Transfer 3.0 mL each of the *Standard preparation*, the *Assay preparation*, and alcohol to provide the blank, to separate 25-mL conical flasks. Evaporate each with gentle warming and with the aid of a current of air just to dryness, and cool in a vacuum desiccator for 30 minutes. Add 15.0 mL of acid-ferric chloride TS to each flask, mix by swirling, and allow the mixtures to stand protected from light, swirling frequently, at a temperature not exceeding 30°, for 15 minutes. Pass each through separate fine glass wool filters. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 590 nm, with a suitable spectrophotometer, using the blank to set the instrument. Repeat the measurements at 2-minute intervals until maximum absorbance readings have been obtained. Calculate the quantity, in mg, of  $C_{47}H_{74}O_{19}$  in the Deslanoside taken by the formula:

$$0.1C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Deslanoside RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the maximum absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Deslanoside Injection

» Deslanoside Injection is a sterile solution of Deslanoside in a suitable solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{47}H_{74}O_{19}$ . It may contain Glycerin.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—  
USP Deslanoside RS

**Identification**—Transfer a volume of Injection, equivalent to about 2 mg of deslanoside, to a small separator, and ex-

tract with 25 mL of a mixture of 7 volumes of chloroform and 3 volumes of alcohol. Transfer the extract to a 10-mL conical flask, evaporate on a steam bath to dryness, and dissolve the residue in 500  $\mu$ L of methanol. Proceed with a 5- $\mu$ L portion of the solution so obtained, as directed in the *Identification* test under *Deslanoside*, beginning with "Apply 5  $\mu$ L of this solution." The specified result is obtained.

**pH** (791): between 5.5 and 7.0.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Standard preparation**—Prepare as directed in the *Assay* under *Deslanoside*.

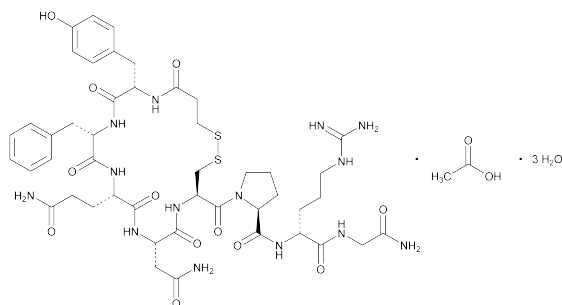
**Assay preparation**—Transfer a volume of Injection, equivalent to about 600  $\mu$ g of deslanoside, to a separator, and add 50 mL of water and 1 mL of 2 N sulfuric acid. Extract with four 30-mL portions of a mixture of 5 volumes of chloroform and 1 volume of *n*-propyl alcohol, washing each portion in a second separator containing 5 mL of water and filtering through cotton that previously has been moistened with chloroform. Combine the extracts, and evaporate on a steam bath, with the aid of a current of air, just to dryness. Transfer the residue, with the aid of a small volume of the chloroform-*n*-propyl alcohol mixture, to a 25-mL conical flask.

**Procedure**—Transfer 3.0 mL each of the *Standard preparation* and of alcohol to provide the blank to separate 25-mL conical flasks. Using these solutions and the *Assay preparation*, proceed as directed for *Procedure* in the *Assay* under *Deslanoside*, beginning with "Evaporate each with gentle warming." Calculate the quantity, in  $\mu$ g, of  $C_{47}H_{74}O_{19}$  in each mL of the Injection taken by the formula:

$$(C / V)(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Deslanoside RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the maximum absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Desmopressin Acetate



$C_{48}H_{68}N_{14}O_{14}S_2$   
 $C_{48}H_{68}N_{14}O_{14}S_2 \cdot xH_2O$   
 (anhydrous) 1129.27 [62288-83-9].  
 Vasopressin, 1-(3-mercaptopropionic acid)-8-D-arginine-, monoacetate (salt).  
 1-(3-Mercaptopropionic acid)-8-D-arginine-vasopressin monoacetate (salt).  
 Trihydrate 1183.31 [62357-86-2].

» Desmopressin Acetate is a synthetic octapeptide hormone having the property of antidiuresis. It is a synthetic analog of vasopressin. It contains not less than 95.0 percent and not more than

105.0 percent of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), calculated on the anhydrous, acetic acid-free basis.

**Packaging and storage**—Preserve in tight containers, preferably of Type I glass, protected from light. Store at a temperature not exceeding 25°, preferably between 2° and 8°.

**Labeling**—Label it to state the potency, in mg, of desmopressin.

**USP Reference standards** (11)—

USP Desmopressin Acetate RS

#### Identification—

**A: Mass spectral analysis—**

**Diluent:** a mixture of water and methanol (1:1).

**Standard solution**—Dissolve an accurately weighed quantity of USP Desmopressin Acetate RS in *Diluent* to obtain a solution having a known concentration of about 5  $\mu$ g per mL.

**Test solution**—Dissolve an accurately weighed quantity of Desmopressin Acetate in *Diluent* to obtain a solution having a known concentration of about 5  $\mu$ g per mL.

[NOTE—The final concentration of the *Standard solution* and the *Test solution* can be adjusted depending on the sensitivity of the mass spectrometer used in the testing.]

**Mass spectrometric system** (see *Mass Spectrometry* (736))—The LC/MS Spectrometer is equipped with an electrospray interface, positive ion mode, infusion system, and MS/MS capability.

**Procedure**—Separately infuse the *Standard solution* and the *Test solution* at about 5  $\mu$ L per minute into the mass spectrometer. Obtain optimized MS and MS/MS spectra of the peak with mass-to-charge ratio 1069. For MS spectra, the major peak with mass-to-charge ratio of 1069 should be observed. For MS/MS spectra, product ions at mass-to-charge ratios of about 641, 742, and 995 are present.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between -72° and -82°, calculated on the anhydrous, acetic acid-free basis.

**Test solution:** 5 mg per mL, in diluted acetic acid.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 100 cfu per g.

**Water, Method 1c** (921): not more than 6.0%.

**Amino acid content** (see *Biotechnology-Derived Articles—Amino Acid Analysis* (1052))—

NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used. The relative proportions of amino acids, however, must be met for any method used.

**Solution A**—Prepare a solution having final concentrations of 20 mM sodium acetate, 0.2% (v/v) triethylamine, and 0.3% (v/v) tetrahydrofuran.

**Solution B**—Prepare a solution containing 20% (v/v) 100 mM sodium acetate, 40% (v/v) methanol, and 40% (v/v) acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Borate buffer**—Transfer 12.4 g of boric acid to a 500-mL volumetric flask, and suspend it in 300 mL of water. Add 100 mL of 1 N potassium hydroxide, and mix. Adjust with 1 N potassium hydroxide to a pH of 10.4, dilute with water to volume, and mix. Store in a closed plastic container.

**Norvaline solution**—Prepare a 4 mM solution of norvaline.

**2% DTDPA solution**—Transfer 2 g of dithiodipropionic acid to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

**Sarcosine solution**—Prepare a 4 mM solution of sarcosine.

**0.1% Phenol**—Prepare a solution containing 0.1% (w/v) phenol in 6 N hydrochloric acid.

**OPA reagent**—Prepare a solution containing 10 mg per mL each of o-phthalaldehyde and 3-mercaptopropionic acid in Borate buffer.

**FMOC reagent**—Prepare a solution containing 2.5 mg per mL of 9-fluorenylmethylchloroformate in acetonitrile.

**Calibration solution**—Prepare a mixture in which the final concentrations of amino acids are as follows: about 2.50 mM glycine; about 2.50 mM for the L-form of the amino acids lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine; and about 1.25 mM L-cystine. Transfer a 1-mL aliquot of this solution to a suitable vial, and add 5  $\mu$ L each of *Norvaline solution* and *2% DTDPA solution*. Evaporate the aliquot to dryness, and add 300  $\mu$ L of *0.1% Phenol*. Mix, and alternate between purging the head space of the vial with nitrogen gas and reducing the pressure to 2 mm of mercury for a total of two purge-vacuum cycles. Purge the sample with nitrogen gas one additional time, and reduce the pressure to 1.5 mm of mercury. Seal, and heat the sample at 110° for 24 hours. Open the vial, and evaporate to dryness. Dissolve the residue in 115  $\mu$ L of *Borate buffer*, and add 5  $\mu$ L of *Sarcosine solution*. Centrifuge for 2 minutes, and transfer the supernatant to a clean vial. Remove a 6- $\mu$ L aliquot, and add 1  $\mu$ L of *OPA reagent*. Mix, and add 1  $\mu$ L of *FMOC reagent*. Mix, add 28  $\mu$ L of water, and mix again.

**Test solution**—Dissolve an accurately weighed quantity of Desmopressin Acetate in water to obtain a solution having a known concentration of about 1.00 mg per mL. Add 5  $\mu$ L each of *Norvaline solution* and *2% DTDPA solution* to a 1-mL aliquot, and prepare as directed in *Calibration solution*, beginning with "Bring the aliquot to dryness and add 300  $\mu$ L of *0.1% Phenol*."

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a thermoregulated autosampler, set at 4°, that is capable of adding and mixing derivatizing agents; a multi-wavelength detector set at 262 nm and 338 nm; and a 2.1-mm  $\times$  20-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 0.45 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–17	100→40	0→60	linear gradient
17–18.10	40→0	60→100	linear gradient
18.10–24	0	100	isocratic
24–25	0→100	100→0	linear gradient
25–35	100	0	re-equilibration

Process and inject the *Calibration solution* and the *Test solution*, and record the peak responses for individual amino acid derivatives as directed for *Procedure*: the order of elution of the amino acid derivatives is aspartic acid, glutamic acid, serine, histidine, glycine, threonine, cysteine (reduced cystine), alanine, arginine, tyrosine, valine, methionine, norvaline, phenylalanine, isoleucine, leucine, lysine, and proline; the resolution,  $R$ , between the amino acid pairs histidine and glycine, alanine and arginine, and valine and methionine in the *Calibration solution* is not less than 1; and the relative standard deviation for triplicate injection of the *Calibration solution* is not greater than 15% for cysteine, lysine and proline, and not greater than 10% for all other amino acids. The peak area of the norvaline peak in the *Test*

*solution* should be not less than 80% and not greater than 120% of that found in the *Calibration solution*.

**Procedure**—Using the autosampler, separately remove equal volumes (about 6  $\mu$ L) of the *Calibration solution* and the *Test solution*, and to each add 1  $\mu$ L of *OPA reagent*. Mix, and to each add 1  $\mu$ L of *FMOC reagent*. Mix, add 28  $\mu$ L of water to each, mix again, and inject the entire volume into the chromatograph. Record the area responses for the main peaks, and identify the amino acids. Using the *Calibration solution* as a standard, express the content of each amino acid in moles. With the content for arginine set to 1, calculate the relative proportions of the amino acids: aspartic acid, glutamic acid, proline, glycine, and phenylalanine are between 0.95 and 1.05; tyrosine is between 0.7 and 1.05; cysteine is between 0.30 and 1.05; lysine, isoleucine, and leucine are absent; and not more than traces of other amino acids are found.

#### Limit of acetic acid—

**Internal standard solution**—Transfer about 16 mL of hydrochloric acid into a 1000-mL volumetric flask containing about 500 mL of water, and mix. Add about 0.5 mL of propionic acid, accurately measured, dilute with acetonitrile to volume, and mix.

**Standard solution**—Transfer about 1.049 g of acetic acid, accurately measured, to a 100-mL volumetric flask. Dilute with *Internal standard solution* to volume, and mix. Transfer 2.5 mL of the resulting solution to a 50-mL volumetric flask. Dilute with *Internal standard solution* to volume, and mix.

**Test solution**—Dissolve about 5 mg of Desmopressin Acetate, accurately weighed, in 0.5 mL of *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm  $\times$  30-m fused silica capillary column coated with a 0.25- $\mu$ m film of phase G35. The carrier gas is helium, flowing at a rate of about 3 mL per minute, and the split flow ratio is 20:3. The column temperature is maintained at 120°, and the injection port and detector temperatures are maintained at 250°. Chromatograph six replicate injections of the *Standard solution*, and record the peak areas as directed for *Procedure*: the order of elution is acetic acid followed by propionic acid; the resolution,  $R$ , between acetic acid and propionic acid is not less than 5.0; the tailing factor for acetic acid is not more than 3.0; and the relative standard deviation of the peak area ratio of acetic acid to propionic acid is not more than 15%.

**Procedure**—Separately inject equal volumes (about 1.0  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the peak responses. Calculate the percentage of acetic acid in the portion of Desmopressin Acetate taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which  $C_S$  is the concentration, in mg per mL, of acetic acid in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of desmopressin acetate in the *Test solution*; and  $R_U$  and  $R_S$  are the peak area ratios of acetic acid to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not less than 3% and not more than 8.0% is found.

#### Chromatographic purity—

**Mobile phase and System suitability solution**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Desmopressin Acetate RS in *Mobile phase* to obtain a solution having a known concentration of about 1  $\mu$ g per mL.

**Test solution**—Dissolve an accurately weighed quantity of Desmopressin Acetate in *Mobile phase* to prepare a solution having a known concentration of about 200  $\mu$ g per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute, and the column is maintained at 30°. Chromatograph the *Standard solution* and the *System suitability solution*, and record the peak areas as directed for *Procedure*: the desmopressin peak elutes before the oxytocin peak; the resolution, *R*, between desmopressin and oxytocin is not less than 1.5; the tailing factor is not greater than 2.0; and the relative standard deviation of the desmopressin peak area for replicate injections of the *Standard solution* is not greater than 5.0%.

**Procedure**—Separately inject equal volumes (about 200 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the response for each peak, except for the main desmopressin peak in the chromatogram of the *Test solution*. Calculate the percentage of each impurity in the portion of Desmopressin Acetate taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

in which *C<sub>S</sub>* is the concentration, in mg per mL, of USP Desmopressin Acetate RS, calculated on the anhydrous, acetic acid-free basis, in the *Standard solution*; *C<sub>U</sub>* is the concentration, in mg per mL, of Desmopressin Acetate, calculated on the anhydrous, acetic acid-free basis, in the *Test solution*; *r<sub>i</sub>* is the peak response of an individual impurity in the chromatogram obtained from the *Test solution*; and *r<sub>S</sub>* is the desmopressin peak response obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found, and the sum of all impurities is less than 1.5%.

#### Assay—

**Buffer solution**—Dissolve 3.4 g of monobasic potassium phosphate and 2.0 g of sodium 1-heptanesulfonic acid in 1000 mL of water. Adjust the pH to 4.50 ± 0.05 with phosphoric acid or sodium hydroxide, as needed. Pass through a filter having a porosity of 0.45-μm.

**Mobile phase**—Mix 780 mL of *Buffer solution* with 220 mL of acetonitrile, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>). [NOTE—The retention time of desmopressin is very sensitive to the composition of the *Mobile phase*.]

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desmopressin Acetate RS in *Mobile phase* to obtain a solution having a known concentration of about 20 μg per mL.

**Assay preparation**—Dissolve an accurately weighed quantity of Desmopressin Acetate in *Mobile phase* to prepare a solution having a known concentration of about 20 μg per mL.

**System suitability solution**—Dissolve about 1 mg of oxytocin, accurately weighed, in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution and 5.0 mL of *Assay preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The column temperature is maintained at 30°. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation* and the *System suitability solution*, and record the peak areas as directed for *Procedure*: the desmopressin peak elutes before the oxytocin peak; the resolution, *R*, between desmopressin and oxytocin is not less than 1.5; the tailing factor is not greater than 2.0; and the relative standard deviation of the desmopressin peak area for replicate injections is not greater than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard preparation* and the *Assay preparation*, both freshly prepared, into the chromatograph, record the chromatograms, and measure the desmopressin peak areas. Cal-

culate the percentage of C<sub>46</sub>H<sub>64</sub>N<sub>14</sub>O<sub>12</sub>S<sub>2</sub> in the portion of Desmopressin Acetate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which *C<sub>S</sub>* is the concentration, in mg per mL, of USP Desmopressin Acetate RS, calculated on the anhydrous, acetic acid-free basis, in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of Desmopressin Acetate, calculated on the anhydrous, acetic acid-free basis, in the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for desmopressin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desmopressin Acetate Injection

» Desmopressin Acetate Injection is a sterile solution of Desmopressin Acetate in a suitable diluent. It may contain suitable preservatives. It possesses, in each mL, an activity of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of desmopressin (C<sub>46</sub>H<sub>64</sub>N<sub>14</sub>O<sub>12</sub>S<sub>2</sub>), calculated on the anhydrous, acetic acid-free basis.

**Packaging and storage**—Preserve in tight containers, protected from light. Store at a temperature between 2° and 8°.

**Labeling**—Label it to state the potency, in mg, of desmopressin.

#### USP Reference standards <11>—

USP Desmopressin Acetate RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 10 USP Endotoxin Units per μg of desmopressin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** <791>: between 3.5 and 6.0.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Container Content* under *Injections* <1>.

#### Assay—

**Buffer solution**—Dissolve 4.9 g of phosphoric acid, accurately weighed, in water. Dilute with water to 1000 mL. Adjust with triethylamine to a pH of 3.5.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (83.5:16.5). Make adjustments, if necessary (see *System Suitability* under *Chromatography* <621>).

**Solution A**—Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, and dissolve in and dilute with water to volume. Adjust with hydrochloric acid to a pH between 3.5 and 5.0.

**Solution B**—Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, dissolve in water, and add 5 g of chlorobutanol. Dilute with water to volume, and adjust with hydrochloric acid to a pH between 3.5 and 5.0.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desmopressin Acetate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Dilute this solution with *Solution A* or *Solution B*, as di-

rected for the *Assay preparation*, to obtain a solution with a concentration of desmopressin equivalent to that in the *Assay preparation*.

**Assay preparation**—For injections at concentrations of desmopressin between 4 µg per mL and 0.1 mg per mL, use undiluted Desmopressin Acetate Injection. For injections at concentrations exceeding 0.1 mg per mL and without preservatives, dilute 1000 µL of Desmopressin Acetate Injection, accurately measured, with 10 mL of *Solution A*. For injections at concentrations exceeding 0.1 mg per mL and containing preservatives, dilute 1000 µL of Desmopressin Acetate Injection, accurately measured, with 10 mL of *Solution B*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph about 50 µL of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.4; and the relative standard deviation for replicate injections is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation*, both freshly prepared, into the chromatograph, and record the chromatograms for a total time of not less than 2.5 times the retention time of the desmopressin peak. Calculate the quantity of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), in mg, in the volume of Injection taken by the formula:

$$CD(r_U / r_S)$$

in which *C* is the concentration of desmopressin, in mg per mL, in the *Standard preparation*; *D* is the dilution factor used to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for desmopressin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desmopressin Nasal Spray Solution

» Desmopressin Nasal Spray Solution is a solution of Desmopressin Acetate in a suitable diluent. It is supplied in a form suitable for nasal administration and contains suitable preservatives. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), calculated on the anhydrous, acetic acid-free basis.

**Packaging and storage**—Preserve in containers suitable for administering the contents by spraying into the nasal cavities in a controlled, individualized dosage. Protect from light, and store at a temperature between 2° and 8°.

**Labeling**—Label it to indicate that it is for intranasal administration only and to state the total number of discharges. Label it also to state that the dosage regulation is described in the package insert.

**USP Reference standards** <11>—  
USP Desmopressin Acetate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—The total aerobic microbial count does not exceed 100 cfu per mL, the total combined molds and yeasts count does not exceed 10 cfu per mL, and it meets the requirements of the test for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**pH** <791>: between 3.5 and 6.0.

**Uniformity of unit spray weight and total number of discharges per container**—Select three Nasal Spray Solution units, and prime each spray pump as directed on the label, but not more than five times. Accurately weigh, by difference, 10 individual deliveries from each unit, weighing the first 3 discharges immediately after priming, weighing 4 discharges from the middle of each unit, and 3 close to the end of each unit. Continue to fire until the unit is empty. For each unit, determine the total number of discharges, including the number of priming deliveries, and calculate the mean weight delivered per discharge: it contains not less than the number of discharges stated on the label; the mean weight delivered per discharge is within 10% of the labeled weight per discharge; and not fewer than 9 tested discharges for each unit are between 85% and 125% of the labeled weight per discharge.

**Assay**—

*Buffer solution*, *Mobile phase*, *Solution A*, *Solution B*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Desmopressin Acetate Injection*.

*Assay preparation*, *Standard preparation*, and *Procedure*—Proceed as directed for products containing preservatives in the *Assay* under *Desmopressin Acetate Injection*.

## Desogestrel and Ethinyl Estradiol Tablets

### DEFINITION

Desogestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST <201>

**Standard solution:** 0.15 mg/mL of USP Desogestrel RS and 0.03 mg/mL of USP Ethinyl Estradiol RS in ether

**Sample solution:** Transfer a number of Tablets, equivalent to 1.5 mg desogestrel and 0.3 mg ethinyl estradiol, to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if necessary, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to about 10 mL.

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Application volume:** 30 µL

**Developing solvent system:** Chloroform and alcohol (96:4)

**Spray reagent:** Methanol and sulfuric acid (1:1)

**Analysis:** Proceed as directed in the chapter, and then air-dry. Spray the plate with the *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.

**Acceptance criteria:** Meet the requirements

- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 20 mM potassium phosphate buffer, pH 6.0

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution A:** 0.3 mg/mL of USP Desogestrel RS in methanol

**Standard stock solution B:** 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol

**Standard solution:** 0.6 µg/mL of USP Desogestrel RS and 0.12 µg/mL of USP Ethinyl Estradiol RS in *Diluent*, prepared by diluting appropriate aliquots of *Standard stock solution A* and *Standard stock solution B* with *Diluent*

**Sample solution:** Transfer 20 Tablets into a 200-mL volumetric flask. Add about 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of the sample, and dilute with *Diluent* to obtain a solution nominally containing 0.6 µg/mL of desogestrel.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

#### Detectors

**Desogestrel analysis:** UV 210 nm

**Ethinyl estradiol analysis:** Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm

#### Columns

**Guard:** 4.6-mm × 12.5-mm; packing L11

**Analytical:** 4.6-mm × 15-cm; packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 200 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and desogestrel are about 0.2 and 1.0, respectively.]

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both ethinyl estradiol and desogestrel

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the relevant analyte from the *Sample solution*

$r_S$  = peak response of the relevant analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the relevant analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

#### Test 1

**Medium:** 0.05% sodium lauryl sulfate with an assay content of NLT 95%; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 20 mM potassium phosphate buffer, pH 6.0

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard stock solution A:** 0.005 mg/mL of USP Desogestrel RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Desogestrel RS in methanol to obtain a solution containing 0.25 mg/mL of USP Desogestrel RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.

**Standard stock solution B:** 0.005 mg/mL of USP Ethinyl Estradiol RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Ethinyl Estradiol RS in methanol to obtain a solution containing 0.25 mg/mL of USP Ethinyl Estradiol RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.

**Standard solution:** 0.3 µg/mL of USP Desogestrel RS and 0.06 µg/mL of USP Ethinyl Estradiol RS in *Medium*, from *Standard stock solution A* and *Standard stock solution B*

**Sample solution:** Sample per *Dissolution* <711>. Centrifuge a portion of the dissolution sample, and use the clear supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

#### Detectors

**Desogestrel analysis:** UV 210 nm

**Ethinyl estradiol analysis:** Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm

#### Columns

**Guard:** 4.6-mm × 12.5-mm; packing L11

**Analytical:** 4.6-mm × 15-cm; packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 200 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and for desogestrel are about 0.2 and 1.0, respectively.]

#### Suitability requirements

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of the relevant analyte from the *Sample solution*

$r_S$  = peak response of the relevant analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of medium, 500 mL

**Tolerances:** NLT 80% (Q) of each of the labeled amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.3% sodium lauryl sulfate; 500 mL

**Apparatus 2:** 100 rpm

**Time:** 30 min

**Analysis:** Determine the amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) dissolved by the chromatographic method used in *Test 1*.

**Tolerances:** NLT 80% (Q) of each of the labeled amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* for both desogestrel and ethinyl estradiol

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

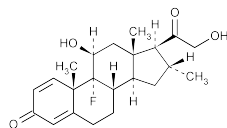
- **USP REFERENCE STANDARDS <11>**

USP Desogestrel RS

USP Ethinyl Estradiol RS



## Desoximetasone



$C_{22}H_{29}FO_4$  376.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-, (11 $\beta$ ,16 $\alpha$ )-

9-Fluoro-11 $\beta$ ,21-dihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione [382-67-2].

» Desoximetasone contains not less than 97.0 percent and not more than 103.0 percent of  $C_{22}H_{29}FO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Desoximetasone RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** Prepare a solution of it in a mixture of chloroform and alcohol (3:1) containing 10 mg per mL. Prepare a solution of USP Desoximetasone RS in the same mixture, containing 10 mg per mL. Apply separately 20  $\mu$ L of each solution to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a saturated chamber containing a mixture of chloroform and ethyl acetate (1:1). Allow the solvent front to move 10 cm beyond the application point. After drying, examine the plate under UV light at 254 nm. Spray the dried plate with a 1 in 5 solution of *p*-toluenesulfonic acid in alcohol. The major spot from the test solution corresponds in  $R_f$  value (about 0.25) and appearance to that obtained from the Standard solution.

**Melting range** (741): between 206° and 218°, but the range between beginning and end of melting does not exceed 4°.

**Specific rotation** (781S): between +107° and +112°.

**Test solution:** 5 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

**Assay**—

**Mobile phase**—Prepare a suitable filtered and degassed solution of methanol, water, and glacial acetic acid (65:35:1), such that the retention time of desoximetasone is about 6 minutes.

**Standard preparation**—On the day of use, weigh accurately about 20 mg of USP Desoximetasone RS, and dissolve in methanol to obtain 50.0 mL. Dilute 10.0 mL of this solution with a mixture of methanol and acetonitrile (1:1) to 100.0 mL.

**Assay preparation**—Weigh accurately 40 mg of Desoximetasone, dissolve in 100.0 mL of methanol, and proceed as directed for *Standard preparation*, beginning with "Dilute 10.0 mL of this solution."

**Procedure**—Using an injection loop, inject 10- $\mu$ L portions of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph equipped with an UV detector capable of monitoring absorption at 254 nm. The instrument is equipped with a 4.6-mm  $\times$  15-cm stainless steel column that contains packing L7 and is operated at a flow rate of about 1 mL per minute. In a suitable chromatogram, five replicate injections of the *Standard preparation*

show a relative standard deviation of not more than 2.0%, and the tailing factor is not more than 1.5. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Desoximetasone taken by the formula:

$$1000C(H_U / H_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*, and  $H_U$  and  $H_S$  are the peak heights of desoximetasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoximetasone Cream

» Desoximetasone Cream is Desoximetasone in an emollient cream base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_4$ .

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

**USP Reference standards** (11)—

USP Desoximetasone RS

**Identification**—Evaporate 25 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 2 mL of acetonitrile. This is the test solution. Prepare a Standard solution of USP Desoximetasone RS in acetonitrile containing 500  $\mu$ g per mL. Using 10  $\mu$ L instead of 20  $\mu$ L of each solution, proceed as directed in *Identification* test *B* under *Desoximetasone*, beginning with "Apply separately 20  $\mu$ L of each." The specified result is observed.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 4.0 and 8.0, in a solution prepared in the following manner. Add 15 mL of boiling water to 3.5 g of the Cream in a 50-mL centrifuge tube, cap the tube, shake vigorously until the cream is uniformly dispersed, then place the tube in a steam bath until the water and oil layers separate completely. Cool, separate the layers, and determine the pH of the aqueous phase.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (65:35:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of ethylparaben in methanol having a concentration of about 0.04 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desoximetasone RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL. Pipet 5 mL of this solution into a 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution*, dilute with methanol quantitatively to 40.0 mL, and mix to obtain the *Standard preparation* having a known concentration of about 0.05 mg per mL.

**Assay preparation**—Transfer an accurately weighed amount of Cream, equivalent to about 2 mg of desoximetasone, to a 50-mL centrifuge tube, and add a few 3-mm glass beads. Add 10.0 mL of *Internal standard solution* and about 30 mL of methanol, and mix. Tightly cap the centrifuge tube, and immerse it for 10 minutes in a bath maintained at a temperature of 65°. Remove the tube from the bath, and immediately vortex at high speed for 30 seconds. Return the tube to the hot water bath for 5 minutes, remove it from the bath, and immediately vortex for 30 seconds. Repeat the procedure one more time, then cool the tube in an ice-bath held at 10° until no further

flocculent precipitation occurs. Centrifuge, and use the supernatant.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1 and 2 for ethylparaben and desoximetasone, respectively; the tailing factor for the analyte peak is not more than 2.0; the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub> in the portion of Cream taken by the formula:

$$40C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoximetasone Gel

» Desoximetasone Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub>.

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

**USP Reference standards** <11>—  
USP Desoximetasone RS

**Identification**—Transfer an amount of Gel, equivalent to 100 µg of desoximetasone, to a 15-mL centrifuge tube. Add 3 mL of acetonitrile, sonicate for approximately 1 minute, centrifuge, and transfer the clear supernatant to another 15-mL centrifuge tube. Evaporate the solution under nitrogen at a temperature between 35° and 45° to dryness. Dissolve the residue in 100 µL of methanol, using a sonicator. Streak separately the entire test solution and 100 µL of a Standard solution of USP Desoximetasone RS in methanol containing 1 mg per mL on a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture and an area of preadsorbent material on which specimens are applied. Allow the streaks to dry, and develop the chromatogram in a saturated chamber containing a mixture of acetone and chloroform (1:1). Allow the solvent front to move not less than 10 cm beyond the origin. After drying, examine the plate under UV light at 254 nm: the *R<sub>f</sub>* value of the principal spot obtained in the chromatogram of the test solution corresponds to that of the Standard solution.

**Minimum fill** <755>: meets the requirements.

**Alcohol content**—Transfer about 2.5 g of Gel, accurately weighed, to a 50-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Determine the alcohol content of the specimen thus prepared by the *Method II—Gas-Liquid Chromatographic Method* (see *Alcohol Determination* <611>), using isopropyl alcohol as the internal standard and using methanol in place of water as the solvent: between 18.0% and 24.0% (w/w) of C<sub>2</sub>H<sub>5</sub>OH is found.

## Assay—

**Mobile phase**—Prepare a suitable filtered and degassed solution of methanol, water, and glacial acetic acid (65:35:1). Adjust the ratio, if necessary, so that the retention time of desoximetasone is about 8 minutes.

**Standard preparation**—Using an accurately weighed quantity of USP Desoximetasone RS, prepare a solution in methanol containing 0.5 mg per mL. Dilute an accurately measured volume of this solution with methanolic calcium chloride dihydrate solution (1.5 in 100) to obtain a *Standard preparation* having a known concentration of about 0.025 mg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Gel, equivalent to about 1.25 mg of desoximetasone, to a 50-mL volumetric flask, add approximately 40 mL of methanolic calcium chloride dihydrate solution (1.5 in 100), and sonicate to disperse the gel. Dilute with the same solution to volume, mix, and centrifuge. Use the clear supernatant.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub> in the portion of Gel taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of desoximetasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoximetasone Ointment

» Desoximetasone Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of desoximetasone (C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub>).

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

**USP Reference standards** <11>—  
USP Desoximetasone RS

**Identification**—Transfer an accurately weighed quantity of Ointment, equivalent to about 5 mg of desoximetasone, to a 50-mL centrifuge tube. Add 20 mL of hexane, heat gently to 60°, and shake until the Ointment is completely dispersed. Add 8 mL of acetonitrile, insert the stopper in the tube, and shake vigorously for 5 minutes. Cool to room temperature, and centrifuge until the lower layer is clear. Transfer the lower layer to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix. Prepare a solution of USP Desoximetasone RS in acetonitrile containing 0.5 mg per mL. Separately apply 5 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the plate in a saturated chamber containing a mixture of ethyl acetate and chloroform (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the

plate, and allow to air-dry. Examine under short-wavelength UV light. Spray the dried plate with a 1 in 5 solution of *p*-toluenesulfonic acid in alcohol. Heat the plate at 100° for 5 minutes, and examine under long-wavelength UV light: the  $R_F$  value and appearance (brownish yellow fluorescent spot) of the principal spot from the test solution, correspond to those of the principal spot from the Standard solution.

**Minimum fill (755):** meets the requirements.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (65:35:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desoximetasone RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute 1 volume of this solution with 9 volumes of a 1:1 mixture of methanol and spectrophotometric acetonitrile that is saturated with *n*-heptane, and mix.

**Assay preparation**—Transfer an accurately weighed amount of Ointment, equivalent to about 2 mg of desoximetasone, to a 50-mL centrifuge tube. Add 20 mL of *n*-heptane that has been previously saturated with spectrophotometric acetonitrile, and heat gently with occasional shaking until the Ointment is completely dispersed. Allow to cool slightly, and extract with a 10-mL portion of spectrophotometric acetonitrile. Shake vigorously, centrifuge, remove the bottom layer of acetonitrile with a syringe and needle, and transfer to a 50-mL volumetric flask. Using the same needle and syringe, extract the desoximetasone with successive 10-mL and 8-mL portions of acetonitrile, combining all acetonitrile layers in the 50-mL flask. Dilute with methanol nearly to volume, mix, and allow the solution to reach room temperature. Dilute with methanol to volume, and mix.

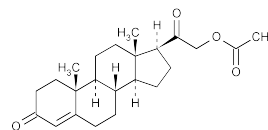
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 2.0, the resolution,  $R$ , between the analyte and solvent peaks is not less than 5.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Ointment taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoxycorticosterone Acetate



$C_{23}H_{32}O_4$  372.50

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-.

11-Deoxycorticosterone acetate [56-47-3].

» Desoxycorticosterone Acetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{23}H_{32}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

#### USP Reference standards (11)—

USP Desoxycorticosterone Acetate RS

#### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 240 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 155° and 161°.

**Specific rotation** (781S): between +171° and +179°.

*Test solution:* 10 mg, undried, per mL, in dioxane.

**Loss on drying** (731)—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

#### Assay—

**Standard preparation**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Desoxycorticosterone Acetate RS.

**Assay preparation**—Accurately weigh about 100 mg of Desoxycorticosterone Acetate, dissolve in sufficient alcohol to make 200.0 mL, and mix. Pipet 5 mL of this solution into a 250-mL volumetric flask, add alcohol to volume, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in the Desoxycorticosterone Acetate taken by the formula:

$$10C(A_U / A_S).$$

## Desoxycorticosterone Acetate Injection

» Desoxycorticosterone Acetate Injection is a sterile solution of Desoxycorticosterone Acetate in vegetable oil. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{23}H_{32}O_4$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, protected from light.

**USP Reference standards** (11)—

USP Desoxycorticosterone Acetate RS

USP Endotoxin RS

**Identification**—Evaporate 25 mL of the *Assay preparation* from the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Using this as the test solution, proceed as directed under *Thin-Layer Chromatographic Identification Test* (201).

**Bacterial endotoxins** (85)—It contains not more than 71.4 USP Endotoxin Units per mg of desoxycorticosterone acetate.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Alcohol-isoctane and Isoctane-alcohol*—Shake equal volumes of 90 percent alcohol and isoctane in a separator for 10 to 15 minutes, and allow to separate. Withdraw the layers into separate containers, designating the lower layer as “alcohol-isoctane” and the upper layer as “isoctane-alcohol.”

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Desoxycorticosterone Acetate RS.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of desoxycorticosterone acetate, to a separator containing 50 mL of *Isoctane-alcohol*. Extract with six 20-mL portions of *Alcohol-isoctane*, receiving the extracts in a 250-mL volumetric flask, dilute with *Alcohol-isoctane* to volume, and mix. Pipet 10 mL of this solution into a glass-stoppered, 50-mL conical flask, evaporate on a steam bath with the aid of a gentle current of air just to dryness, and dissolve the residue in 20.0 mL of alcohol.

*Procedure*—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in each mL of the Injection taken by the formula:

$$0.5(C/V)(A_U/A_S)$$

in which *V* is the volume, in mL, of Injection taken.

**Desoxycorticosterone Acetate Pellets**

» Desoxycorticosterone Acetate Pellets are sterile pellets composed of Desoxycorticosterone Acetate in compressed form, without the presence of any binder, diluent, or excipient. They contain not less than 97.0 percent and not more than 103.0 percent of  $C_{23}H_{32}O_4$ .

**Packaging and storage**—Preserve in tight containers suitable for maintaining sterile contents, holding one pellet each.

**USP Reference standards** (11)—

USP Desoxycorticosterone Acetate RS

**Solubility in alcohol**—A solution of 25 mg of powdered Pellets in 1 mL of alcohol is clear and practically free from insoluble residue.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* alcohol.

Absorptivities at 240 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 155° and 161°.

**Specific rotation** (781S): between +171° and +179°.

*Test solution:* 10 mg, undried, per mL, in dioxane.

**Sterility** (71): meet the requirements.

**Weight variation**—Weigh 5 Pellets singly, and calculate the average weight. The average weight is not less than 95% and not more than 105% of the labeled weight of  $C_{23}H_{32}O_4$ , and each Pellet weighs not less than 90% and not more than 110% of the labeled weight of  $C_{23}H_{32}O_4$ .

**Assay**—

*Standard preparation*—Prepare as directed under *Assay for Steroids* (351), using USP Desoxycorticosterone Acetate RS.

*Assay preparation*—Weigh and finely powder not fewer than 10 Pellets. Weigh accurately about 100 mg of the powder, dissolve it in sufficient alcohol to make 200.0 mL, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL conical flask.

*Procedure*—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in the portion of Pellets taken by the formula:

$$10C(A_U/A_S)$$

**Desoxycorticosterone Pivalate** $C_{26}H_{38}O_4$  414.58

Pregn-4-ene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-11-Deoxycorticosterone pivalate [808-48-0].

» Desoxycorticosterone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{26}H_{38}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Desoxycorticosterone Pivalate RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 20 µg per mL.

*Medium:* methanol.

Absorptivities at 241 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 200° and 206°.

**Specific rotation** (781S): between +155° and +163°.

*Test solution:* 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Transfer about 100 mg of desoxycorticosterone acetate to a 50-mL volumetric flask, add methanol to volume, and mix.

*Standard preparation*—Transfer about 12.5 mg of USP Desoxycorticosterone Pivalate RS, accurately weighed, to a 25-mL volumetric flask, add 20 mL of methanol, and mix. Add 2.5 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known

concentration of about 0.5 mg of USP Desoxycorticosterone Pivalate RS per mL.

**Assay preparation**—Transfer about 50 mg of Desoxycorticosterone Pivalate, accurately weighed, to a 100-mL volumetric flask, add 80 mL of methanol, and mix. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for desoxycorticosterone acetate and 1.0 for desoxycorticosterone pivalate. Calculate the quantity, in mg, of  $C_{26}H_{38}O_4$  in the portion of Desoxycorticosterone Pivalate taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Desoxycorticosterone Pivalate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoxycorticosterone Pivalate Injectable Suspension

» Desoxycorticosterone Pivalate Injectable Suspension is a sterile suspension of Desoxycorticosterone Pivalate in an aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{26}H_{38}O_4$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label Suspension to indicate that it is for veterinary use only.

**USP Reference standards** <11>—  
USP Desoxycorticosterone Pivalate RS  
USP Endotoxin RS

**Identification**—Centrifuge a portion of Suspension, decant the supernatant, wash the residue by stirring with several successive portions of water, centrifuging and decanting each time, and finally dry the residue at 105°: the desoxycorticosterone pivalate so obtained melts between 198° and 204°, and when about 5 mg of the residue is dissolved in 2 mL of sulfuric acid, the solution is yellowish, with a greenish fluorescence. Dilute the solution with 2 mL of water: the color changes to a dark red-blue, and on further dilution with 2 mL of water it is discharged.

**Bacterial endotoxins** <85>—It contains not more than 2.78 USP Endotoxin Units per mg of desoxycorticosterone pivalate.

**pH** <791>: between 5.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections* <1>.

## Assay—

*Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the Assay under *Desoxycorticosterone Pivalate*.

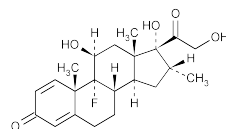
**Assay preparation**—Using a “to contain” pipet, transfer an accurately measured volume of Suspension, equivalent to about 125 mg of desoxycorticosterone pivalate, to a 250-mL volumetric flask. Add about 200 mL of methanol, and sonicate to dissolve. Add 25.0 mL of the *Internal standard solution*, dilute with methanol to volume, and mix. Centrifuge a 20-mL portion at high speed for about 5 minutes. Filter the supernatant through a 5- $\mu$ m disk, discarding the first 5 mL of the filtrate.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Desoxycorticosterone Pivalate*. Calculate the quantity, in mg, of  $C_{26}H_{38}O_4$  in each mL of the Suspension taken by the formula:

$$250(C/V)(R_U/R_S)$$

in which  $V$  is the volume, in mL, of Suspension taken, and the other terms are as previously defined.

## Dexamethasone



$C_{22}H_{29}FO_5$  392.46  
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11 $\beta$ ,16 $\alpha$ )-  
9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione [50-02-2].

» Dexamethasone contains not less than 97.0 percent and not more than 102.0 percent of  $C_{22}H_{29}FO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Dexamethasone RS

## Identification—

**A: Infrared Absorption** <197K>.

**B: Ultraviolet Absorption** <197U>—

*Solution*: 10  $\mu$ g per mL.

*Medium*: methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** <781S>: between +72° and +80°.

*Test solution*: 10 mg per mL, in dioxane.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.2% from 250 mg.

## Chromatographic purity—

*Formate buffer*—Dissolve 1.32 g of ammonium formate in 1 L of water, adjust with formic acid to a pH of 3.6, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of *Formate buffer* and acetonitrile (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Test solution**—Transfer about 180 mg of Dexamethasone, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer about 33 mL of this solution to a 100-mL volumetric flask, dilute with *Formate buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates.

**Procedure**—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the sum of the responses of all peaks: not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a suitable degassed solution of water and acetonitrile (about 7:3) such that at an approximate flow rate of 2 mL per minute, the retention time of Dexamethasone is about 7 minutes.

**Standard preparation**—Prepare a solution of USP Dexamethasone RS in methanol having a known concentration of about 7.5 mg per mL. Dilute an accurately measured volume of this solution with the *Mobile phase* to obtain a *Standard preparation* having a known concentration of about 0.3 mg per mL.

**Assay preparation**—Using 30 mg of Dexamethasone, proceed as directed for *Standard preparation*.

**Procedure**—Introduce equal volumes (between 15 and 30 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* <621>) operated at room temperature by means of a suitable microsyringe or sampling valve, adjusting the operating parameters such that the peak obtained with the *Standard preparation* is 60% full-scale. Typically, the apparatus is fitted with a 4-mm × 25-cm column containing packing L7, is equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder, and is operated at about 1000 psi. Five replicate injections of the *Standard preparation* show a relative standard deviation of not more than 3.0%. Determine the peak responses, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Dexamethasone taken by the formula:

$$100C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Topical Aerosol

» Dexamethasone Topical Aerosol is Dexamethasone in a suitable lotion base mixed with suitable propellants in a pressurized container. Dexamethasone Topical Aerosol delivers not less

than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in pressurized containers, and avoid exposure to excessive heat.

**USP Reference standards** <11>—  
USP Dexamethasone RS

**Identification**—Evaporate 5 mL of the *Assay preparation*, obtained as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Apply 100 µL of this solution and 10 µL of a solution of USP Dexamethasone RS in chloroform containing 500 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and diethylamine (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a heat lamp until spots appear: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Other requirements**—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* <601>.

#### Assay—

**Standard preparation**—Prepare as directed under *Assay for Steroids* <351>, using USP Dexamethasone RS.

**Assay preparation**—Shake the Topical Aerosol container gently, and invert, immersing the valve in about 75 mL of alcohol contained in a 400-mL beaker. Actuate the valve by pushing against the bottom of the beaker. Remove the container at 15-second intervals, shake gently, and allow the container to warm to room temperature. Continue spraying until the contents of the container are exhausted. Transfer the alcohol solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of this solution, equivalent to about 1 mg of dexamethasone, with alcohol to 100.0 mL, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL flask.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* <351>, except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each container taken by the formula:

$$(10C / V)(A_u / A_s)$$

in which  $V$  is the volume, in mL, of assay solution taken for the second dilution.

## Dexamethasone Elixir

» Dexamethasone Elixir contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—  
USP Dexamethasone RS

**Identification**—Evaporate 9 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 2 mL of a mixture of methylene chloride and methanol (1:1). Apply separately 5  $\mu$ L of this solution and 5  $\mu$ L of a solution of USP Dexamethasone RS in the mixture of methylene chloride and methanol (1:1), containing 0.5 mg per mL, to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* <621>). Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, acetone, and glacial acetic acid (80:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by viewing under short-wavelength UV light: the  $R_F$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Alcohol content**, *Method II* <611>: between 3.8% and 5.7% of  $C_2H_5OH$ , *n*-propyl alcohol being used as the internal standard.

#### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2), and dilute quantitatively, and stepwise if necessary, with dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Elixir, freshly mixed and free from air bubbles, equivalent to about 1 mg of dexamethasone, to a 10-mL volumetric flask, dilute with water to volume, mix, and filter through a suitable membrane filter.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (between 5  $\mu$ L and 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Elixir taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, *V* is the volume, in mL, of Elixir taken, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Gel

» Dexamethasone Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in collapsible tubes. Keep tightly closed. Avoid exposure to temperatures exceeding 30°.

**USP Reference standards** <11>—  
USP Dexamethasone RS

**Identification**—Evaporate 25 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 0.5 mL of chloroform. The chloroform extract responds to the *Thin-Layer Chromatographic Identification Test* <201>, 10  $\mu$ L of the chloroform extract and 10  $\mu$ L of a Standard solution containing about 500  $\mu$ g per mL of USP Dexamethasone RS being applied, and a mixture of chloroform and diethylamine (2:1) being used for development. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating.

**Minimum fill** <755>: meets the requirements.

#### Assay—

*Mobile solvent*—Dilute 100 mL of methylene chloride with isooctane to one L.

*Chromatographic columns*—Tamp a pledget of glass wool at the constriction of a glass chromatographic tube measuring about 30-  $\times$  1.5-cm, equipped with a polytetrafluoroethylene stopcock. Fill the tube about half-full with *Mobile solvent*. Mix 8 g of chromatographic siliceous earth with 8 mL of methanol and water (1:1). Transfer successive portions of the mixture to the column, emptying and adding *Mobile solvent* after each addition to pack the column. Finally drain the column to a layer of *Mobile solvent* about 1 cm above the absorbant. Pack a second tube to provide a blank and proceed as directed for *Assay preparation*, but omit the specimen.

*Standard preparation*—Prepare a solution of USP Dexamethasone RS in alcohol to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

*Assay preparation*—Accurately weigh an amount of Gel, equivalent to about 0.5 mg of Dexamethasone in a 100-mL beaker, add 1 g of chromatographic siliceous earth, and mix. Transfer the mixture to the column, wash the beaker with small portions of *Mobile solvent*, adding them to the column. Adjust the flow rate to about 2 mL per minute, discarding the eluate. Elute with four additional 25-mL portions of *Mobile solvent*, and discard. Rinse the sample beaker with two 25-mL portions of methylene chloride and transfer to the column, collecting the eluate in a suitable beaker. Elute the column with six additional 25-mL portions of methylene chloride, combining the eluates and evaporating with a gentle stream of air to dryness, dissolve the residue in alcohol, and transfer to a 50-mL volumetric flask. Wash the beaker with successive 5-mL portions of alcohol, collecting the washings in the flask, dilute with alcohol to volume, and mix. Centrifuge or filter and then pipet 10 mL of this solution, 10 mL of the *Standard preparation*, 10 mL of the column blank solution, and 10 mL of alcohol to provide a reagent blank to separate flasks. Proceed as directed under *Assay for Steroids* <351>, except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Gel taken by the formula:

$$(0.05C)(A_U - A_{CB} / A_S - A_{RB})$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Dexamethasone RS in the *Standard preparation*; and  $A_U$ ,  $A_{CB}$ ,  $A_S$ , and  $A_{RB}$  are the absorbances of the solutions from the *Assay preparation*, column blank preparation, *Standard preparation*, and reagent blank preparation, respectively.

## Dexamethasone Injection

» Dexamethasone Injection is a sterile solution of Dexamethasone in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in light-resistant single-dose or multiple-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Dexamethasone RS

USP Endotoxin RS

**Identification**—

**A: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution**—Transfer a quantity of Injection, equivalent to about 5 mg of dexamethasone, to a 50-mL separator, add 10 mL of water, and extract with two 20-mL portions of chloroform. Filter the lower layers through chloroform-saturated cotton into a 50-mL conical flask, and evaporate to dryness. Dissolve the residue in 10 mL of chloroform.

**Developing solvent system:** a mixture of methylene chloride and methanol (180:16).

**Procedure**—Visualize the spots using a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1), followed by heat.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 21.0 USP Endotoxin Units per mg of dexamethasone.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 5.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Mobile phase* containing in each mL about 0.3 mg of USP Dexamethasone RS, 1.35 mg of benzyl alcohol, 0.27 mg of methylparaben, and 0.03 mg of propylparaben.

**Standard preparation**—Quantitatively dissolve an accurately weighed amount of USP Dexamethasone RS in methanol to obtain a stock solution having a known concentration of about 7.5 mg per mL. Transfer 4.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.3 mg of USP Dexamethasone RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 30 mg of dexamethasone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for benzyl alcohol, 0.5 for methylparaben, 1.0 for dexamethasone, and 1.4 for propylparaben; and the resolution, *R*<sub>s</sub> between the neighboring peaks for benzyl alcohol and methylparaben, methylparaben and dexamethasone, and dexamethasone and propylparaben is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the peak responses for dexamethasone. Calculate the quantity, in mg, of dexamethasone (C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r*<sub>U</sub> and *r*<sub>S</sub> are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Ophthalmic Suspension

» Dexamethasone Ophthalmic Suspension is a sterile, aqueous suspension of dexamethasone containing a suitable antimicrobial preservative. It may contain suitable buffers, stabilizers, and suspending and viscosity agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dexamethasone RS

**Identification**—Transfer a volume of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a test tube, add 5 mL of chloroform, and shake. Centrifuge, and apply 10 μL of the chloroform layer and 10 μL of a Standard solution of USP Dexamethasone RS in chloroform containing 500 μg per mL on a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in *Solvent A* as directed under *Single-steroid Assay* (511). Mark the solvent front, and locate the spots on the plate by spraying with a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of 9 volumes of alcohol and 1 volume of propylene glycol, and heating until spots appear. The *R*<sub>F</sub> value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Sterility** (71): meets the requirements.

**pH** (791): between 5.0 and 6.0.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.12 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 3 mg of dexamethasone, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1750 theoretical plates; the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 3.0%.



**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Ophthalmic Suspension taken by the formula:

$$25(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Suspension taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Oral Solution

» Dexamethasone Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label concentrated Oral Solution to state that the term **Concentrate** is to appear apart from and immediately after the official title in prominent boldface type. Label concentrated Oral Solution also to indicate that it is to be diluted to appropriate strength with a suitable diluent prior to administration unless produced for dispensing with instructions for administration by a calibrated dropper or syringe.

**USP Reference standards** (11)—

USP Dexamethasone RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Transfer a quantity of Oral Solution, equivalent to about 5 mg of dexamethasone, to a 50-mL separator, add 10 mL of water, and extract with two 20-mL portions of chloroform. Filter the lower layers through chloroform-saturated cotton into a 50-mL conical flask, and evaporate to dryness. Dissolve the residue in 10 mL of chloroform.

**Developing solvent system:** a mixture of methylene chloride and methanol (180:16).

**Procedure**—Visualize the spots, using a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1) followed by heat.

**pH** (791): 2.7 to 4.0.

**Alcohol content, Method II** (611) (if present): between 27.0% and 33.0%.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (55:43:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluting solution**—Prepare a mixture of methanol and water (1:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 0.04 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 4 mg of dexamethasone, to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg per mL, of dexamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Oral Solution taken by the formula:

$$LC/D(r_U/r_S)$$

in which *L* is the labeled quantity, in mg per mL, of dexamethasone in the Oral Solution; *D* is the concentration, in mg per mL, of dexamethasone in the *Assay preparation* on the basis of the labeled quantity and the extent of dilution; *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Tablets

» Dexamethasone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dexamethasone RS

**Identification**—Evaporate 10 mL of the methanol extract of Tablets obtained as directed under *Assay preparation* in the Assay on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Apply 10  $\mu$ L of this solution and 20  $\mu$ L of a solution of Dexamethasone RS in chloroform containing 500  $\mu$ g per mL on a thin-layer chromatographic plate (see *System Suitability* under *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in *Solvent A* as directed under *Single-Steroid Assay* (511). Mark the solvent front, and locate the spots on the plate by visualizing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Dissolution** (711)—

**Medium:** dilute hydrochloric acid (1 in 100); 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Standard solution**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Dexamethasone RS.

**Procedure**—Extract a filtered aliquot of *Dissolution Medium*, equivalent to about 200  $\mu$ g of dexamethasone, with three 15-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath just to dryness, cool, and dissolve the residue in 20 mL of alcohol. Proceed as directed for *Procedure* under *Assay for Steroids* (351), ex-

cept to allow to stand in the dark for 45 minutes. Calculate the portion, in mg, of  $C_{22}H_{29}FO_5$  dissolved by the formula:

$$10(C/V)(A_U/A_S)$$

in which  $V$  is the volume, in mL, of the aliquot extracted with chloroform.

**Tolerances**—Not less than 70% ( $Q$ ) of the labeled amount of  $C_{22}H_{29}FO_5$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—

**Standard solution**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Dexamethasone RS.

**Test solution**—Place 1 Tablet in a separator with 15 mL of water, and swirl to disintegrate the Tablet completely. Extract with four 10-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 50-mL volumetric flask, add chloroform to volume, and mix. Pipet a volume of this solution, equivalent to about 200  $\mu$ g of dexamethasone into a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol. Use this where *Assay Preparation* is specified in the *Procedure*.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351), except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of total steroids, as  $C_{22}H_{29}FO_5$ , in the Tablet by the formula:

$$(C/V)(A_U/A_S)$$

in which  $V$  is the volume, in mL, of the aliquot taken to prepare the *Test solution*.

#### Assay—

**Mobile solvent**—Prepare a suitable aqueous solution of acetonitrile, approximately 1 in 3, such that the retention time of dexamethasone is between 3 minutes and 6 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of dexamethasone, transfer to a 50-mL volumetric flask, and add 30 mL of dilute methanol (1 in 2). Sonicate the flask for about 2 minutes, shake by mechanical means for 30 minutes, and dilute with the same solvent to volume. Filter a portion of the mixture through a suitable filter to obtain a clear filtrate.

**Procedure**—Introduce equal volumes (between 5  $\mu$ L and 25  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at room temperature, by means of a loop injector, adjusting the specimen size and other operating parameters such that the peak obtained with the *Standard preparation* is about 0.6 full scale. Typically, the apparatus is fitted with a 4.6-mm  $\times$  30-cm column packed with packing L1 and is equipped with a UV detector capable of monitoring absorption at 254 nm and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%. Measure the responses of the peaks, at identical retention times, obtained with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Acetate

$C_{24}H_{31}FO_6 \cdot H_2O$  452.51

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-16-methyl-, (11 $\beta$ ,16 $\alpha$ )-monohydrate.

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 21-acetate monohydrate [55812-90-3].

Anhydrous 434.51 [1177-87-3].

» Dexamethasone Acetate contains one molecule of water of hydration or is anhydrous. It contains not less than 97.0 percent and not more than 102.0 percent of  $C_{24}H_{31}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate whether it is hydrous or anhydrous.

**USP Reference standards** (11)—

USP Dexamethasone Acetate RS

#### Identification—

**A: Infrared Absorption** (197M).

**B: Ultraviolet Absorption** (197U)—

*Solution*: 15  $\mu$ g per mL.

*Medium*: methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +82° and +88°.

*Test solution*: 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it in vacuum at 105° for 3 hours: the hydrous form loses between 3.5% and 4.5%, and the anhydrous form not more than 0.4%, of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 0.002%.

#### Chromatographic purity—

**Format buffer**—Dissolve 1.32 g of ammonium formate in 1 L of water, adjust with formic acid to a pH of 3.6, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Formate buffer* and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Test solution**—Transfer about 200 mg of Dexamethasone Acetate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer about 40 mL of this solution to a 100-mL volumetric flask, dilute with *Formate buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5400 theoretical plates.

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone Acetate taken by the formula:

$$100(r_i/r_S)$$

in which  $r_i$  is the peak response for each impurity; and  $r_S$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (550:450). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**pH 6.0 Buffer solution**—Transfer 3 mL of 1 N sodium hydroxide, 138 mL of 0.5 N potassium chloride, and 50 mL of 0.5 M monobasic potassium phosphate to a 1-L volumetric flask, dilute with water to volume, and mix.

**Diluent**—Prepare a mixture of acetonitrile and pH 6.0 Buffer solution (1:1).

**Standard preparation**—Transfer about 25 mg of USP Dexamethasone Acetate RS, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 25 mg of Dexamethasone Acetate, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing 10-μm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, is not less than 2.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>24</sub>H<sub>31</sub>FO<sub>6</sub> in the portion of Dexamethasone Acetate taken by the formula:

$$250C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Acetate Injectable Suspension

» Dexamethasone Acetate Injectable Suspension is a sterile suspension of Dexamethasone Acetate in Water for Injection. It contains an amount of dexamethasone acetate monohydrate (C<sub>24</sub>H<sub>31</sub>FO<sub>6</sub> · H<sub>2</sub>O) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone (C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** <11>—

USP Dexamethasone Acetate RS  
USP Endotoxin RS

**Identification, Infrared Absorption** <197M>—Obtain the test specimen as follows. Transfer the contents of a well-shaken container of Injectable Suspension to a fine-porosity, sintered-glass vacuum filter, filter, and wash with several 10-mL portions of water. Remove the powder from the filter

and allow to air-dry. [NOTE—Do not use heat to dry the specimen. Total or partial dehydration may occur. Use a similar undried preparation of USP Dexamethasone Acetate RS.]

**Bacterial endotoxins** <85>—It contains not more than 21.7 USP Endotoxin Units per mg of dexamethasone acetate.

**pH** <791>: between 5.0 and 7.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay—**

**Mobile phase, pH 6.0 Buffer solution, Diluent, and Chromatographic system**—Proceed as directed in the Assay under *Dexamethasone Acetate*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone Acetate RS in *Diluent* to obtain a solution having a known concentration of about 0.09 mg per mL.

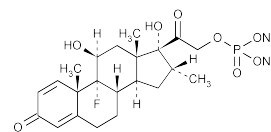
**Assay preparation**—Transfer an accurately measured volume of well-shaken Injectable Suspension, equivalent to about 40 mg of dexamethasone, to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dexamethasone (C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>) in each mL of the Injectable Suspension taken by the formula:

$$(392.47 / 434.51)(500C / V)(r_U / r_S)$$

in which 392.47 and 434.51 are the molecular weights of dexamethasone and anhydrous dexamethasone acetate, respectively; *C* is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation*; *V* is the volume, in mL, of Injectable Suspension taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Sodium Phosphate



C<sub>22</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>8</sub>P 516.40

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonoxy)-, disodium salt, (11β,16α)-9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium salt [2392-39-4].

» Dexamethasone Sodium Phosphate contains not less than 97.0 percent and not more than 102.0 percent of C<sub>22</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>8</sub>P, calculated on the water-free and alcohol-free basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Dexamethasone RS  
USP Dexamethasone Phosphate RS

**Identification—**

**A: pH 9 Buffer with magnesium**—Mix 3.1 g of boric acid and 500 mL of water in a 1-L volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.

**Alkaline phosphatase solution**—Transfer  $95 \pm 5$  mg of alkaline phosphatase enzyme to a 50-mL volumetric flask, dissolve by adding pH 9 Buffer with magnesium to volume, and mix. Prepare this solution fresh daily.

**Standard solution**—Weigh 15 mg of USP Dexamethasone RS into a 5-mL volumetric flask. Dissolve in and dilute with ethyl acetate to volume. [NOTE—Sonication may be required to ensure dissolution.]

**Test solution**—Weigh 20 mg of Dexamethasone Sodium Phosphate into a 15-mL centrifuge tube. Add 5.0 mL of Alkaline phosphatase solution, shake vigorously, and allow to stand for 30 minutes. Add 5.0 mL of ethyl acetate, shake vigorously, centrifuge, and use the upper, ethyl acetate layer.

**Procedure**—Apply 10- $\mu$ L portions of the Test solution and the Standard solution to a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a mobile phase consisting of a mixture of chloroform, methanol, and water (180:15:1) to a distance of three-fourths of the length of the plate. Air-dry the plate and observe under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the Test solution corresponds to that obtained from the Standard solution.

**B:** The residue from the ignition of it meets the requirements of the tests for Phosphate (191) and for Sodium (191).

**Specific rotation** (781S): between  $+74^\circ$  and  $+82^\circ$ , calculated on the water-free and alcohol-free basis.

**Test solution:** 10 mg per mL, in water.

**pH** (791): between 7.5 and 10.5, in a solution (1 in 100).

**Water, Method I** (921)—Determine the water content. The sum of the percentages of water content, and alcohol content, determined as directed in the test for Alcohol, does not exceed 16.0%.

**Limit of phosphate ions—**

**Standard phosphate solution**—Dissolve 143.3 mg of dried monobasic potassium phosphate,  $\text{KH}_2\text{PO}_4$ , in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate ( $\text{PO}_4$ ) in each mL.

**Phosphate reagent A**—Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.

**Phosphate reagent B**—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.

**Procedure**—Dissolve about 50 mg of Dexamethasone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of Phosphate reagent A and Phosphate reagent B, dilute with water to 25 mL, mix, and allow to stand at room temperature for 30 minutes. Similarly and concomitantly, prepare a standard solution, using 5.0 mL of Standard phosphate solution instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the standard solution. The limit is 1.0% of phosphate ( $\text{PO}_4$ ).

**Limit of free dexamethasone—**

**Mobile phase**—Prepare a solution containing 7.5 mL of triethylamine in 1 L of water. Adjust by the addition of phosphoric acid to a pH of 5.4. Prepare a filtered and degassed mixture of 74 parts of the resulting solution with 26 parts of methanol. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in Mobile phase to obtain a solution containing about 0.5 mg per mL. Prepare a second solution by dissolving an accurately weighed quantity of USP Dexamethasone RS in a mixture of methanol and water (1:1) to obtain a solution containing about 50  $\mu$ g per mL. Transfer 10.0 mL of the first solution and 1.0 mL of the second solution to a 100-mL volumetric flask. Dilute with Mobile phase to volume, and mix to obtain a solution having known concentrations of 50  $\mu$ g of USP Dexamethasone Phosphate RS per mL and 0.5  $\mu$ g of USP Dexamethasone RS per mL.

**Test solution**—Transfer about 50 mg of Dexamethasone Sodium Phosphate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix. Further dilute 5.0 mL of this solution with Mobile phase to 50.0 mL.

**System suitability solution**—Prepare a solution in Mobile phase containing in each mL 0.05 mg of USP Dexamethasone Phosphate RS and 0.02 mg of USP Dexamethasone RS.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.5-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L11. The flow rate is about 1.2 mL per minute. Chromatograph the Standard solution and the System suitability solution, record the peak responses as directed for Procedure, and determine the chromatographic characteristics from chromatograms obtained from the System Suitability: the column efficiency determined from the analyte peak is not less than 900 theoretical plates; the tailing factor for the analyte peak is not more than 1.6; the resolution,  $R$ , between dexamethasone phosphate and dexamethasone is not less than 1.8; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the dexamethasone peaks. Calculate the quantity, in  $\mu$ g, of dexamethasone ( $\text{C}_{22}\text{H}_{29}\text{FO}_5$ ) in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$1000C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Dexamethasone RS in the Standard solution; and  $r_U$  and  $r_S$  are the peak responses obtained from the Test solution and the Standard solution, respectively: not more than 1.0% is found.

**Chromatographic purity—**

**Acetate buffer**—Dissolve 7 g of ammonium acetate in 1 L of water, adjust with glacial acetic acid to a pH of 4.0, and mix.

**Solution A**—Prepare a filtered and degassed mixture of methanol, water, and Acetate buffer (7:7:6). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Solution B**—Prepare a filtered and degassed mixture of methanol and Acetate buffer (7:3). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Mobile phase**—Use variable mixtures of Solution A and Solution B as directed for Chromatographic system.

**Test solution**—Transfer about 25 mg of Dexamethasone Sodium Phosphate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with Solution A to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at  $40^\circ$ . The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration
0–3.5	90	10	isocratic
3.5–23.5	90→60	10→40	linear gradient
23.5–34.5	60→5	40→95	linear gradient
34.5–59.5	5	95	isocratic
59.5–60	5→90	95→10	linear gradient

Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the resolution between the major peak and the nearest impurity is not less than 1.0; and the relative standard deviation for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all peaks: not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

**Alcohol content, Method II** (611)—Proceed as directed in the chapter except to use column packing S8 and to use the following modifications.

**Internal standard solution**—Pipet 1 mL of isopropyl alcohol into a 100-mL volumetric flask, add water to volume, and mix.

**Standard stock solution**—Prepare a solution of alcohol in water (1 in 50). Determine the specific gravity at 25° (see *Specific* (841)), and obtain the percentage of C<sub>2</sub>H<sub>5</sub>OH by reference to the *Alcoholometric Table* in the section *Reference Tables*.

**Standard solution**—Into a 10-mL volumetric flask pipet 4 mL of *Standard stock solution* and 5 mL of *Internal standard solution*, add water to volume, and mix. Inject 2 µL of this solution into the gas chromatograph.

**Test solution**—Transfer about 500 mg of Dexamethasone Sodium Phosphate, accurately weighed, into a 10-mL volumetric flask. Pipet 5 mL of *Internal standard solution* into the flask, and mix to dissolve. Add water to volume, and mix. Inject 2 µL of this solution into the gas chromatograph.

**Calculation**—Calculate the percentage of alcohol in the Dexamethasone Sodium Phosphate taken by the formula:

$$4(S / W)(Z / Y)$$

in which  $S$  is the percentage of alcohol in the *Standard stock solution*;  $W$  is the weight, in g, of Dexamethasone Sodium Phosphate used in the *Test solution*; and  $Y$  and  $Z$  are the ratios of the alcohol peak heights to the internal standard peak heights for the *Standard solution* and the *Test solution*, respectively. The content of C<sub>2</sub>H<sub>5</sub>OH is not more than 8.0%.

#### Assay—

**Buffer solution**—Dissolve 7.0 g of ammonium acetate in 1 L of water, adjust with glacial acetic acid to a pH of 4.00 ± 0.05, and mix.

**Solution A**—Prepare a filtered and degassed mixture of methanol, water, and *Buffer solution* (350:350:300).

**Solution B**—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (700:300).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in *Solution A*

to obtain a solution having a known concentration of about 0.92 mg per mL.

**Assay preparation**—Dissolve an accurately weighed quantity of Dexamethasone Sodium Phosphate in *Solution A*, and mix to obtain a solution having a concentration of about 1.0 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The column temperature is maintained at about 40°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration
0–3.5	90	10	isocratic
3.5–24	90→60	10→40	linear gradient
24–35	60→5	40→95	linear gradient
35–60	5	95	isocratic
60–60.1	5→90	95→10	linear gradient
60.1–65	90	10	isocratic

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%. Chromatograph the *Assay preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between dexamethasone phosphate and the nearest impurity eluting after it is not less than 1.0.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>8</sub>P in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$(516.41 / 472.45)C(r_U / r_S)$$

in which 516.41 and 472.45 are the molecular weights of dexamethasone sodium phosphate and dexamethasone phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Dexamethasone Phosphate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Sodium Phosphate Inhalation Aerosol

» Dexamethasone Sodium Phosphate Inhalation Aerosol is a suspension, in suitable propellants and alcohol, in a pressurized container, of dexamethasone sodium phosphate (C<sub>22</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>8</sub>P) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone phosphate (C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P).

**Packaging and storage**—Preserve in tight, pressurized containers, and avoid exposure to excessive heat.

#### USP Reference standards (11)—

USP Dexamethasone RS

**Identification**—Prepare a pH 9.0 buffer solution by dissolving 3.1 g of boric acid, 203 mg of magnesium chloride, and 860 mg of sodium hydroxide in water to make 1000 mL. Dissolve 50 mg of alkaline phosphatase enzyme in 50 mL of the pH 9.0 buffer solution, and transfer 5 mL of

the resulting solution to a glass-stoppered, 50-mL tube containing 5 mL of the *Assay preparation* prepared as directed in the *Assay*. Incubate at 37° for 45 minutes, add 25 mL of methylene chloride, and shake for 2 minutes: the methylene chloride extract so obtained responds to the *Identification* test under *Dexamethasone Sodium Phosphate Injection*, beginning with "Evaporate 15 mL of the methylene chloride extract."

**Alcohol content**, *Method II* (611): between 1.7% and 2.3% of C<sub>2</sub>H<sub>5</sub>OH.

**Delivered dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

PROCEDURE FOR DOSE UNIFORMITY—

**Standard solution**—Transfer about 10 mg of USP Dexamethasone RS, accurately weighed, to a 10-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix to obtain a solution having a known concentration of about 10 µg per mL.

**Test solution**—Discharge the minimum recommended dose into the sampling apparatus, and detach the inhaler as directed. Rinse the apparatus (filter and interior) with two 5.0-mL portions of 0.1 N sulfuric acid, and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube containing 15 mL of methylene chloride that was previously chilled in a dry ice-acetone bath for a few minutes. Insert the stopper in the centrifuge tube, and shake cautiously, releasing the pressure occasionally. Allow the phases to separate, and equilibrate to room temperature. The aqueous phase is the *Test solution*.

**Procedure**—Transfer the *Test solution* and 10.0 mL of the *Standard solution* into separate flasks. Add 2.0 mL of 0.1 N sulfuric acid to each, and swirl to mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in µg, of dexamethasone phosphate (C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P) contained in the minimum dose by the formula:

$$10(472.45 / 392.47)(CN)(A_U / A_S)$$

in which 472.45 and 392.47 are the molecular weights of dexamethasone phosphate and dexamethasone, respectively; C is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard solution*; N is the number of sprays discharged to obtain the minimum recommended dose; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively.

#### Assay—

**Standard preparation**—Transfer about 40 mg of USP Dexamethasone RS, accurately weighed, to a 50-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Weigh accurately a filled Inhalation Aerosol container, and record the weight (W<sub>1</sub>). Place the container in a dry ice-acetone bath, and cool for 60 minutes. Remove the container from the bath, and carefully remove the valve with wire cutters, taking precautions to save all pieces of the valve and cap. With the aid of four 5-mL portions of 0.1 N sulfuric acid, transfer the contents of the container to a beaker previously chilled in the bath. Dry the rinsed empty container and all of its parts in an oven at 105° for 2 hours, cool, and weigh (W<sub>2</sub>). Allow the contents of the beaker to warm to room temperature. After the bulk of the propellant has evaporated, quantitatively transfer the contents of the beaker, with the aid of several mL of 0.1 N

sulfuric acid, to a 200-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix. Transfer about 20 mL of this solution to a centrifuge tube, add 10 mL of methylene chloride, shake vigorously for 1 minute, and centrifuge. Pipet 10 mL of the clear supernatant into a 100-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of dexamethasone phosphate (C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P) in each g of Inhalation Aerosol taken by the formula:

$$2(472.45 / 392.47)(A_U / A_S)[C / (W_1 - W_2)]$$

in which 472.45 and 392.47 are the molecular weights of dexamethasone phosphate and dexamethasone, respectively, A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, C is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard preparation*, and W<sub>1</sub> and W<sub>2</sub> are the weights, in g, as previously defined.

## Dexamethasone Sodium Phosphate Cream

» Dexamethasone Sodium Phosphate Cream contains an amount of dexamethasone sodium phosphate (C<sub>22</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>8</sub>P) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dexamethasone phosphate (C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P).

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

**USP Reference standards** (11)—

USP Dexamethasone RS

USP Dexamethasone Phosphate RS

**Identification**—Prepare a pH 9.0 buffer solution by dissolving 3.1 g of boric acid, 203 mg of magnesium chloride, and 860 mg of sodium hydroxide in water to make 1000 mL. Dissolve 50 mg of alkaline phosphatase enzyme in 50 mL of the pH 9.0 buffer solution, and transfer 5 mL of the resulting solution to a glass-stoppered, 50-mL tube containing 5 mL of the *Assay preparation*, prepared as directed in the *Assay*. Incubate at 37° for 45 minutes, then add 25 mL of methylene chloride, and shake for 2 minutes. The methylene chloride extract so obtained responds to the *Identification* test under *Dexamethasone Sodium Phosphate Injection*, beginning with "Evaporate 15 mL of the methylene chloride extract."

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

#### Assay—

**Alcohol-aqueous phosphate buffer**—Dissolve 0.29 g of dibasic sodium phosphate in 450 mL of water, add 550 mL of alcohol, and mix.

**0.05 M Phosphate buffer**—In a 1-L volumetric flask, dissolve 6.9 g of monobasic sodium phosphate in 500 mL of water, dilute with water to volume, and mix.

**Mobile phase**—Prepare a suitable degassed solution of methanol and 0.05 M Phosphate buffer (52:48) which, at ambient temperature and at a flow rate of 1.5 mL per min-

ute, gives a retention time of about 8.5 minutes for dexamethasone phosphate.

**Standard preparation**—Using an accurately weighed quantity of USP Dexamethasone Phosphate RS, prepare a solution in *Alcohol-aqueous phosphate buffer* having a known concentration of about 30 µg per mL. Prepare this solution fresh.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 3 mg of dexamethasone phosphate, to a 150-mL beaker. Add 65 mL of *Alcohol-aqueous phosphate buffer*, and heat just to boiling. Pour the contents of the beaker into a 125-mL separator containing 45 mL of isooctane. After shaking for 1 minute, decant the lower layer into a 100-mL volumetric flask with the aid of a glass funnel. Rinse the 150-mL beaker with two 15-mL portions of *Alcohol-aqueous phosphate buffer*, extracting the remaining isooctane in the separator with each portion and decanting the lower layer from each extraction into the 100-mL volumetric flask. Dilute with *Alcohol-aqueous phosphate buffer* to volume, and mix. Filter through a membrane filter before injecting.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%.

**Procedure**—By means of a suitable sampling valve, separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P in the portion of Cream taken by the formula:

$$0.1 C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Dexamethasone Phosphate RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses at equivalent retention times obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Sodium Phosphate Injection

» Dexamethasone Sodium Phosphate Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dexamethasone phosphate (C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P), present as the disodium salt.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** <11>—

USP Dexamethasone RS

USP Dexamethasone Phosphate RS

USP Endotoxin RS

**Identification**—Pipet a volume of Injection, equivalent to 10 mg of dexamethasone phosphate, into a 100-mL volumetric flask, add water to volume, and mix. Pipet 5 mL of this solution into a 125-mL separator, and wash with two 10-mL portions of water-washed methylene chloride, discarding the washings. Transfer the solution into a glass-stoppered, 50-mL tube, and add 5 mL of alkaline phosphatase

solution, prepared by dissolving 50 mg of alkaline phosphatase enzyme in 50 mL of *pH 9 Buffer with magnesium* (prepared as directed in *Identification test A* under *Dexamethasone Sodium Phosphate*). Allow to stand at 37° for 45 minutes, and extract with 25 mL of methylene chloride. Evaporate 15 mL of the methylene chloride extract on a steam bath to dryness, and dissolve the residue in 1 mL of methylene chloride. Apply 5 µL of this solution and 5 µL of a solution of USP Dexamethasone RS in methylene chloride containing 300 µg per mL to a 20- × 20-cm, thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a tank completely lined with a strip of filter paper, using a solvent system consisting of a mixture of 50 parts of chloroform, 50 parts of acetone, and 1 part of water, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, mark the solvent front, and allow the spots to dry. Spray the plate with dilute sulfuric acid (1 in 2), and heat at 105° until brown or black spots appear: the  $R_F$  value of the principal spot obtained from the test specimen corresponds to that obtained from the Reference Standard.

**Bacterial endotoxins** <85>—It contains not more than 31.3 USP Endotoxin Units per mg of dexamethasone phosphate.

**pH** <791>: between 7.0 and 8.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

**Mobile phase**—Prepare a suitable degassed solution of 0.01 M monobasic potassium phosphate in a mixture of methanol and water (1:1) which, at ambient temperature and at a flow rate of about 1.6 mL per minute, gives a retention time of about 5 minutes for dexamethasone phosphate.

**Standard preparation**—[NOTE—Prepare this solution at the time of use.] Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in *Mobile phase* to obtain a solution having a known concentration of about 80 µg per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 8 mg of dexamethasone phosphate, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%.

**Procedure**—By means of a suitable sampling valve, separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>30</sub>FO<sub>8</sub>P in each mL of the Injection taken by the formula:

$$0.1(C / V)(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Dexamethasone Phosphate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and  $r_U$  and  $r_S$  are the peak responses at equivalent retention times obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Sodium Phosphate Ophthalmic Ointment

» Dexamethasone Sodium Phosphate Ophthalmic Ointment is a sterile ointment containing an amount of dexamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dexamethasone phosphate ( $C_{22}H_{30}FO_8P$ ).

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

### USP Reference standards (11)—

USP Dexamethasone RS

USP Dexamethasone Phosphate RS

**Identification**—The *Assay preparation*, prepared as directed in the *Assay*, responds to the *Identification* test under *Dexamethasone Sodium Phosphate Cream*.

**Minimum fill** (755): meets the requirements.

**Sterility** (71): meets the requirements.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

### Assay—

*Alcohol-aqueous phosphate buffer, 0.05 M Phosphate buffer, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dexamethasone Sodium Phosphate Cream*.

*Assay preparation*—Using an accurately weighed portion of Ophthalmic Ointment, prepare as directed in the *Assay* under *Dexamethasone Sodium Phosphate Cream*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dexamethasone Sodium Phosphate Cream*. Calculate the quantity, in mg, of  $C_{22}H_{30}FO_8P$  in the portion of Ophthalmic Ointment taken by the formula:

$$0.1C(r_U / r_S).$$

## Dexamethasone Sodium Phosphate Ophthalmic Solution

» Dexamethasone Sodium Phosphate Ophthalmic Solution is a sterile, aqueous solution of Dexamethasone Sodium Phosphate. It contains an amount of dexamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dexamethasone phosphate ( $C_{22}H_{30}FO_8P$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Dexamethasone RS

USP Dexamethasone Phosphate RS

**Identification**—The *Assay preparation*, prepared as directed in the *Assay*, responds to the *Identification* test under *Dexamethasone Sodium Phosphate Cream*.

**pH** (791): between 6.6 and 7.8.

**Sterility** (71): meets the requirements.

### Assay—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dexamethasone Sodium Phosphate Injection*.

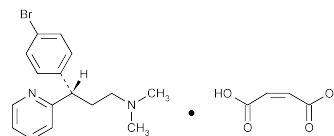
*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 8 mg of dexamethasone phosphate, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dexamethasone Sodium Phosphate Injection*. Calculate the quantity, in mg, of  $C_{22}H_{30}FO_8P$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.1(C / V)(r_U / r_S)$$

in which V is the volume, in mL, of Ophthalmic Solution taken.

## Dexbrompheniramine Maleate



$C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  435.31

2-Pyridinepropanamine,  $\gamma$ -(4-bromophenyl)-*N,N*-dimethyl-, (S)-, (Z)-2-butenedioate (1:1).

(+)-2-[*p*-Bromo- $\alpha$ -[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1) [2391-03-9].

» Dexbrompheniramine Maleate contains not less than 98.0 percent and not more than 100.5 percent of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Dexbrompheniramine Maleate RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 35  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 261 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +35.0° and +38.5°.

*Test solution:* 50 mg per mL, in dimethylformamide.

**Loss on drying** (731)—Dry it at 65° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

### Related compounds—

*Test solution*—Dissolve about 200 mg of Dexbrompheniramine Maleate in 5 mL of methylene chloride, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.2-m glass column containing 3% phase G3 on support S1AB. The column temperature is maintained at about 190°, and the injection port and detector temperatures are both maintained at about 250°. The carrier gas is dry helium, flowing at a rate adjusted to obtain a retention time of 6 to 7 minutes for the main peak.



Chromatograph the *Test solution*, record the chromatogram, and determine the peak area as directed under *Procedure*: the tailing factor for the dexbrompheniramine maleate peak is not more than 1.8.

*Procedure*—Inject a volume (about 1  $\mu$ L) of the *Test solution* into the chromatograph. Record the chromatogram for a total time of not less than twice the retention time of the dexbrompheniramine peak, and measure the areas of the peaks. The total relative area of all extraneous peaks (except that of the solvent peak and maleic acid, if observed) does not exceed 2.0%.

**Assay**—Dissolve about 400 mg of Dexbrompheniramine Maleate, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.77 mg of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ .

## Dexbrompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution

» Dexbrompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of dexbrompheniramine maleate ( $C_{10}H_{15}BrN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

### USP Reference standards (11)—

USP Dexbrompheniramine Maleate RS

USP Pseudoephedrine Sulfate RS

### Identification—

**A:** The retention time of the major peak for dexbrompheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** The retention time of the major peak for pseudoephedrine sulfate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** A solution of it responds to the test for *Sulfate* (191).

**D:** Transfer a volume of Oral Solution, equivalent to about 6 mg of dexbrompheniramine maleate, to a separatory funnel, add 0.5 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare a Standard solution in methanol containing 1.2 mg of USP Dexbrompheniramine Maleate RS and a second Standard solution in methanol containing 9 mg of USP Pseudoephedrine Sulfate RS per mL. Separately apply 5  $\mu$ L of the test solution and each Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl ether, methanol, and ammonium hydroxide (16:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the respective Standard solutions.

### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

### Assay—

*Mobile phase*—Prepare a mixture of water, acetonitrile, methanol, and tetrahydrofuran (55:32:8:5). Transfer 0.1 mL of phosphoric acid, followed by 0.433 g of sodium lauryl sulfate, to each 100 mL of this mixture, and mix. Adjust with ammonium hydroxide to a pH of  $3.50 \pm 0.05$ , filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—The pH of the *Mobile phase* is critical, and may cause differences of 1 to 4 minutes in the retention times of the internal standard and dexbrompheniramine.]

*Internal standard solution*—Dissolve an accurately weighed quantity of naphazoline hydrochloride in *Mobile phase* to obtain a solution containing 0.5 mg per mL.

*Dexbrompheniramine standard solution*—Dissolve an accurately weighed quantity of USP Dexbrompheniramine Maleate RS in *Mobile phase* to obtain a solution having a known concentration of about 6000  $\mu$ g per mL,  $J$  being the ratio of the labeled amount, in mg, of dexbrompheniramine maleate to the labeled amount, in mg, of pseudoephedrine sulfate per mL of the Oral Solution.

*Standard preparation*—Transfer about 30 mg of USP Pseudoephedrine Sulfate RS, accurately weighed, to a 25-mL volumetric flask, add 5.0 mL each of *Dexbrompheniramine standard solution* and *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 1.2  $J$  mg of USP Dexbrompheniramine Maleate RS per mL and about 1.2 mg of USP Pseudoephedrine Sulfate RS per mL.

*Assay preparation*—Using a “to contain” pipet, transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of pseudoephedrine sulfate, to a 25-mL volumetric flask. Rinse the pipet with about 5 mL of *Mobile phase*, collecting the rinsing in the volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

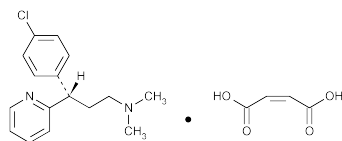
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for pseudoephedrine, 1.5 for naphazoline, and 2.5 for dexbrompheniramine; the resolution,  $R$ , between the pseudoephedrine and naphazoline peaks is not less than 3; the resolution,  $R$ , between the dexbrompheniramine and naphazoline peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg per mL, of dexbrompheniramine maleate ( $C_{10}H_{15}BrN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in the portion of Oral Solution taken by the formula:

$$25CV(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the corresponding analyte to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexchlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  390.86

2-Pyridinepropanamine,  $\gamma$ -(4-chlorophenyl)-*N,N*-dimethyl-, (S)-, (Z)-2-butenedioate (1:1).

(+)-2-[*p*-Chloro- $\alpha$ -[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1) [2438-32-6].

» Dexchlorpheniramine Maleate, dried at 65° for 4 hours, contains not less than 98.0 percent and not more than 100.5 percent of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Dexchlorpheniramine Maleate RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 40  $\mu$ g per mL.

*Medium:* water.

**Melting range**, Class I (741): between 110° and 115°.

**Specific rotation** (781S): between +39.5° and +43.0°.

*Test solution:* 50 mg per mL, in dimethylformamide.

**pH** (791): between 4.0 and 5.0, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 65° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

### Related compounds—

*Test solution*—Dissolve about 200 mg of Dexchlorpheniramine Maleate in 5 mL of methylene chloride, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 1.2-m  $\times$  4-mm glass column containing 3% phase G3 on support S1AB. The column temperature is maintained at about 190°, and the injection port and detector temperatures are both maintained at about 250°. The carrier gas is dry helium, flowing at a rate adjusted to obtain a retention time of 4 to 5 minutes for the main peak. Chromatograph the *Test solution*, record the chromatogram, and determine the peak area as directed under *Procedure*: the tailing factor for the dexchlorpheniramine maleate peak is not more than 1.8.

*Procedure*—Inject a volume (about 1  $\mu$ L) of the *Test solution* into the chromatograph. Record the chromatogram for a total time of not less than twice the retention time of the dexchlorpheniramine peak, and measure the areas of the peaks. The total relative area of all extraneous peaks (except that of the solvent peak and maleic acid, if observed) does not exceed 2.0%.

**Assay**—Dissolve about 400 mg of Dexchlorpheniramine Maleate, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.54 mg of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

## Dexchlorpheniramine Maleate Oral Solution

» Dexchlorpheniramine Maleate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexchlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Dexchlorpheniramine Maleate RS

### Identification—

**A:** Evaporate the remaining extract from the Assay on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, and mix: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is between +0.06° and +0.11° (distinction from chlorpheniramine maleate).

**B:** Ultraviolet Absorption (197U): Assay preparation compared to Standard preparation from Assay.

**Alcohol content** (611): between 5.0% and 7.0% of  $C_2H_5OH$ .

### Assay—

*Standard preparation*—Transfer about 40 mg of USP Dexchlorpheniramine Maleate RS, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with two 50-mL portions of solvent hexane, shaking each portion for 2 minutes before separating the phases, and combining the hexane extracts in a second separator. Extract the hexane solution with two 40-mL portions of dilute hydrochloric acid (1 in 120), combine the acid extracts in a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 120) to volume, and mix. Filter the solution into a glass-stoppered conical flask, discarding the first few mL of the filtrate. The concentration of USP Dexchlorpheniramine Maleate RS in the *Standard preparation* is about 40  $\mu$ g per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 40 mg of dexchlorpheniramine maleate, to a 250-mL separator, using a pipet calibrated “to contain” the required volume. Rinse the pipet with small portions of water, add the rinsings to the separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with five 70-mL portions of solvent hexane, combine the hexane extracts in a 500-mL separator, and wash the hexane solution with two 10-mL portions of sodium hydroxide solution (1 in 250). Extract the combined alkaline washings with two 20-mL portions of solvent hexane, and add these extracts to the bulk of the alkali-washed hexane solution. Filter the hexane solution through a pledget of cotton that previously has been saturated with solvent hexane into a 500-mL volumetric flask, rinse the separator with portions of solvent hexane, pass the rinsings through the filter to add to volume, and mix. Transfer 50.0 mL of this solution to a separator (retain the remaining extract for *Identification* test A), and proceed as directed for *Standard preparation*, beginning with “Extract the hexane solution.”

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of dexchlorphenir-

amine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each mL of the Oral Solution taken by the formula:

$$(C / V)(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Dexchlorpheniramine Maleate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Dexchlorpheniramine Maleate Tablets

» Dexchlorpheniramine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Dexchlorpheniramine Maleate RS

### Identification—

A: Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

B: Shake a quantity of finely powdered Tablets, equivalent to about 150 mg of dexchlorpheniramine maleate, with 100 mL of 1 N acetic acid for 10 minutes, filter through a sintered-glass funnel into a suitable vessel, adjust the filtrate with sodium hydroxide solution (1 in 10) to a pH of 11, and extract the solution with six 100-mL portions of solvent hexane, filtering each hexane extract using suitable means to effect separation of the hexane layer from the aqueous layer. Concentrate the combined extracts on a steam bath to a small volume, transfer to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, mix, and centrifuge if necessary: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is between  $+0.24^\circ$  and  $+0.35^\circ$  (*distinction from chlorpheniramine maleate*).

### Dissolution (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Determine the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved, using the following procedure.

**Internal standard solution**—Prepare a solution of Dexbrompheniramine Maleate in water having a final concentration of about 90  $\mu\text{g}$  per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Dexchlorpheniramine Maleate RS in water, and dilute quantitatively and stepwise with water to obtain a stock solution having a known concentration of about 12.5  $\mu\text{g}$  per mL. Pipet 5 mL of this stock solution into a 50-mL centrifuge tube, add 10.0 mL of water and 1.0 mL of *Internal standard solution*, and mix. Adjust with sodium hydroxide solution (1 in 2) to a pH of  $11 \pm 0.1$ , and add 3.0 mL of chromatographic solvent hexane. Insert the stopper in the tube, shake by mechanical means for 3 minutes, centrifuge, and use the clear supernatant hexane layer.

**Test solution**—Pipet 15 mL of a portion of the solution under test into a 50-mL centrifuge tube, add 1.0 mL of *Internal standard solution*, and mix. Proceed as directed for *Standard solution*, beginning with "Adjust with sodium hydroxide solution (1 in 2)."

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm  $\times$  1.8-m column that contains a packing consisting of 1.2 percent phase G16 and 0.5 percent potassium hydroxide on support S1AB. The carrier gas is helium maintained at a flow rate of about 60 mL per minute. The column, injector, and detector temperatures are maintained at 205°, 250°, and 250°, respectively. Chromatograph replicate injections of the *Standard solution*, and record the peak response as directed for *Procedure*: the relative retention times are about 0.7 for dexchlorpheniramine and 1.0 for dexbrompheniramine; the resolution,  $R$ , between dexchlorpheniramine and dexbrompheniramine is not less than 1.9; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 2  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved by comparison of the peak response ratios.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

**Standard preparation**—Prepare as directed in the *Assay under Dexchlorpheniramine Maleate Oral Solution*. The concentration of USP Dexchlorpheniramine Maleate RS in the *Standard preparation* is about 40  $\mu\text{g}$  per mL.

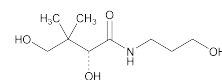
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 8 mg of dexchlorpheniramine maleate, to a 250-mL separator, mix with 50 mL of water for 10 minutes, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75-mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50-mL portions of dilute hydrochloric acid (1 in 120), combining the acid extracts in a 200-mL volumetric flask. Add dilute hydrochloric acid (1 in 120) to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of dexchlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$0.2C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Dexchlorpheniramine Maleate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Dexpanthenol



$C_9H_{19}NO_4$  205.25  
Butanamide, 2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl-, (R)-;  
D-(-)-2,4-Dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutyramide [81-13-0].

**DEFINITION**

Dexpanthenol contains NLT 98.0% and NMT 102.0% of dexpanthenol ( $C_9H_{19}NO_4$ ), calculated on the anhydrous basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197F)• **B.**

**Sample solution:** 100 mg/mL of Dexpanthenol

**Analysis:** To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.

**Acceptance criteria:** A deep blue color develops.

• **C.**

**Sample solution:** 10 mg/mL of Dexpanthenol

**Analysis:** To 1 mL of the *Sample solution* add 1 mL of 1 N hydrochloric acid, and heat on a steam bath for 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, and add 5 mL of 1 N sodium hydroxide. Allow to stand for 5 min, then adjust with 1 N hydrochloric acid to a pH of 2.5–3.0, and add 1 drop of ferric chloride TS.

**Acceptance criteria:** A purplish red color develops.

**ASSAY**• **PROCEDURE**

**0.1 M potassium biphthalate:** Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.

**Sample:** 400 mg of Dexpanthenol

**Blank:** Proceed as directed in the *Analysis*, omitting the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N perchloric acid VS

**Back-titrant:** 0.1 M potassium biphthalate

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask, add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination.

Calculate the percentage of dexpanthenol ( $C_9H_{19}NO_4$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

$V_B$  = *Back-titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back-titrant* volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 205.3 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**• **RESIDUE ON IGNITION** (281): NMT 0.1%• **LIMIT OF AMINOPROPANOL**

**Sample:** 5 g of Dexpanthenol

**Blank:** 10 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 10 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination. Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 75.11 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* (781S)

**Sample solution:** 50 mg/mL in water

**Acceptance criteria:** +29.0° to +31.5°

• **REFRACTIVE INDEX** (831): 1.495–1.502, at 20°• **WATER DETERMINATION**, *Method I* (921): NMT 1.0%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS** (11)

USP Dexpanthenol RS

**Dexpanthenol Preparation****DEFINITION**

Dexpanthenol Preparation contains NLT 94.5% and NMT 98.5% of dexpanthenol ( $C_9H_{19}NO_4$ ), and NLT 2.7% and NMT 4.2% of pantolactone, both calculated on the anhydrous basis.

**IDENTIFICATION**• **A.** The IR absorption spectrum of a thin film of Preparation exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dexpanthenol RS, except that there is an additional maximum at 5.6  $\mu\text{m}$  due to pantolactone.• **B.**

**Sample solution:** 100 mg/mL of Preparation

**Analysis:** To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.

**Acceptance criteria:** A deep blue color develops.

**COMPOSITION**• **CONTENT OF DEXPANTHENOL**

**0.1 M potassium biphthalate:** Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.

**Sample:** 400 mg of Preparation

**Blank:** Proceed as directed in the *Analysis*, omitting the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N perchloric acid VS

**Back-titrant:** 0.1 M potassium biphthalate

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture

from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination. Calculate the percentage of dexpanthenol ( $C_9H_{19}NO_4$ ) in the *Sample* taken:

$$\text{Result} = \{(V_B - V_S) \times M \times F\} / W \times 100$$

$V_B$  = *Back-titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back-titrant* volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 205.3 mg/mM

$W$  = weight of the *Sample* (mg)

**Acceptance criteria:** 94.5%–98.5% on the anhydrous basis

#### • CONTENT OF PANTOLACTONE

**Internal standard solution:** 100 mg/mL of 2,6-dimethylphenol in toluene

**Standard stock solution:** 10 mg/mL of USP Pantolactone RS in methylene chloride

**Standard solution:** Pipet 0.4 mL of *Standard stock solution* into a suitable small vial. Evaporate the solvent by means of a steady stream of dry air, and add 50.0  $\mu$ L of *Internal standard solution*. Add 1 mL of a mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane (9:3:1), immediately close the vial, and shake vigorously for 30 s.

**Sample solution:** Transfer 100 mg of Preparation to a suitable small vial, and proceed as directed for the *Standard solution*, beginning with "add 50.0  $\mu$ L of *Internal standard solution*".

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2.0-mm  $\times$  1.8-m, packed with 5% liquid phase G2 on support S1A

**Temperature**

**Column:** 170°, isothermal

**Injector:** 180°

**Flow rate:** Using a suitable carrier gas, adjust the flow rate so that the derivatized pantolactone elutes in 4 min.

**Injection size:** 0.5  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention times of the derivatized pantolactone and derivatized internal standard are 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between the derivatized pantolactone and derivatized internal standard

**Relative standard deviation:** NMT 2.0% for the ratios of the derivatized pantolactone peak response to the derivatized internal standard peak response

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of pantolactone in the portion of Preparation taken:

$$\text{Result} = (R_U/R_S) \times (V/W) \times C_S \times 100$$

$R_U$  = internal standard ratio (peak response of derivatized pantolactone/peak response of derivatized internal standard) from the *Sample solution*

$R_S$  = internal standard ratio (peak response of derivatized pantolactone/peak response of derivatized internal standard) from the *Standard solution*

$V$  = volume of the *Standard stock solution* taken to prepare the *Standard solution*, 0.4 mL

$W$  = weight of Preparation taken to prepare the *Sample solution* (mg)

$C_S$  = concentration of USP Pantolactone RS in the *Standard stock solution* (mg/mL)

**Acceptance criteria:** 2.7%–4.2% on the anhydrous basis

#### IMPURITIES

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

• **LIMIT OF AMINOPROPANOL**

**Sample:** 5 g of Preparation

**Blank:** 10 mL of water

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 10 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination. Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F\} / W \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 75.11 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** NMT 1.0%

#### SPECIFIC TESTS

• **OPTICAL ROTATION**, *Specific Rotation* <781S>

**Sample solution:** 50 mg/mL in water

**Acceptance criteria:** +27.5° to +30.0°

• **REFRACTIVE INDEX** <831>: 1.495–1.502, at 20°

• **WATER DETERMINATION**, *Method I* <921>: NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** <11>

USP Dexpanthenol RS

USP Pantolactone RS

## Dextran 1

» Dextran 1 is a low molecular weight fraction of dextran, consisting of a mixture of isomalto-oligosaccharides. It is obtained by controlled hydrolysis and fractionation of dextrans produced by fermentation of *Leuconostoc mesenteroides* (strain NRRL B-512; CIP 78.59, or its sub-strains, for example *L. mesenteroides* B-512F; NCTC, 10817), in the presence of sucrose. It is a glucose polymer in which the linkages between glucose units are almost exclusively  $\alpha$ -1,6. Its weight-average molecular weight is about 1000.

**Packaging and storage**—Store in well-closed containers at a temperature between 4° and 30°.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** <11>—

USP Dextran 1 RS

USP Endotoxin RS

**Identification—**

**A: Infrared Absorption** (197K)—To 1 to 2 mg each of USP Dextran 1 RS and the sample add one to two drops of water, grind in an agate mortar for 1 to 2 minutes, add about 300 mg of potassium bromide, and mix to a slurry. [NOTE—Do not grind.] Dry under vacuum at 40° for 15 minutes, and if it is not dry, continue drying for another 15 minutes. Crush the residue, prepare a disk, and run the IR spectrum with a blank potassium bromide disk in the reference beam.

**B:** It meets the requirements of the test for *Molecular weight distribution and average molecular weight*.

**Absorbance** (851)—The absorbance of a 15% solution in water at 375 nm is not more than 0.12, water being used as the blank.

**Specific rotation** (781S): between +148° and +164° at 20°, for a solution in water, on the dried basis (dry at 70° under vacuum to constant weight), and corrected for the content of sodium chloride.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 10<sup>2</sup> cfu per g, determined by plate count; and the total combined molds and yeasts count does not exceed 10 cfu per g.

**Bacterial endotoxins** (85) (where it is labeled as intended for use in the preparation of injectables): not more than 25.0 USP Endotoxin Units per g.

**pH** (791): between 4.5 and 7.0, in a 15% solution in water.

**Loss on drying** (731)—Dry it at 100° to 105° for 5 hours: it loses not more than 5.0% of its weight.

**Heavy metals, Method II** (231): not more than 5 µg per g.

**Limit of alcohol and related impurities—**

**Test solution**—Proceed as directed for *Test solution* in the test for *Limit of alcohol and related impurities* under *Dextran 40*, except to use 5.0 g of Dextran 1.

**Standard solution, Chromatographic system, and Procedure**—Proceed as directed in the test for *Limit of alcohol and related impurities* under *Dextran 40*. The total area of peaks from impurities in the *Test solution* does not exceed the area of the *n*-propyl alcohol solution peak.

**Limit of sodium chloride**—Dissolve 5 g of Dextran 1, accurately weighed, in 100 mL of water. Add 0.2 mL of potassium chromate TS, and titrate with 0.1 N silver nitrate VS (see *Titrimetry* (541)). Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride: not more than 1.5% of sodium chloride is found.

**Limit of nitrogenous impurities** (461) (where it is labeled as intended for use in the preparation of injectables)—

**Sulfate solution**—To 1000 mL sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°.

[NOTE—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting proportions accordingly.]

**Indicator**—Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.

**Procedure**—Transfer 0.2 g Dextran 1, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*. Heat until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, cautiously add 30 mL of water, mix, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask with three 5-mL portions of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and start steam distillation immediately. Receive the distillate in 1 mL of *Indicator* and sufficient water to cover the end of the condensing tube. Upon completion of the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate.

Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet. Perform a blank determination, and make any necessary correction. The corrected volume of 0.010 N hydrochloric acid required to change the color does not exceed 0.15 mL (110 ppm of nitrogen).

**Molecular weight distribution and average molecular weight—**

**Mobile phase**—Prepare a filtered and degassed solution of sodium chloride containing 2.9 g per L.

**Calibration solution**—Prepare a solution containing about 0.45 mg of isomaltotriose (3 glucose units) and 0.60 mg of sodium chloride per mL.

**Reference solution**—Prepare a solution of USP Dextran 1 RS in *Mobile phase* containing 6.0 to 6.5 mg per mL.

**Test solution**—Prepare a solution of Dextran 1 in *Mobile phase* containing 6.0 to 6.5 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a differential refractive index detector and two 10-mm × 30-cm columns in series that contain packing L54 and are maintained at 20–25°. The flow rate is 0.07 to 0.08 mL per minute, maintained constant to ±1%.

**Procedure**—Inject about 100 µL of the *Calibration solution*, record the chromatogram, and note the retention times of the peaks. Separately inject equal volumes (about 100 µL) of the *Reference solution* and *Test solution*, and record the chromatograms. Using the retention times in the chromatogram of *Calibration solution*, identify the peaks due to isomaltotriose and sodium chloride in the chromatograms of *Reference solution* and *Test solution*. Disregard the peak due to sodium chloride in *Reference solution* and *Test solution*. Calculate the weight-average molecular weight,  $M_w$ , by the formula:

$$\sum W_i M_i$$

in which  $W_i$  is the weight proportion of oligosaccharide  $i$ ; and  $M_i$  is the molecular weight of oligosaccharide  $i$ . Use the following molecular weight values for calculation:

Glucose	180
Isomaltose	342
Isomaltotriose	504
Isomaltotetraose	666
Isomaltopentaose	828
Isomaltohexaose	990
Isomaltoheptaose	1152
Isomaltooctaose	1314
Isomaltnonaose	1476
Isomaltodecaose	1638
Isomaltoundecaose	1800
Isomaltododecaose	1962
Isomaltotridecaose	2124
Isomaltotetradecaose	2286
Isomaltopentadecaose	2448
Isomaltohexadecaose	2610
Isomaltoheptadecaose	2772
Isomaltooctadecaose	2934
Isomaltnonadecaose	3096

Calculate the amounts of the fractions with fewer than 3 and with more than 9 glucose units for the *Reference solution* and the *Test solution*: the  $M_w$  and amounts of the fractions obtained for the *Reference solution* are within the values stated in the data sheet that accompanies USP Dextran 1 RS. The  $M_w$  of Dextran 1 is between 850 and 1150. The fraction with fewer than 3 units of glucose is less than 15%,

and the fraction with more than 9 units of glucose is less than 20%.

## Dextran 40

Dextrans.

Dextrans [9004-54-0].

» Dextran 40 is derived by controlled hydrolysis and fractionation of polysaccharides elaborated by the fermentative action of certain strains of *Leuconostoc mesenteroides* (NRRL, B.512 F; NCTC, 10817) on a sucrose substrate. It is a glucose polymer in which the linkages between glucose units are almost entirely of the  $\alpha$ -1:6 type. Its weight average molecular weight is in the 35,000 to 45,000 range.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards (11)—

USP Dextran 40 RS  
USP Dextran 4 Calibration RS  
USP Dextran 10 Calibration RS  
USP Dextran 40 Calibration RS  
USP Dextran 70 Calibration RS  
USP Dextran 250 Calibration RS  
USP Dextran Vo Marker RS  
USP Dextran 40 System Suitability RS  
USP Endotoxin RS

**Color of solution**—The absorbance of a solution in water (1 in 10), measured in a 4-cm cell determined at 375 nm against a water blank, is not greater than 0.20.

### Identification—

**A: Infrared Absorption** (197K).

**B:** Prepare four *Test solutions* of Dextran 40 in water, in such a manner that the concentrations are accurately known and approximately evenly distributed in the range of 2% to 0.5%. Using a capillary tube viscometer having dimensions such that the flow time of water is not less than 100 seconds, measure the flow times of water and of the *Test solution* at 20°. Calculate the viscosity numbers of each of the *Test solutions* by the formula:

$$\{\ln[(R_D)(t/t_0)]\} / C$$

in which  $R_D$  is the ratio of the density of the individual *Test solution* to that of water;  $t$  and  $t_0$  are the flow times for the *Test solution* and water, respectively; and  $C$  is the concentration, in g per mL, of Dextran 40 in the *Test solution*. Plot the viscosity numbers of each of the *Test solutions* against their respective concentrations, and draw the straight line of best fit through the points and extrapolate to zero concentration: the value of the intercept is between 18 and 23 mL per g.

**Specific rotation** (781S): between +195° and +203°.

*Test solution:* 20 mg per mL, heated, if necessary, on a water bath to dissolve.

**Bacterial endotoxins** (85) (where it is labeled as intended for use in the preparation of injectables)—When tested in Sodium Chloride Injection (1 in 10), it contains not more than 1.0 USP Endotoxin Unit per mL.

**Safety**—Inject intravenously 1.0 mL of a sterile 1 in 10 solution of 10% Dextran 40 in saline TS into each of five mice

weighing 18 to 20 g. The injection period is not less than 10 seconds and not greater than 15 seconds. If there are no deaths within 72 hours, it meets the requirements of the test. If 1 or more animals die, continue the test using 10 mice weighing  $20 \pm 0.5$  g. If all animals survive for 72 hours, the requirements of the test are met.

**pH** (791): between 4.5 and 7.0, in a solution (1 in 10).

**Loss on drying** (731)—Dry it at 105° for 5 hours: it loses not more than 7.0% of its weight.

**Sulfate** (221)—A 1.5-g portion shows no more sulfate than corresponds to 0.45 mL of 0.020 N sulfuric acid (0.03%).

**Heavy metals, Method II** (231): 5 µg per g.

**Limit of nitrogenous impurities** (where it is labeled as intended for use in the preparation of injectables)—

*Sulfate solution*—To 1000 mL of sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°. [NOTE—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting the proportions accordingly.]

*Indicator*—Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.

*Procedure*—Transfer 0.2 g, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*. Heat until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask three times with 5 mL of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and commence steam distillation without delay. Receive the distillate in 1 mL of *Indicator* in a 100-mL flask, keeping the end of the condensing tube below the liquid surface for 5 minutes and above the liquid surface for 1 minute. Upon completion of the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate. Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet. Perform a blank determination, and make any necessary correction. The corrected volume of 0.010 N hydrochloric acid titrated does not exceed 0.14 mL (0.01%, as N).

### Limit of alcohol and related impurities—

*Test solution*—Dissolve without heating 5.0 g in 100 mL of water, and distill the solution, collecting the first 45 mL of the distillate. Dilute the distillate with water to 50.0 mL, and mix.

*Standard solution*—To 25.0 mL of the *Test solution* add 0.5 mL of a 2.5% (w/v) solution of *n*-propyl alcohol.

*Chromatographic system*—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm  $\times$  1.8-m column packed with support S3. The column temperature is maintained at about 160°, the injection port temperature is maintained at about 240°, and the detector is maintained at about 210°. The carrier gas is nitrogen, flowing at a rate of about 25 mL per minute. [NOTE—Injector seals may deteriorate after multiple injections of the *Standard* and *Test solutions*. Inspect the seals before making a series of injections.]

*Procedure*—Separately inject equal volumes (about 1 µL) of the *Test solution*, the *Standard solution*, and a 0.05% (w/v) solution of *n*-propyl alcohol and water, and measure the peak responses. After corrections for any impurities in the *n*-propyl alcohol solution and water, the total area of peaks from impurities in the *Test solution* does not exceed the area of the *n*-propyl alcohol solution peak.

**Antigenic impurities** (where it is labeled as intended for use in the preparation of injectables)—Prepare a sterile solution containing 100 mg per mL in Sodium Chloride Injection. At intervals of about 48 hours, inject three 0.5-mL doses into the peritoneal cavities of each of 6 guinea pigs.

At 14 days after the first intraperitoneal injection, inject 0.20 mL intravenously into each of 3 of the guinea pigs, and at 21 days treat the other 3 guinea pigs similarly. Observe the animals for 30 minutes after each intravenous injection and again 24 hours later. The animals exhibit no evidence of anaphylactoid reactions, such as coughing, bristling of hair, or respiratory distress.

#### Molecular weight distribution and weight and number average molecular weights—

**Mobile phase**—Prepare a suitable degassed and filtered solution containing 7.1 g of anhydrous sodium sulfate per L in water.

**Calibration solutions**—Separately dissolve USP Dextran 4 Calibration RS, USP Dextran 10 Calibration RS, USP Dextran 40 Calibration RS, USP Dextran 70 Calibration RS, and USP Dextran 250 Calibration RS in *Mobile phase* to obtain solutions each containing 20 mg per mL.

**Marker solution**—Prepare a solution in *Mobile phase* containing 3 mg of dextrose and 3 mg of USP Dextran V<sub>0</sub> Marker RS per mL.

**System suitability solution**—Prepare a solution of USP Dextran 40 System Suitability RS in *Mobile phase* containing 20 mg per mL.

**Test solution**—Prepare a solution of Dextran 40 in *Mobile phase* containing 20 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a refractive index detector and three 7.5-mm × 30-cm columns containing packing L38, and maintained at a constant temperature. Chromatograph the *Marker solution*, and record the peak responses as directed for *Procedure*: the elution profile shows two peaks, the first due to the V<sub>0</sub> marker, the second due to dextrose. Determine the void volume, V<sub>0</sub>, of the system as the inflection point of the ascending part of the first peak. Determine the total volume, V<sub>T</sub>, of the system as the maximum of the second peak; the tailing factor, t<sub>f</sub>, of the dextrose peak is not more than 1.3; and the relative standard deviation of the ratio V<sub>0</sub>/V<sub>T</sub> is not more than 1%. Chromatograph each of the *Calibration solutions* separately, and record the peak responses as directed for *Procedure*. Divide each profile into at least 60 vertical sections of equal volume increments. (The actual number of sections is represented by the variable *a* in the equations below.) Record y<sub>i</sub>, the height above the baseline, corresponding to each value of v<sub>i</sub>, the volume eluted at that section. For each value of v<sub>i</sub>, calculate the distribution coefficient, K<sub>i</sub>, by the formula:

$$(v_i - V_0) / (V_T - V_0).$$

Find appropriate values of b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, b<sub>4</sub>, and b<sub>5</sub>, using a suitable method,\* that, when substituted in the equation:

$$M_i = b_5 + e^{(b_4 + b_1 K_i + b_2 K_i^2 + b_3 K_i^3)}$$

and the resulting values of M<sub>i</sub> substituted, along with their corresponding values of y<sub>i</sub>, in the equation:

$$\bar{M}_w = \frac{\sum_{i=1}^a (y_i M_i)}{\sum_{i=1}^a y_i}$$

give values of weight average molecular weight,  $\bar{M}_w$ , within 5% of the labeled values for each of the *Calibration solutions* and 180 ± 2 for dextrose. Chromatograph the *System suitability solution*, and record the peak responses as directed for

\* The Gauss-Newton method, modified by Hartley [see D. Hartley *Technometrics*, 3 (1961)], and the G. Nilsson and K. Nilsson method [see G. Nilsson and K. Nilsson *J. Chromat.*, 101, 137 (1974)] are suitable methods. A curve-fitting program capable of nonlinear regression may be used.

*Procedure*. Calculate  $\bar{M}_w$  of the total molecular weight distribution using the same method as directed for the *Calibration solutions*, but inserting the now known values of b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, b<sub>4</sub>, and b<sub>5</sub>. It is between 39,000 and 46,000.

Similarly, calculate  $\bar{M}_w$  of the high-fraction dextran eluted through section *n* by the formula:

$$\frac{\sum_{i=1}^n (y_i M_i)}{\sum_{i=1}^n y_i}$$

in which *n* is defined by the relations:

$$\sum_{i=1}^n y_i \leq 0.1 \left( \sum_{i=1}^a y_i \right) \text{ and } \sum_{i=1}^{n+1} y_i > 0.1 \left( \sum_{i=1}^a y_i \right)$$

It is between 111,000 and 135,000.

Similarly, calculate  $\bar{M}_w$  of the low-fraction dextran eluted in and after section *m* by the formula:

$$\frac{\sum_{i=m}^a (y_i M_i)}{\sum_{i=m}^a y_i}$$

in which *m* is defined by:

$$\sum_{i=m}^a y_i \leq 0.1 \left( \sum_{i=1}^a y_i \right) \text{ and } \sum_{i=m-1}^a y_i > 0.1 \left( \sum_{i=1}^a y_i \right).$$

It is between 6000 and 9000.

*Procedure*—Chromatograph a 50-μL volume of the *Test solution*, and record the peak responses. Calculate values of the weight average molecular weight,  $\bar{M}_w$ , of the total molecular weight distribution of the high-fraction dextran, and of the low-fraction dextran as directed for *System Suitability* under *Chromatography* <621> the values are between 35,000 and 45,000, not more than 120,000, and not less than 5,000, respectively. With the values of b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, b<sub>4</sub>, and b<sub>5</sub>, obtained with the *Calibration solutions* under *Chromatographic system*, calculate the number average molecular weight,  $\bar{M}_n$ , of the total molecular weight distribution of the *Test solution* by substituting the corresponding values of M<sub>i</sub>, along with their corresponding values of y<sub>i</sub>, in the equation:

$$\bar{M}_n = \frac{\sum_{i=1}^a y_i}{\sum_{i=1}^a (y_i / M_i)}$$

The number average molecular weight,  $\bar{M}_n$ , is between 16,000 and 30,000. Where Dextran 40 is labeled as intended for use in the preparation of injectables, the ratio  $\bar{M}_w / \bar{M}_n$  is in the 1.4 to 1.9 range.



## Dextran 40 in Dextrose Injection

» Dextran 40 in Dextrose Injection is a sterile solution of Dextran 40 and Dextrose in Water for Injection. It contains in each 100 mL not less than 9.0 g and not more than 11.0 g of Dextran 40 and not less than 4.1 g and not more than 5.0 g of dextrose ( $C_6H_{12}O_6$ ). It contains no bacteriostatic agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Dextrose RS

USP Endotoxin RS

**Color of solution**—Its absorbance, determined at 375 nm against a water blank, is not greater than 0.06.

**Identification**—Dilute a portion quantitatively with dextrose solution (4.5 in 100) to a concentration of about 10 mg of Dextran 40 per mL. Using a capillary tube viscometer having dimensions such that the flow time of water is not less than 100 seconds, measure the flow times of the diluted Injection and that of a dextrose solution (4.5 in 100) at 20°. Calculate the intrinsic viscosity by the formula:

$$\{\ln[(R_D)(t/t_0)]\} / C$$

in which  $R_D$  is the ratio of the density of the diluted Injection to that of the dextrose solution;  $t$  and  $t_0$  are the flow times for the diluted Injection and the dextrose solution, respectively; and  $C$  is the concentration, in g per mL, of Dextran 40 in the diluted Injection: the intrinsic viscosity is between 18 and 23 mL per g.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 3.0 and 7.0.

**Heavy metals, Method II** (231): 5 µg per mL.

**Limit of 5-hydroxymethylfurfural and related substances**—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of  $C_6H_{12}O_6 \cdot H_2O$ , with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, using water as the blank: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements for *Injections* (1) and for *Particulate Matter in Injections* (788).

**Assay for dextrose**—

*Mobile phase*—Use filtered and degassed 0.01 N sulfuric acid.

*System suitability preparation*—Prepare a solution in water containing 5 mg each of dextrose and xylitol per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextrose RS in water to obtain a solution having a known concentration of about 5 mg of dextrose monohydrate per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 250 mg of dextrose monohydrate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index

detector and a 7.8-mm × 30-cm column containing packing L17. The column and, if necessary, the detector are maintained at a constant temperature of about 40°, and the flow rate is about 0.6 mL per minute. Chromatograph the *System suitability preparation*, and measure the peak responses as directed for *Procedure*: the resolution,  $R$ , between the dextrose and xylitol peaks is not less than 2.5. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5% for dextrose.

*Procedure*—Inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextrose peaks. Calculate the concentration, in g per 100 mL, of dextrose monohydrate ( $C_6H_{12}O_6 \cdot H_2O$ ) in the volume of Injection taken by the formula:

$$(198.17 / 180.16)C(r_U / r_S)$$

in which  $C$  is the concentration, in g per 100 mL, of USP Dextrose RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextran 40**—To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide, and mix. Determine the optical rotation (see *Optical Rotation* (781)), and calculate the concentration, in g per 100 mL, of Dextran 40 in the Injection taken by the formula:

$$(1 / 197.5)[(100a / l) - (52.75C_d)]$$

in which 197.5 and 52.75 represent average values for the specific rotation of Dextran 40 and dextrose, respectively;  $a$  is the observed optical rotation in degrees;  $l$  is the length, in dm, of the polarimeter tube; and  $C_d$  is the concentration, in g per 100 mL, of dextrose as determined in the *Assay for dextrose*.

## Dextran 40 in Sodium Chloride Injection

» Dextran 40 in Sodium Chloride Injection is a sterile solution of Dextran 40 and Sodium Chloride in Water for Injection. It contains in each 100 mL not less than 9.0 g and not more than 11.0 g of dextran 40; and not less than 0.81 g and not more than 0.99 g of sodium chloride. It contains no bacteriostatic agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

**Color of solution**—Its absorbance, determined at 375 nm against a water blank, is not greater than 0.05.

**Identification**—Dilute a portion of Injection quantitatively with sodium chloride solution (0.9 in 100) to a concentration of about 10 mg of Dextran 40 per mL. Using a capillary tube viscometer with a flow time for water of not less than 100 seconds, measure the flow times of the diluted Injection

and of sodium chloride solution (0.9 in 100) at 20°. Calculate the intrinsic viscosity by the formula:

$$\{\ln[(R_D)(t/t_0)]\} / C$$

in which  $R_D$  is the ratio of the density of the diluted Injection to that of the sodium chloride solution;  $t$  and  $t_0$  are the flow times for the diluted Injection and the sodium chloride solution, respectively; and  $C$  is the concentration, in g per mL, of Dextran 40 in the diluted Injection: the intrinsic viscosity is between 18 and 23 mL per g.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 3.5 and 7.0.

**Heavy metals, Method II** (231): 5 µg per mL.

**Other requirements**—It meets the requirements for *Injections* (1) and for *Particulate Matter in Injections* (788).

**Assay for sodium chloride**—Pipet a volume of Injection, equivalent to about 90 mg of chloride, into a porcelain casserole, and add 100 mL of water and 1 mL of dichlorofluorescein TS. Mix, and titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride.

**Assay for Dextran 40**—To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide, and mix. Determine the optical rotation (see *Optical Rotation* (781)), and calculate the concentration, in g per 100 mL, of dextran 40 in the volume of Injection taken by the formula:

$$100(a/l)(1/197.5)$$

in which 197.5 represents an average value for the specific rotation of dextran 40,  $a$  is the observed optical rotation, in degrees, and  $l$  is the length, in dm, of the polarimeter tube.

## Dextran 70

Dextrans.  
Dextrans.

» Dextran 70 is derived by controlled hydrolysis and fractionation of polysaccharides elaborated by the fermentative action of certain appropriate strains of *Leuconostoc mesenteroides* (NRRL, B-512F; NCTC, 10817) on a sucrose substrate. It is a glucose polymer in which the linkages between glucose units are almost entirely of the  $\alpha$ -1:6 type. Its weight average molecular weight is in the 63,000 to 77,000 range.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Dextran 70 RS  
USP Dextran 4 Calibration RS  
USP Dextran 10 Calibration RS  
USP Dextran 40 Calibration RS  
USP Dextran 70 Calibration RS  
USP Dextran 250 Calibration RS  
USP Dextran Vo Marker RS

USP Dextran 70 System Suitability RS  
USP Endotoxin RS

**Color of solution**—The absorbance of a solution in water (6 in 100), measured in a 4-cm cell determined at 375 nm against a water blank, is not greater than 0.15.

**Identification**—

**A: Infrared Absorption** (197K).

**B:** Proceed as directed under *Dextran 40*, except to use Dextran 70 to prepare the *Test solutions*: the value of the results at the intercept is between 24 and 29 mL per g.

**Specific rotation** (781S): between +195° and +203°.

*Test solution:* 20 mg per mL, heated, if necessary, on a water bath to dissolve.

**Bacterial endotoxins** (85) (where it is labeled as intended for use in the preparation of injectables)—When tested in *Sodium Chloride Injection* (0.6 in 10), it contains not more than 0.5 USP Endotoxin Unit per mL.

**Safety**—Inject intravenously 1.0 mL of a sterile solution of 6% Dextran 70 in saline TS into each of five mice weighing 18 to 20 g. The injection period is not less than 10 seconds and not greater than 15 seconds. If there are no deaths within 72 hours, it meets the requirements of the test. If 1 or more animals die, continue the test using 10 mice weighing  $20 \pm 0.5$  g. If all animals survive for 72 hours, the requirements of the test are met.

**pH** (791): between 4.5 and 7.0, in a solution (6 in 100).

**Loss on drying** (731)—Dry it at 105° for 5 hours: it loses not more than 7.0% of its weight.

**Sulfate** (221)—A 1.5-g portion shows no more sulfate than corresponds to 0.45 mL of 0.020 N sulfuric acid (0.03%).

**Heavy metals, Method II** (231): 5 µg per g.

**Limit of nitrogenous impurities** (where it is labeled as intended for use in the preparation of injectables)—

*Sulfate solution and Indicator*—Proceed as directed under *Dextran 40*.

*Procedure*—Proceed as directed under *Dextran 40*, except to use 0.2 g of Dextran 70.

**Limit of alcohol and related impurities**—

*Test solution*—Proceed as directed under *Dextran 40*, except to use 5.0 g of Dextran 70.

*Standard solution, Chromatographic system, and Procedure*—Proceed as directed under *Dextran 40*.

**Antigenic impurities** (where it is labeled as intended for use in the preparation of injectables)—Prepare a sterile solution containing 60 mg per mL of Dextran 70 in *Sodium Chloride Injection*, and proceed as directed under *Dextran 40* beginning with "At intervals of about 48 hours."

**Molecular weight distribution and weight and number average molecular weights**—

*Mobile phase, Calibration solutions, and Marker solution*—Proceed as directed under *Dextran 40*.

*System suitability solution*—Prepare a solution of USP Dextran 70 System Suitability RS in *Mobile phase* containing 20 mg per mL.

*Test solution*—Prepare a solution of Dextran 70 in *Mobile phase* containing 20 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed under *Dextran 40*:  $M_w$  for the total molecular weight distribution is between 65,000 and 74,000;  $M_w$  for the high-fraction dextran is between 180,000 and 240,000; and  $M_w$  for the low-fraction dextran is between 7,000 and 11,000.

*Procedure*—Chromatograph a 50-µL volume of the *Test solution*, and record the peak responses. Calculate values of  $M_w$  of the total molecular weight distribution, of the high-fraction dextran, and of the low-fraction dextran as directed for the *System suitability solution* under *Chromatographic system*. The  $M_w$  values are between 63,000 and 77,000, not more than 195,000, and not less than 13,000, respectively.

With the values of  $b_1$ ,  $b_2$ ,  $b_3$ ,  $b_4$ , and  $b_5$ , obtained with the *Calibration solutions* under *Chromatographic system*, calculate the number average molecular weight,  $\bar{M}_n$ , of the total molecular weight distribution of the *Test solution* by substituting the corresponding values of  $M_i$ , along with their corresponding values of  $y_i$ , in the equation:

$$\bar{M}_n = \sum_{i=1}^a y_i / \sum_{i=1}^a (y_i / M_i)$$

The number average molecular weight,  $\bar{M}_n$ , is between 34,000 and 48,000. Where Dextran 70 is labeled as intended for use in the preparation of injectables, the ratio  $\bar{M}_w / \bar{M}_n$  is in the 1.4 to 1.9 range.

## Dextran 70 in Dextrose Injection

» Dextran 70 in Dextrose Injection is a sterile solution of Dextran 70 and Dextrose in Water for Injection. It contains in each 100 mL not less than 5.4 g and not more than 6.6 g of dextran 70 and not less than 4.1 g and not more than 5.0 g of dextrose ( $C_6H_{12}O_6$ ). It contains no bacteriostatic agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Dextrose RS

USP Endotoxin RS

**Color of solution**—Its absorbance, determined at 375 nm against a water blank, is not greater than 0.05.

**Identification**—Dilute a portion quantitatively with dextrose solution (4.5 in 100) to a concentration of about 10 mg of dextran 70 per mL. Using a capillary tube viscometer having dimensions such that the flow time of water is not less than 100 seconds, measure the flow times of the diluted Injection and of a dextrose solution (4.5 in 100) at 20°. Calculate the intrinsic viscosity by the formula:

$$\{\ln[(R_D)(t / t_0)]\} / C$$

in which  $R_D$  is the ratio of the density of the diluted Injection to that of the dextrose solution;  $t$  and  $t_0$  are the flow times for the diluted Injection and the dextrose solution, respectively; and  $C$  is the concentration, in g per mL, of dextran 70 in the diluted Injection: the intrinsic viscosity is between 24 and 29 mL per g.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 3.5 and 7.0.

**Heavy metals, Method II** (231): 5 µg per mL.

**Limit of 5-hydroxymethylfurfural and related substances**—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of  $C_6H_{12}O_6 \cdot H_2O$ , with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, using water as the blank: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements for *Injections* (1) and for *Particulate Matter in Injections* (788).

**Assay for dextrose**—Proceed as directed in the *Assay for dextrose* under *Dextran 40 in Dextrose Injection* except that the *Assay preparation* for Dextran 70 in Dextrose Injection is obtained by dilution with water (10 mL in 25 mL of water). Adjust the calculations of the dextrose content to take into consideration the dilution factor.

**Assay for dextran 70**—To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide, and mix. Determine the optical rotation (see *Optical Rotation* (781)) in a suitable polarimeter and calculate the concentration, in g per 100 mL, of dextran 70 in the Injection by the formula:

$$(1 / 197.5)[(100a / l) - (52.75C_d)]$$

in which 197.5 and 52.75 represent average values for the specific rotation of dextran 70 and dextrose, respectively,  $a$  is the observed optical rotation in degrees,  $l$  is the length, in dm, of the polarimeter tube, and  $C_d$  is the concentration of dextrose, in g per 100 mL, from the *Assay for dextrose*.

## Dextran 70 in Sodium Chloride Injection

» Dextran 70 in Sodium Chloride Injection is a sterile solution of Dextran 70 and Sodium Chloride in Water for Injection. It contains in each 100 mL not less than 5.4 g and not more than 6.6 g of dextran 70 and not less than 0.81 g and not more than 0.99 g of sodium chloride. It contains no bacteriostatic agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

**Color of solution**—Its absorbance, determined at 375 nm against a water blank, is not greater than 0.04.

**Identification**—Quantitatively dilute a portion of Injection with sodium chloride solution (0.9 in 100) to a concentration of about 10 mg of dextran 70 per mL. Using a capillary tube viscometer having dimensions such that the flow time of water is not less than 100 seconds, measure the times of the diluted Injection and sodium chloride solution (0.9 in 100) at 20°. Calculate the intrinsic viscosity by the formula:

$$\{\ln[(R_D)(t / t_0)]\} / C$$

in which  $R_D$  is the ratio of the density of the diluted Injection to that of the sodium chloride solution;  $t$  and  $t_0$  are the flow times for the diluted Injection and the sodium chloride solution, respectively; and  $C$  is the concentration, in g per mL, of dextran 70 in the diluted Injection: the intrinsic viscosity is between 24 and 29 mL per g.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 7.0.

**Heavy metals, Method II** (231): 5 µg per mL.

**Other requirements**—It meets the requirements for *Injections* (1) and for *Particulate Matter in Injections* (788).

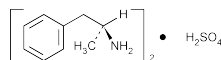
**Assay for sodium chloride**—Pipet a volume of Injection, equivalent to about 90 mg of chloride, into a porcelain casserole, and add 100 mL of water and 1 mL of dichlorofluorescein TS. Mix, and titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride.

**Assay for dextran 70**—To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide, and mix. Determine the optical rotation (see *Optical Rotation* (781)) in a suitable polarimeter and calculate the concentration, in g per 100 mL, of dextran 70 in the Injection taken by the formula:

$$100(a/l)(1/197.5)$$

in which 197.5 represents an average value for the specific rotation of dextran 70;  $a$  is the observed optical rotation, in degrees; and  $l$  is the length, in dm, of the polarimeter tube.

## Dextroamphetamine Sulfate



(C<sub>9</sub>H<sub>13</sub>N)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> 368.49  
Benzeneethanamine,  $\alpha$ -methyl-, (*S*)-, sulfate (2:1).  
(+)- $\alpha$ -Methylphenethylamine sulfate (2:1) [51-63-8].

» Dextroamphetamine Sulfate, the dextrorotatory isomer of amphetamine sulfate, contains not less than 98.0 percent and not more than 102.0 percent of (C<sub>9</sub>H<sub>13</sub>N)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Dextroamphetamine Sulfate RS

USP Dextroamphetamine Related Compound A RS  
1-Phenyl-2-propanol.

C<sub>9</sub>H<sub>12</sub>O 136.20 [CAS-14898-87-4].

USP Dextroamphetamine Related Compound B RS  
Phenyl acetone.

C<sub>9</sub>H<sub>10</sub>O 134.18 [CAS-103-79-7].

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between +20° and +23.5°.

*Test solution:* 40 mg per mL, in water.

**pH** (791): between 5.0 and 6.0, in a solution (1 in 20).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

### Related compounds—

*Solution A, Solution B, Mobile phase, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. Identify the impurities based on the relative retention times given in *Table 1*, and measure the peak responses. Calculate the percentage

of each dextroamphetamine related compound in the portion of Dextroamphetamine Sulfate taken by the formula:

$$100(C_s / C_U)(r_U/r_s)(1/F)$$

in which  $C_s$  and  $C_U$  are the concentrations, in mg per mL, of dextroamphetamine sulfate in the *Standard solution* and the *Test solution*, respectively;  $r_U$  is the peak area of each impurity obtained from the *Test solution*;  $r_s$  is the peak area of dextroamphetamine obtained from the *Standard solution*; and  $F$  is the relative response factor for each of the impurities relative to dextroamphetamine.

**Table 1**

Related Compound	Relative Retention Time (RRT)	Relative Response Factor (F)	Limit (w/w %)
Cathinone	0.81	55.6	NMT 0.25
Dextroamphetamine	1.0	1.0	—
Benzaldehyde	1.73	105.3	NMT 0.25
Dextroamphetamine related compound A	1.88	1.5	NMT 0.25
Dextroamphetamine related compound B	2.05	1.8	NMT 0.25
Individual unspecified impurity	—	1.0	NMT 0.1
Total	—	—	NMT 1.0

### Assay—

*Solution A*—Add 5.0 mL of trifluoroacetic acid to 900 mL of water; adjust with ammonium hydroxide to a pH of 2.2 $\pm$ 0.1; then add 100 mL of acetonitrile. Mix well, and degas.

*Solution B*—Use degassed acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextroamphetamine Sulfate RS in a suitable volumetric flask, and dilute quantitatively with *Solution A* to obtain a solution having a known concentration of about 2.0 mg per mL.

*Resolution solution*—Transfer about 40 mL of the *Standard preparation* to a 50-mL volumetric flask. Using a microliter syringe, add 1  $\mu$ L each of USP Dextroamphetamine Related Compound A RS and USP Dextroamphetamine Related Compound B RS. Dilute with *Standard preparation* to volume.

*Assay preparation*—Dissolve an accurately weighed quantity of Dextroamphetamine Sulfate in a suitable volumetric flask, and dilute quantitatively with *Solution A* to obtain a solution having a known concentration of about 2.0 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 257-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. The column is maintained at 40°. The chromatograph is programmed as follows, and data is collected for 30 minutes.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–15	100→65	0→35	linear gradient
15–20	65→0	35→100	linear gradient
20–22	0	100	isocratic
22–23	0→100	100→0	linear gradient
23–30	100	0	re-equilibration

Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*. Identify the peaks using the relative retention times given in *Table 1*: the resolution,  $R$ , between dextroamphetamine related compound A and dextroamphetamine related compound B is not less than 3.0; the tailing factor for dextroamphetamine sulfate is not more than 3.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%. [NOTE—For identification purposes, the approximate relative retention times are given in *Table 1*.]

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextroamphetamine peak. Calculate the quantity, in percent of  $(C_9H_{13}N)_2 \cdot H_2SO_4$ , in the portion of Dextroamphetamine Sulfate taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  and  $C_U$  are the concentrations of dextroamphetamine sulfate, in mg per mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses for dextroamphetamine sulfate in the *Assay preparation* and the *Standard preparation*, respectively.

## Dextroamphetamine Sulfate Capsules

» Dextroamphetamine Sulfate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Dextroamphetamine Sulfate RS

### Identification—

**A:** Mix an amount of the Capsule contents, equivalent to about 50 mg of dextroamphetamine sulfate, with about 10 mL of water for 30 minutes, and filter into a small flask. Cool the filtrate to about 15°, add 3 mL of 1 N sodium hydroxide, then add 1 mL of a mixture of 1 volume of benzoyl chloride and 2 volumes of anhydrous ethyl ether, and shake for 2 minutes. Filter the precipitate, wash with about 15 mL of cold water, and recrystallize twice from diluted alcohol: the benzoyl derivative of dextroamphetamine so obtained, after being dried at 105° for 1 hour, melts between 154° and 160°.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* is the same as that of the *Standard preparation* obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium:* water; 500 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Mobile phase*—Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 450 mL of methanol. Adjust dropwise, if necessary, with glacial acetic acid to a pH of  $3.3 \pm 0.1$ . Filter through a 0.5- $\mu$ m membrane filter. The volume of

methanol may be adjusted so that the retention time for dextroamphetamine is about 5 minutes.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextroamphetamine Sulfate RS in 0.12 N phosphoric acid to obtain a solution having a known concentration of about 0.3 mg per mL.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh. Transfer an accurately weighed portion of the mixed powder, equivalent to about 15 mg of dextroamphetamine sulfate, to a 50-mL volumetric flask. Add 40 mL of 0.12 N phosphoric acid, and sonicate for 15 minutes. Dilute with 0.12 N phosphoric acid to volume, and mix. Filter through a 0.5- $\mu$ m membrane filter, discarding the first 20 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable automatic injector or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  in the portion of Capsules taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dextroamphetamine Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dextroamphetamine Sulfate Tablets

» Dextroamphetamine Sulfate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Dextroamphetamine Sulfate RS

### Identification—

**A:** Transfer a portion of finely ground Tablets, equivalent to about 50 mg of dextroamphetamine sulfate, to a suitable centrifuge tube. Add 25 mL of water, shake vigorously, and centrifuge until clear. Decant the clear solution into a 250-mL separator, add 5 mL of 2.5 N sodium hydroxide, mix, and extract with 60 mL of ether. Wash the ether extract with two 5-mL portions of 0.25 N sodium hydroxide, and discard the washings. Filter the ether extract through a pledget of cotton, previously saturated with ether, into a 100-mL beaker, and evaporate on a steam bath in a current of air to about 1 mL. Dissolve the residue in 3 mL of alcohol, and transfer to a glass-stoppered, 125-mL conical flask containing 25 mL of water. Rinse the beaker with 3 mL of alcohol, and transfer to the flask. Cool to about 15°, add 3 mL of 1 N sodium hydroxide, then add 1 mL of a mixture of 1 volume of benzoyl chloride and 2 volumes of anhydrous ethyl ether, and shake for 2 minutes. Filter the precipitate, wash with about 15 mL of cold water, and recrystallize twice from diluted alcohol: the benzoyl derivative of dextro-

amphetamine so obtained, after being dried at 105° for 1 hour, melts between 154° and 160°.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium:* water; 500 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

Determine the amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  dissolved by employing the following method.

*Mobile phase*—Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 400 mL of methanol. Adjust by the dropwise addition of glacial acetic acid to a pH of  $3.3 \pm 0.1$ , if necessary, filter, and degas the solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Inject a volume (about 100  $\mu$ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  dissolved in comparison with a *Standard solution* having a known concentration of USP Dextroamphetamine Sulfate RS in the same *Medium* and similarly chromatographed.

*Tolerances*—Not less than 75% (*Q*) of the labeled amount of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Isomeric purity**—Pack a pledget of fine glass wool in the base of a 200- $\times$  25-mm chromatographic tube, with the aid of a tamping rod. Add 5 g of chromatographic siliceous earth, and tamp firmly to compress the material to a uniform mass.

Finely powder a number of Tablets, equivalent to about 300 mg of dextroamphetamine sulfate, mix the powder in a mortar with 5 g of chromatographic siliceous earth, add 1 mL of methanol and 0.5 mL of ammonium hydroxide, and triturate to a uniform mixture. Transfer the mixture without delay to the chromatographic tube, and tamp as before. Wipe the mortar and pestle with a small amount of glass wool, and insert it into the tube on top of the column. Arrange a 125-mL separator containing 35 mL of 0.1 N sulfuric acid to receive the effluent. Pass 60 mL of chloroform through the column. Shake the separator vigorously for 1 minute, allow the layers to separate, and discard the chloroform. Add to the aqueous phase in the separator 2.5 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe, rapidly inject 1.0 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased, releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with 50 mL of chloroform, shaking vigorously for 1 minute. Filter the chloroform extract through a pledget of filter cotton into a 100-mL beaker, rinse the cotton with a small amount of chloroform, and evaporate on a steam bath in a current of air or nitrogen to dryness. Heat and triturate the residue until the odor of chloroform is no

longer perceptible. Allow the residue to cool, inducing it to crystallize. Reduce the crystals to a fine powder, heat at 80° for 30 minutes, and cool: the specific rotation of the acetilamphetamine so obtained, determined in a solution in chloroform containing 20 mg per mL, a 200-mm semimicro polarimeter tube being used, is between  $-37.5^\circ$  and  $-44.0^\circ$ .

**Assay**—

*Mobile phase*—Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 450 mL of methanol. Adjust dropwise, if necessary, with glacial acetic acid to a pH of  $3.3 \pm 0.1$ . Filter through a 0.5- $\mu$ m membrane filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextroamphetamine Sulfate RS in 0.12 N phosphoric acid to obtain a solution having a known concentration of about 0.3 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the mixed powder, equivalent to about 15 mg of dextroamphetamine sulfate, to a 50-mL volumetric flask. Add 40 mL of 0.12 N phosphoric acid, and sonicate for 15 minutes. Dilute with 0.12 N phosphoric acid to volume, and mix. Filter through a 0.5- $\mu$ m membrane filter, discarding the first 20 mL of the filtrate.

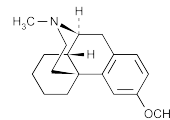
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 3, and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dextroamphetamine Sulfate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dextromethorphan



$C_{18}H_{25}NO$  271.40

Morphinan, 3-methoxy-17-methyl-, (9 $\alpha$ ,13 $\alpha$ ,14 $\alpha$ )-3-Methoxy-17-methyl-9 $\alpha$ ,13 $\alpha$ ,14 $\alpha$ -morphinan [125-71-3].

» Dextromethorphan contains not less than 98.0 percent and not more than 101.0 percent of  $C_{18}H_{25}NO$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dextromethorphan RS

**Identification—****A:** *Infrared Absorption* (197K).**B:** *Ultraviolet Absorption* (197U)—*Solution:* 100 µg per mL.*Medium:* dilute hydrochloric acid (1 in 120).

Absorptivities at 278 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**Melting range, Class I** (741): between 109.5° and 112.5°.**Specific rotation** (781S)—*Test solution:* 100 mg per mL, in chloroform. Its specific rotation does not differ from that of the similarly prepared solution of USP Dextromethorphan RS by more than 1.0%.**Water, Method Ia** (921): not more than 0.5%.**Residue on ignition** (281): not more than 0.1%.**Heavy metals, Method II** (231): 0.002%.**Limit of *N,N*-dimethylaniline—***Standard solution*—Transfer about 50 mg of *N,N*-dimethylaniline to a 100-mL volumetric flask, add 70.0 mL of water, insert the stopper tightly, shake for 20 minutes using a mechanical wrist-action shaker or equivalent, dilute with water to volume, and mix. Transfer 1.0 mL to a 100-mL volumetric flask, dilute with water to volume, transfer 1.0 mL of this solution to a 25-mL volumetric flask, and add 19 mL of water.*Test solution*—Transfer about 500 mg of Dextromethorphan, accurately weighed, to a 25-mL volumetric flask, add 19 mL of water and 1 mL of 3 N hydrochloric acid, dissolve by warming on a steam bath, and cool.*Procedure*—Add 2 mL of 1 N acetic acid and 1 mL of sodium nitrite solution (1 in 100) to the *Test solution*, dilute with water to volume, and mix. This solution shows no more color than the straw yellow to greenish yellow color of the solution obtained from the *Standard solution* similarly treated: not more than 0.001% of *N,N*-dimethylaniline is found.**Limit of phenolic compounds**—Dissolve about 10 mg in 2 mL of 3 N hydrochloric acid, and add 2 drops of ferric chloride TS. Mix, add 2 drops of potassium ferricyanide TS, and observe after 2 minutes: no blue-green color develops.**Assay**—Dissolve about 700 mg of Dextromethorphan, accurately weighed, in 60 mL of glacial acetic acid, warming slightly, if necessary, to dissolve. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.14 mg of C<sub>18</sub>H<sub>25</sub>NO.**Dextromethorphan Hydrobromide**C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O 370.32

Morphinan, 3-methoxy-17-methyl-, (9α,13α,14α)-, hydrobromide, monohydrate.

3-Methoxy-17-methyl-9α,13α,14α-morphinan hydrobromide monohydrate [6700-34-1].

Anhydrous 352.32 [125-69-9].

» Dextromethorphan Hydrobromide contains not less than 98.0 percent and not more than 102.0 percent of C<sub>18</sub>H<sub>25</sub>NO · HBr, calculated on the anhydrous basis.**Packaging and storage**—Preserve in tight containers.**USP Reference standards** (11)—

USP Dextromethorphan Hydrobromide RS

**Identification—****A:** *Infrared Absorption* (197K)—*Test specimen*—Dry in vacuum over phosphorus pentoxide for 4 hours.**B:** *Ultraviolet Absorption* (197U)—*Solution:* 100 µg per mL.*Medium:* 0.1 N hydrochloric acid.

Absorptivities at 278 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**C:** To 5 mL of a solution (1 in 200) add 5 drops of 2 N nitric acid and 2 mL of silver nitrate TS: a yellowish white precipitate is formed.**Specific rotation** (781S)—*Test solution:* 18 mg per mL (warm it, if necessary, to dissolve). Its specific rotation, determined photoelectrically at 325 nm, does not differ from that of the similarly prepared solution of USP Dextromethorphan Hydrobromide RS by more than 1.0%.**pH** (791): between 5.2 and 6.5 in a solution (1 in 100).**Water, Method I** (921): between 3.5% and 5.5%.**Residue on ignition** (281): not more than 0.1%.**Limit of *N,N*-dimethylaniline**—Proceed as directed in the test for *Limit of *N,N*-dimethylaniline* under *Dextromethorphan*, except to use Dextromethorphan Hydrobromide.**Limit of phenolic compounds**—To about 5 mg add 1 drop of 3 N hydrochloric acid, 1 mL of water, and 2 drops of ferric chloride TS. Mix, add 2 drops of potassium ferricyanide TS, and observe after 2 minutes: no blue-green color develops.**Assay—***Mobile phase*—Prepare a filtered and degassed solution containing 0.007 M docusate sodium and 0.007 M ammonium nitrate in a mixture of acetonitrile and water (70:30), and adjust the solution with glacial acetic acid to a pH of 3.4. [NOTE—Dissolve the docusate sodium in the acetonitrile and water mixture before adding the ammonium nitrate.]*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a stock solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.*Assay preparation*—Transfer about 100 mg of Dextromethorphan Hydrobromide, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the major peak is not more than 2.5; and the relative standard deviation is not more than 2.0%.*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>25</sub>NO · HBr in the portion of Dextromethorphan Hydrobromide taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dextromethorphan Hydrobromide Oral Solution

» Dextromethorphan Hydrobromide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Dextromethorphan Hydrobromide RS

### Identification—

**A:** Transfer about 50 mL of Oral Solution to a 250-mL separator, add 20 mL of water, 5 mL of 2.5 N sodium hydroxide, and 40 mL of solvent hexane, and shake thoroughly. Remove the solvent hexane layer, and filter through anhydrous sodium sulfate into a 150-mL beaker. Repeat the solvent hexane extraction, using two 40-mL portions and collecting the extracts in the beaker after filtering. Evaporate the combined extracts at 50° under nitrogen to dryness, and dissolve the residue in, and dilute with, 10 mL of chloroform: the solution is dextrorotatory (see *Optical Rotation* (781)). Retain the chloroform solution for *Identification* test B.

**B:** Evaporate the chloroform solution from *Identification* test A on a steam bath to dryness, dissolve the residue in 2 mL of 2 N sulfuric acid, and add 1 mL of a freshly prepared solution of mercuric nitrate (prepared by dissolving 700 mg of mercuric nitrate in 4 mL of water, adding 100 mg of sodium nitrate, mixing, and filtering): no red color is produced immediately, but after heating, a yellow to red color develops in about 15 minutes.

### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

### Assay—

*Mobile phase and Standard preparation*—Prepare as directed in the Assay under *Dextromethorphan Hydrobromide*.

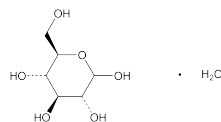
*Assay preparation*—Pipet, using a to-contains pipet, a volume of Oral Solution, equivalent to about 10 mg of dextromethorphan hydrobromide, into a 100-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system and Procedure* (see *Chromatography* (621))—Proceed as directed in the Assay under *Dextromethorphan Hydrobromide*. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the volume of Oral Solution taken by the formula:

$$(370.32 / 352.32)(100C)(r_U / r_S)$$

in which 370.32 and 352.32 are the molecular weights of dextromethorphan hydrobromide and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS, on the anhydrous basis, in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dextrose



$C_6H_{12}O_6 \cdot H_2O$  198.17

$C_6H_{12}O_6$  180.16

D-Glucose monohydrate [5996-10-1].  
Anhydrous [50-99-7].

### DEFINITION

Dextrose is a sugar usually obtained by the hydrolysis of starch. It contains one molecule of water of hydration or is anhydrous.

### IDENTIFICATION

- **A.**  
**Sample solution:** 1 in 20  
**Analysis:** Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.  
**Acceptance criteria:** A copious red precipitate of cuprous oxide is formed.

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **CHLORIDE AND SULFATE, Chloride (221)**  
**Control:** 0.50 mL of 0.020 N hydrochloric acid  
**Sample:** 2.0 g  
**Acceptance criteria:** 0.018%; the *Sample* shows no more chloride than the *Control*.
- **CHLORIDE AND SULFATE, Sulfate (221)**  
**Control:** 0.50 mL of 0.020 N sulfuric acid  
**Sample:** 2.0 g  
**Acceptance criteria:** 0.025%; the *Sample* shows no more sulfate than the *Control*.
- **ARSENIC, Method I (211):** NMT 1 µg/g
- **HEAVY METALS (231)**  
**Test preparation:** 4.0 g of Dextrose in water to make 25 mL  
**Acceptance criteria:** NMT 5 µg/g

### SPECIFIC TESTS

- **COLOR OF SOLUTION**  
**Control:** Mix 1.0 mL of cobaltous chloride CS, 3.0 mL of ferric chloride CS, and 2.0 mL of cupric sulfate CS with water to make 10 mL, and dilute 3 mL of this solution with water to 50 mL.  
**Sample solution:** 25 g of Dextrose in water to make 50.0 mL  
**Analysis:** Make the comparison by viewing the solutions downward in matched color-comparison tubes against a white surface.  
**Acceptance criteria:** The *Sample solution* has no more color than the *Control*.
- **ACIDITY**  
**Sample solution:** Dissolve 5.0 g in 50 mL of carbon dioxide-free water. Add phenolphthalein TS.  
**Analysis:** Titrate with 0.020 N sodium hydroxide to the production of a distinct pink color.  
**Acceptance criteria:** NMT 0.30 mL
- **WATER DETERMINATION, Method III (921)**  
**Analysis:** Dry at 105° for 16 h.  
**Acceptance criteria**  
Hydrous form: 7.5%–9.5%  
Anhydrous form: NMT 0.5%
- **OPTICAL ROTATION, Specific Rotation (781S)**  
**Sample solution:** 100 mg/mL of Dextrose in 0.012 N ammonium hydroxide



- Acceptance criteria:  $+52.6^{\circ}$  to  $+53.2^{\circ}$
- **DEXTRIN**  
Sample: 1 g, finely powdered  
Analysis: Reflux the *Sample* with 20 mL of alcohol.  
Acceptance criteria: The *Sample* dissolves completely.
- **SOLUBLE STARCH, SULFITES**  
Sample solution: 1 g of Dextrose in 10 mL of water  
Analysis: To the *Sample solution* add 1 drop of iodine TS.  
Acceptance criteria: The liquid is yellow.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label to indicate whether it is hydrous or anhydrous.

**Dextrose Injection**

» Dextrose Injection is a sterile solution of Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ). Dextrose Injection contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL for Injections containing less than 5% of dextrose and not more than 10.0 USP Endotoxin Units per g for Injections containing between 5% and 70% of dextrose. [NOTE—Prior to analysis, dilute Injections containing more than 10% of dextrose to a concentration of 10% of dextrose.]

**Identification**—It responds to the *Identification* test under *Dextrose*.

**pH** (791): between 3.2 and 6.5, determined on a portion to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of dextrose.

**Particulate matter** (788): meets the requirements.

**Heavy metals** (231)—Transfer a volume of Injection, equivalent to 4.0 g of dextrose, to a suitable vessel, and adjust the volume to 25 mL by evaporation or addition of water, as necessary: the limit is 0.0005C%, in which C is the labeled amount, in g, of  $C_6H_{12}O_6 \cdot H_2O$  per mL of Injection.

**Limit of 5-hydroxymethylfurfural and related substances**—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of  $C_6H_{12}O_6 \cdot H_2O$ , with water to 250.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumet-

ric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; A is 100 mm divided by the length of the polarimeter tube, in mm; and R is the observed rotation, in degrees.

**Dextrose and Sodium Chloride Injection**

» Dextrose and Sodium Chloride Injection is a sterile solution of Dextrose and Sodium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) and of sodium chloride (NaCl). It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Identification**—It responds to the *Identification* test under *Dextrose*, and to the tests for *Sodium* (191) and for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 10.0 USP Endotoxin Units per g of dextrose.

**pH** (791): between 3.2 and 6.5, determined on a portion diluted with water, if necessary, to a concentration of not more than 5% of dextrose.

**Limit of 5-hydroxymethylfurfural and related substances**—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of  $C_6H_{12}O_6 \cdot H_2O$  with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing from 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

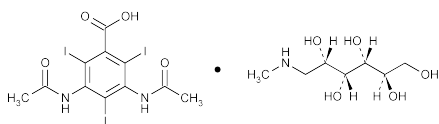
$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose

monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

**Assay for sodium chloride**—Transfer an accurately measured volume of Injection, equivalent to about 90 mg of sodium chloride, to a conical flask, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

## Diatrizoate Meglumine



$C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$  809.13  
Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, compd.  
with 1-deoxy-1-(methylamino)-D-glucitol (1:1).  
1-Deoxy-1-(methylamino)-D-glucitol 3,5-diacetamido-2,4,  
6-triiodobenzoate (salt) [131-49-7].

» Diatrizoate Meglumine contains not less than 98.0 percent and not more than 102.0 percent of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$  571.88

### Identification—

**A:** It responds to the *Thin-Layer Chromatographic Identification Test* (201), the test solution and the Standard solution of USP Diatrizoic Acid RS being prepared at a concentration of 1 mg per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**Specific rotation** (781S): between  $-5.65^\circ$  and  $-6.37^\circ$ .

*Test solution:* 100 mg per mL, in water.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

### Iodine and iodide—

*Test preparation*—Transfer 2.0 g to a 50-mL centrifuge tube provided with a stopper, dilute with water to 24 mL, and shake to dissolve.

*Procedure*—Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge: any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (1 in 4000) and 22 mL of water is substituted for the solution under test (0.02% of iodide).

### Heavy metals (231)—

*Standard preparation*—Transfer 2.0 mL of *Standard Lead Solution* (20 µg of Pb) to a 50-mL color-comparison tube, add 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix.

*Test preparation*—Dissolve 1.0 g of Diatrizoate Meglumine in 20 mL of water and 5 mL of 1 N sodium hydroxide, transfer the solution to a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

*Procedure*—To each of the tubes containing the *Standard preparation* and the *Test preparation* add 10 mL of sodium sulfide TS, mix, allow to stand for 5 minutes, and view downward over a white surface: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (0.002%).

**Free aromatic amine**—Transfer 1.0 g to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. To a third 50-mL volumetric flask add 5 mL of water and 10 mL of 0.1 N sodium hydroxide to provide a blank.

Treat each flask as follows. Add 25 mL of dimethyl sulfoxide, insert the stopper, and mix by swirling gently. Chill in an ice bath in the dark for 5 minutes. [NOTE—In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible until all of the reagents have been added.] Slowly add 2 mL of hydrochloric acid, mix, and allow to stand for 5 minutes. Add 2 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 1 mL of sulfamic acid solution (2 in 25), shake, and allow to stand for 5 minutes. [Caution—Considerable pressure is produced.] Add 2 mL of a 1 in 1000 solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in dilute propylene glycol (7 in 10), and mix.

Remove the flasks from the ice bath and from storage in the dark, and allow to stand in a water bath at 22° to 25° for 10 minutes. Shake gently and occasionally during this period, releasing the pressure by loosening the stopper. Dilute with water to volume, and mix.

Within 5 minutes from the time of diluting the solutions in all three flasks to 50 mL, concomitantly determine the absorbances of the solution from the substance under test and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 465 nm, with a suitable spectrophotometer, versus the prepared blank. The absorbance of the solution from the Diatrizoate Meglumine is not greater than that of the Standard solution (0.05%).

**Assay**—Transfer about 400 mg of Diatrizoate Meglumine, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ .

## Diatrizoate Meglumine Injection

» Diatrizoate Meglumine Injection is a sterile solution of Diatrizoate Meglumine in Water for In-

jection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate meglumine ( $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Diatrizoate Meglumine Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve Injection intended for intravascular injection either in single-dose containers, preferably of Type I or Type III glass, protected from light or, where intended for administration with a pressure injector through a suitable transfer connection, in similar glass 500-mL or 1000-mL bottles, protected from light. Injection packaged for other than intravascular use may be packaged in 100-mL multiple-dose containers, preferably of Type I or Type III glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection, where packaged in single-dose containers, to direct the user to discard any unused portion remaining in the container or, where packaged in bulk bottles to state, "Bulk Container—only for sterile filling of pressure injectors," to state that it contains no antimicrobial preservatives, and to direct the user to discard any unused portion remaining in the container after 6 hours. Indicate also in the labeling of bulk bottles that a pressure injector is to be charged with a dose just prior to administration of the Injection. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$  571.88

USP Endotoxin RS

**Identification**—

**A:** Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate meglumine, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 1.1 USP Endotoxin Units per mL for Injections containing less than 50% of diatrizoate meglumine, and less than 5.0 USP Endotoxin Units per mL for Injections containing 50% or more of diatrizoate meglumine.

**pH** (791): between 6.0 and 7.7.

**Iodine and iodide**—Dilute a volume of Injection, equivalent to 2.0 g of diatrizoate meglumine, with 24 mL of water in a 50-mL centrifuge tube provided with a stopper. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge: any red color in the toluene layer is not darker than that obtained when a volume of potassium iodide solution (1 in 4000), containing a quantity of iodide corresponding to 0.02% of the weight of diatrizoate meglumine in the vol-

ume of Injection taken, is diluted with water to 24 mL and substituted for the solution under test (0.02% of iodide).

**Heavy metals** (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of diatrizoate meglumine, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Free aromatic amine**—Transfer a volume of Injection, accurately measured and equivalent to 1 g of diatrizoate meglumine, to a glass-stoppered, 50-mL volumetric flask. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Into a second 50-mL volumetric flask pipet a known volume of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. The volume of Standard solution used contains a quantity of free aromatic amine corresponding to 0.05% of the weight of diatrizoate meglumine in the volume of Injection taken. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a third 50-mL volumetric flask add 5 mL of water".

**Meglumine content**—Determine the angular rotation (see *Optical Rotation* (781)) of the Injection, using a 10-cm cell and a suitable polarimeter. Calculate the content, in mg per mL, of meglumine in the Injection by the formula:

$$1000a / 24.9$$

in which *a* is the observed angular rotation, in degrees, corrected for the blank, and the factor, 24.9, is the average specific rotation, in degrees, of meglumine. The meglumine content is between 22.9% and 25.3% of the labeled amount of diatrizoate meglumine.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Pipet a volume of Injection, or a suitable dilution of it, equivalent to about 600 mg of diatrizoate meglumine, into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ .

## Diatrizoate Meglumine and Diatrizoate Sodium Injection

» Diatrizoate Meglumine and Diatrizoate Sodium Injection is a sterile solution of Diatrizoate Meglumine and Diatrizoate Sodium in Water for Injection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Sodium Hydroxide and Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of diatrizoate meglumine ( $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ ) and of iodine (I). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium

or Edetate Disodium as a stabilizer. Diatrizoate Meglumine and Diatrizoate Sodium Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve either in single-dose containers, preferably of Type I or Type III glass, protected from light or, where intended for administration with a pressure injector through a suitable transfer connection, in similar glass 500-mL or 1000-mL bottles, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection, where packaged in single-dose containers, to direct the user to discard any unused portion remaining in the container or, where packaged in bulk bottles to state, "Bulk Container—only for sterile filling of pressure injectors," to state that it contains no antimicrobial preservatives, and to direct the user to discard any unused portion remaining in the container after 6 hours. Indicate also in the labeling of bulk bottles that a pressure injector is to be charged with a dose just prior to administration of the Injection. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_{13}N_2O_3$  571.88

USP Endotoxin RS

**Identification**—

**A:** Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate meglumine and diatrizoate sodium, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 1.8 USP Endotoxin Units per mL for Injections containing less than 60% of diatrizoate meglumine, and not more than 3.6 USP Endotoxin Units for Injections containing 60% or more of diatrizoate meglumine.

**pH** (791): between 6.0 and 7.7.

**Free aromatic amine**—Transfer an accurately measured volume of Injection, equivalent to about 1 g of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a third 50-mL volumetric flask add 5 mL of water".

**Iodine and iodide**—Transfer an accurately measured volume of Injection, equivalent to about 2.0 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL centrifuge tube provided with a stopper. Dilute with water to 24 mL. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake,

and centrifuge: any red color in the toluene layer is not darker than that obtained when a volume of potassium iodide solution (1 in 4000), containing a quantity of iodide corresponding to 0.02% of the weight of diatrizoate meglumine and diatrizoate sodium in the volume of Injection taken is diluted with water to 24 mL and substituted for the solution under test (0.02% of iodide).

**Heavy metals** (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of the total of diatrizoate meglumine and diatrizoate sodium, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for diatrizoate meglumine**—Pipet 5 mL of Injection into a 10-mL volumetric flask, add water to volume, and mix. Determine the angular rotation (see *Optical Rotation* (781)) of the diluted Injection, using a 100-mm tube. Calculate the content, in mg per mL, of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$  in the Injection by the formula:

$$2000a / 6.01$$

in which  $a$  is the observed angular rotation, in degrees, corrected for the blank, and the factor 6.01 is the specific rotation, in degrees, of diatrizoate meglumine.

**Assay for iodine**—Transfer an accurately measured volume of Injection, equivalent to about 4 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine.

## Diatrizoate Meglumine and Diatrizoate Sodium Solution

» Diatrizoate Meglumine and Diatrizoate Sodium Solution is a solution of Diatrizoic Acid in Purified Water prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of diatrizoate meglumine ( $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ ) and of iodine (I). It may contain small amounts of suitable buffers, Edetate Disodium, and flavoring agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label the container to indicate that the contents are not intended for parenteral use.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

**Identification**—

**A:** Dilute a volume of Solution, if necessary, with an 0.8 in 1000 solution of sodium hydroxide in methanol, to ob-

tain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-Layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Evaporate a volume of Solution, equivalent to about 500 mg of diatrizoate meglumine and diatrizoate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.

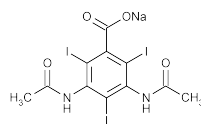
**pH** (791): between 6.0 and 7.6.

**Iodine and iodide**—Using as the *Test preparation* a volume of Solution equivalent to about 2 g of diatrizoate meglumine and diatrizoate sodium and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Diatrizoate Meglumine*.

**Assay for diatrizoate meglumine**—Pipet a volume of Solution, equivalent to about 1.5 g of diatrizoate meglumine and diatrizoate sodium, into a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of the solution so obtained into a glass-stoppered, 250-mL flask. Add 4 mL of 2 N sulfuric acid and 20 mL of sodium metaperiodate solution (1 in 200). Insert the stopper, and set aside in the dark for 1 hour. Add 50 mL of water, mix, and add 10 mL of potassium iodide TS. Insert the stopper quickly, mix by swirling for 20 seconds, and immediately titrate with 0.1 N sodium thiosulfate VS, using 3 mL of starch TS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 10.11 mg of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ .

**Assay for iodine**—Transfer an accurately measured volume of Solution, equivalent to about 4 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine.

## Diatrizoate Sodium



$C_{11}H_8I_3N_2NaO_4$  635.90

Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, monosodium salt.

Monosodium 3,5-diacetamido-2,4,6-triiodobenzoate [737-31-5].

» Diatrizoate Sodium contains not less than 98.0 percent and not more than 102.0 percent of  $C_{11}H_8I_3N_2NaO_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers. Store at room temperature.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$  571.88

**Identification**—

**A:** It responds to the *Thin-layer Chromatographic Identification Test* (201), the test solution and the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**C:** It responds to the flame test for *Sodium* (191).

**Water, Method I** (921): not more than 10.0%.

**Free aromatic amine**—Transfer 1.0 g to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a second 50-mL volumetric flask transfer 4 mL of water."

**Iodine and iodide**—

*Test preparation*—Transfer 2.0 g to a 50-mL centrifuge tube provided with a stopper, dilute with water to 24 mL, and shake to dissolve.

*Procedure*—Proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Diatrizoate Meglumine*.

**Heavy metals** (231)—Dissolve 1.0 g of Diatrizoate Sodium in 20 mL of water and 5 mL of 1 N sodium hydroxide, transfer the solution to a 50-mL color-comparison tube, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Assay**—Transfer about 300 mg of Diatrizoate Sodium, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of  $C_{11}H_8I_3N_2NaO_4$ .

## Diatrizoate Sodium Injection

» Diatrizoate Sodium Injection is a sterile solution of Diatrizoate Sodium in Water for Injection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate sodium ( $C_{11}H_8I_3N_2NaO_4$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Diatrizoate Sodium Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve Injection intended for intravascular injection in single-dose containers, preferably of Type I or Type III glass, protected from light. Injection intended for other than intravascular use may be packaged in 100-mL multiple-dose containers, preferably of Type I or Type III glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$  571.88

USP Endotoxin RS

**Identification**—

**A:** Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 5.6 USP Endotoxin Units per mL for Injections containing 20% of diatrizoate sodium; not more than 1.3 USP Endotoxin Units per mL for Injections containing 25% of diatrizoate sodium; and not more than 5.0 USP Endotoxin Units per mL for Injections containing 50% of diatrizoate sodium.

**pH** (791): between 6.0 and 7.7.

**Iodine and iodide**—Using a volume of Injection equivalent to 2.0 g of diatrizoate sodium, and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Diatrizoate Meglumine*.

**Heavy metals** (231)—In a 50-mL color-comparison tube mix a volume of Injection, equivalent to 1.0 g of diatrizoate sodium, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Free aromatic amine**—Transfer a volume of Injection, equivalent to 1.0 g of diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a second 50-mL volumetric flask transfer 4 mL of water."

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Pipet into a glass-stoppered 125-mL conical flask a volume of Injection, equivalent to about 500 mg of diatrizoate sodium. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, and reflux the mixture for 1 hour. Cool to room temperature, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the color of the yellow precipitate just changes to green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of  $C_{11}H_8I_3N_2NaO_4$ .

## Diatrizoate Sodium Solution

» Diatrizoate Sodium Solution is a solution of Diatrizoate Sodium in Purified Water, or a solution of Diatrizoic Acid in Purified Water prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate sodium ( $C_{11}H_8I_3N_2NaO_4$ ). It may contain a suitable preservative.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label the container to indicate that the contents are not intended for parenteral use.

**USP Reference standards** (11)—

USP Diatrizoic Acid RS

**Identification**—

**A:** Dilute a volume of Solution, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

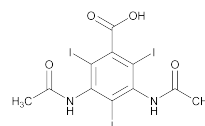
**B:** Evaporate a volume of Solution, equivalent to about 500 mg of diatrizoate sodium, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

**pH** (791): between 4.5 and 7.5.

**Iodine and iodide**—Using as the *Test preparation* a volume of Solution equivalent to 2.0 g of diatrizoate sodium and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Diatrizoate Meglumine*.

**Assay**—Pipet a volume of Solution, equivalent to about 400 mg of diatrizoate sodium, into a 125-mL conical flask. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of  $C_{11}H_8I_3N_2NaO_4$ .

## Diatrizoic Acid



$C_{11}H_8I_3N_2O_4$  (anhydrous) 613.91

$C_{11}H_8I_3N_2O_4 \cdot 2H_2O$  649.95

Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-;  
3,5-Diacetamido-2,4,6-triiodobenzoic acid [117-96-4].  
Dihydrate [50978-11-5].

**DEFINITION**

Diatrizoic Acid is anhydrous or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 102.0% of diatrizoic acid ( $C_{11}H_9I_3N_2O_4$ ), calculated on the anhydrous basis.

**IDENTIFICATION****• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Solution A:** Sodium hydroxide in methanol (0.8 in 1000)

**Standard solution:** 1 mg/mL in *Solution A*

**Sample solution:** 1 mg/mL in *Solution A*

**Developing solvent system:** Chloroform, methanol, and ammonium hydroxide (20:10:2)

**Analysis:** Use short-wavelength UV light to locate the spots.

**Acceptance criteria:** Meets the requirements

**• B.**

**Sample:** 500 mg

**Analysis:** Heat the *Sample* in a suitable crucible.

**Acceptance criteria:** Violet vapors are evolved.

**ASSAY****• PROCEDURE**

**Sample:** 300 mg

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 N silver nitrate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS. Titrate the *Sample* with *Titrant* until the yellow precipitate just turns green. Each mL of *Titrant* is equivalent to 10.23 mg of diatrizoic acid ( $C_{11}H_9I_3N_2O_4$ ).

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****• RESIDUE ON IGNITION (281):** NMT 0.1%**• HEAVY METALS (231)**

**Standard solution:** To 2.0 mL of *Standard Lead Solution* (20 µg of Pb) in a 50-mL color-comparison tube add 5 mL of 1 N sodium hydroxide, and dilute with water to 40 mL.

**Sample solution:** Transfer 2.0 mL of solution, prepared as directed for *Sample solution* in the test for *Iodine and Iodide*, to a 50-mL color-comparison tube. Add 5 mL of 1 N sodium hydroxide, and dilute with water to 40 mL.

**Analysis:** To each of the tubes containing the *Standard solution* and the *Sample solution* add 10 mL of sodium sulfide TS, allow to stand for 5 min, and view downward over a white surface.

**Acceptance criteria:** 20 ppm; the color of the *Sample solution* is not darker than that of the *Standard solution*.

**• FREE AROMATIC AMINE**

**Standard stock solution:** Dissolve a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain 500 µg/mL.

**Standard solution:** Transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a *Standard stock solution* to a 50-mL volumetric flask.

**Sample solution:** Transfer 1.0 g to a 50-mL volumetric flask, and add 12.5 mL of water and 2.5 mL of 0.1 N sodium hydroxide.

**Blank:** Transfer 5 mL of water and 10 mL of 0.1 N sodium hydroxide to a 50-mL volumetric flask.

**Analysis:** Treat each flask as follows. Add 25 mL of dimethyl sulfoxide, insert the stopper, and mix by swirling gently. Chill in an ice bath in the dark for 5 min. In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible until all of the reagents have been added.

Slowly add 2 mL of hydrochloric acid, and allow to stand for 5 min. Add 2 mL of sodium nitrite solution (20 mg/mL), and allow to stand for 5 min. Add 1 mL of sulfamic acid solution (80 mg/mL), shake, and allow to stand for 5 min. [NOTE—Considerable pressure is produced.] Add 2 mL of a solution (1 mg/mL) of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in dilute propylene glycol (700 mg/mL). Remove the flasks from the ice bath and from storage in the dark, and allow to stand in a water bath at 22°–25° for 10 min. Shake gently and occasionally during this period, releasing the pressure by loosening the stopper. Dilute with water to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Vis

**Analytical wavelength:** 465 nm

**Cell:** 1 cm

**Analysis:** Within 5 min from the time of diluting the solutions in all three flasks to 50 mL, concomitantly determine the absorbances of the solutions.

**Acceptance criteria:** 0.05%; the absorbance of the *Sample solution* is NMT that of the *Standard solution*.

**SPECIFIC TESTS****• WATER DETERMINATION, Method I (921):** NMT 1.0% (anhydrous form), and between 4.5% and 7.0% (hydrous form)**• IODINE AND IODIDE**

**Sample solution:** Suspend 10.0 g in 10 mL of water, and add in small portions, with stirring, 1.5 mL of sodium hydroxide solution (400 mg/mL). When the solution is complete, adjust to a pH between 7.0 and 7.5 with a dilute solution (8 mg/mL) of sodium hydroxide or hydrochloric acid, and dilute with water to 20 mL.

**Analysis:** Dilute 4.0 mL of *Sample solution* with 20 mL of water in a 50-mL centrifuge tube provided with a stopper, add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (20 mg/mL), shake, and centrifuge.

**Acceptance criteria:** NMT 0.02% of iodide; any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (0.25 mg/mL) and 22 mL of water is substituted for the solution under test.

**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.**• LABELING:** Label it to indicate whether it is anhydrous or hydrous.**• USP REFERENCE STANDARDS (11)**

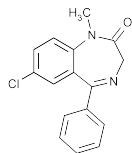
USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$  571.88

## Diazepam



$C_{16}H_{13}ClN_2O$  284.74

2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-

7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one [439-14-5].

» Diazepam contains not less than 95.0 percent and not more than 105.0 percent of  $C_{16}H_{13}ClN_2O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Diazepam RS

USP Diazepam Related Compound A RS  
2-Methylamino-5-chlorobenzophenone.  
 $C_{14}H_{12}ClNO$  245.71

USP Diazepam Related Compound B RS  
3-Amino-6-chloro-1-methyl-4-phenylcarbostyryl.  
 $C_{16}H_{13}ClN_2O$  284.74

USP Nordazepam RS  
7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one.  
 $C_{15}H_{11}ClN_2O$  270.72

### Identification—

**A:** Infrared Absorption (197K).

**B:** Thin-Layer Chromatographic Identification Test (201)—  
Test solution: 5 mg per mL, in acetone.

Developing solvent system: a mixture of ethyl acetate and n-heptane (1:1).

Procedure—Proceed as directed in the chapter except use an unsaturated developing chamber.

**Melting range**, Class I (741): between 131° and 135°.

**Loss on drying** (731)—Dry it in vacuum over phosphorus pentoxide at 60° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, Method II (231): 0.002%.

### Related compounds—

Mobile phase, System suitability solution, and Chromatographic system—Proceed as directed in the Assay.

Standard solution—Dissolve accurately weighed quantities of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, and USP Nordazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 1 µg per mL, 0.1 µg per mL, and 3 µg per mL, respectively.

Test solution—Transfer about 10 mg of Diazepam, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of diazepam related compound B, diazepam related com-

pound A, and nordazepam in the portion of Diazepam taken by the formula:

$$(C_R / W)(r_U / r_S)$$

in which  $C_R$  is the concentration, in µg per mL, of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, or USP Nordazepam RS in the Standard solution;  $W$  is the weight, in mg, of Diazepam taken to prepare the Test solution; and  $r_U$  and  $r_S$  are the peak responses obtained from the Test solution and the Standard solution, respectively: not more than 0.01% of diazepam related compound A, not more than 0.1% of diazepam related compound B, and not more than 0.3% of nordazepam are found.

Calculate the percentage of any other impurity in the portion of Diazepam taken by the formula:

$$(C_S / W)(r_i / r_S)$$

in which  $C_S$  is the concentration, in µg per mL, of USP Diazepam Related Compound B RS in the Standard solution;  $r_i$  is the peak response for any other impurity obtained from the Test solution; and  $r_S$  is the peak response of USP Diazepam Related Compound B RS obtained from the Standard solution: not more than 0.1% of any other impurity is found; and not more than 1.0% of the total impurities is found.

### Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and methanol (2:2:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

System suitability solution—Dissolve suitable quantities of USP Nordazepam RS and USP Diazepam RS in methanol, using sonication if necessary, to obtain a solution containing about 0.1 mg of each per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer about 10 mg of Diazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.76 for nordazepam and 1.0 for diazepam; the resolution,  $R$ , between nordazepam and diazepam is not less than 4; the column efficiency is not less than 5000 theoretical plates for the diazepam peak; the tailing factor for diazepam is not more than 2.0; and the relative standard deviation for the diazepam peak for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{16}H_{13}ClN_2O$  in the portion of Diazepam taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Diazepam RS in the Standard preparation; and  $r_U$  and  $r_S$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.



## Diazepam Capsules

» Diazepam Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diazepam ( $C_{16}H_{13}ClN_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Diazepam RS

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one.

$C_{15}H_{11}ClN_2O$  270.72

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer an accurately weighed amount of Capsule contents, equivalent to about 10 mg of diazepam, to a 50-mL centrifuge tube, and add 2 mL of acetone. Place the centrifuge tube in an ultrasonic bath for 5 minutes, and centrifuge. Using 100  $\mu$ L of the supernatant as the test solution, and 100  $\mu$ L of a solution of USP Diazepam RS in acetone containing 5 mg per mL as the Standard solution, proceed as directed for *Identification test B* under *Diazepam*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{16}H_{13}ClN_2O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Diazepam RS in the same *Medium*.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $C_{16}H_{13}ClN_2O$  is dissolved in 45 minutes.

**Uniformity of dosage units (905):** meet the requirements.

### Assay—

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Diazepam*.

*Assay preparation*—Weigh and mix the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the capsule contents, equivalent to about 10 mg of diazepam, to a 100-mL volumetric flask. Add 50 mL of methanol, sonicate for 5 minutes, shake by mechanical means for 5 minutes, dilute with methanol to volume, mix, and filter, discarding the first few mL of the filtrate.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diazepam ( $C_{16}H_{13}ClN_2O$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazepam Extended-Release Capsules

» Diazepam Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diazepam ( $C_{16}H_{13}ClN_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Diazepam RS

USP Ethylparaben RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**B:** Accurately weigh a quantity of Capsule contents, equivalent to 10 mg of diazepam, place in a 50-mL centrifuge tube, and add 2 mL of acetone. Place the centrifuge tube in an ultrasonic bath for 5 minutes, remove from the ultrasonic bath, and centrifuge. Using 100  $\mu$ L of the supernatant as the test solution, and 100  $\mu$ L of a solution of USP Diazepam RS in acetone containing 5 mg per mL as the Standard solution, proceed as directed in *Identification test B* under *Diazepam*.

### Dissolution (711)—

*Medium:* simulated gastric fluid TS, prepared without enzymes; 900 mL.

*Apparatus 1:* 100 rpm.

*Times:* 1, 4, 8, and 12 hours.

*Mobile phase*—Prepare a suitable degassed and filtered mixture of methanol and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Buffer solution*—Dissolve 77.1 g of ammonium acetate in water to make 1000 mL of solution, and adjust with ammonium hydroxide to a pH of 8.7.

*Standard solution*—Dissolve an accurately weighed quantity of USP Diazepam RS in *Medium*, dilute quantitatively with *Medium* to obtain a solution having a known concentration of about 0.15 mg per mL, and mix. Transfer 2.0-, 5.0-, 8.0-, and 10.0-mL aliquots of this solution to separate 100-mL volumetric flasks, add *Medium* to volume, and mix. Pipet 1.0 mL of each solution and 1.0 mL of *Buffer solution* into individual small vials, mix, and allow to stand at room temperature for about 10 minutes.

*Test solution*—Wrap each Capsule in a coil made from a 10-cm piece of 18-gauge copper wire weighing approximately 750 mg, so that the wire encircles the Capsule 4 times. The Capsule enclosed in the coil remains at the bottom of the basket (it should not float). Pass a portion of the solution under test, obtained at each time interval, through a suitable filter having a 0.6- $\mu$ m porosity. Pipet 1.0 mL of each solution and 1.0 mL of *Buffer solution* into individual small vials, mix, and allow to stand at room temperature for about 10 minutes.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm  $\times$  10-cm column that contains packing L1. The flow rate is about 5.0 mL per minute. Chromatograph the appropriate *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Determine the

amount of  $C_{16}H_{13}ClN_2O$  dissolved from peak responses of diazepam obtained from the *Test solution* and the *Standard solution*.

**Tolerances**—The percentage of the labeled amount of  $C_{16}H_{13}ClN_2O$  dissolved is within the range stated at each of the following times.

Time (hours)	Amount dissolved
1	between 15% and 27%
4	between 49% and 66%
8	between 76% and 96%
12	between 85% and 115%

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay* under *Diazepam*.

**Internal standard solution**—Dissolve an accurately weighed quantity of USP Ethylparaben RS in methanol to obtain a solution having a known concentration of about 1.5 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, quantitatively dilute with methanol to obtain a solution having a known concentration of about 1 mg per mL, and mix. Transfer 15.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

**Assay preparation**—Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 15 mg of diazepam, to a 100-mL volumetric flask. Add 5.0 mL of *Internal standard solution* and about 45 mL of methanol. Shake by mechanical means for 30 minutes, dilute with methanol to volume, and mix. Centrifuge about 30 mL of this solution for 5 minutes, and filter.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The tailing factor for diazepam is not more than 2.0; and the relative standard deviation for the diazepam peak for replicate injections is not more than 2.0%. [NOTE—For the purpose of identification, the relative retention times are about 0.5 for ethylparaben and 1.0 for diazepam.] Calculate the quantity, in mg, of diazepam ( $C_{16}H_{13}ClN_2O$ ) in the portion of Capsules taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazepam Injection

» Diazepam Injection is a sterile solution of Diazepam in a suitable medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{13}ClN_2O$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

#### USP Reference standards (11)—

USP Diazepam RS

USP Endotoxin RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**B:** Transfer a volume of Injection, equivalent to about 10 mg of diazepam, to a separator, add 20 mL of water, and shake. Add 20 mL of chloroform, and shake vigorously for 2 minutes. Filter the chloroform layer through about 5 g of anhydrous granular sodium sulfate into a beaker. Wash the sodium sulfate with 20 mL of chloroform, collecting the washing in the beaker. Evaporate the chloroform extract on a steam bath with the aid of a current of air to a volume of about 5 mL. Remove the beaker from the steam bath, and evaporate the chloroform extract with the aid of a current of air to dryness. Dissolve the residue in 20 mL of anhydrous ether, filter, and evaporate the filtrate to dryness using a current of air. Vigorously scrape the resulting oily film with a spatula, and dry in vacuum over phosphorus pentoxide at 60° for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Diazepam RS.

**Bacterial endotoxins** (85)—It contains not more than 11.6 USP Endotoxin Units per mg of diazepam.

**pH** (791): between 6.2 and 6.9.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—[NOTE—Prepare fresh daily.] Prepare a solution of *p*-tolualdehyde in methanol containing about 0.3  $\mu$ L per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.2 mg of USP Diazepam RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of diazepam, to a 50-mL volumetric flask. Pipet 10 mL of *Internal standard solution* into the flask, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.5 for *p*-tolualdehyde and 1.0 for diazepam, the tailing factor for the diazepam peak is not more than 2.5, the resolution,  $R$ , between the *p*-tolualdehyde and diazepam peaks is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (between 10  $\mu$ L and 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Cal-

culate the quantity, in mg, of  $C_{16}H_{13}ClN_2O$  in each mL of the Injection taken by the formula:

$$50C / V(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*,  $V$  is the volume, in mL, of Injection taken, and  $R_U$  and  $R_S$  are the ratios of the peak responses of diazepam to that of *p*-tolualdehyde obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazepam Tablets

» Diazepam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diazepam ( $C_{16}H_{13}ClN_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Diazepam RS

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.

$C_{15}H_{11}ClN_2O$  270.72

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

**B:** Accurately weigh an amount of Tablet mass, equivalent to 10 mg of diazepam, place in a 50-mL centrifuge tube, and add 2 mL of acetone. Place the centrifuge tube in an ultrasonic bath for 5 minutes, and centrifuge. Using 100  $\mu$ L of the supernatant as the test solution, 100  $\mu$ L of a solution of USP Diazepam RS in acetone containing 5 mg per mL as the Standard solution, and a solvent system consisting of equal volumes of ethyl acetate and *n*-heptane, proceed as directed in *Identification test B* under *Diazepam*. The specified result is observed.

### Dissolution (711)—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{13}ClN_2O$  dissolved by employing UV absorption at a wavelength of about 242 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Diazepam RS in the same *Medium*.

**Tolerances**—Not less than 85% ( $Q$ ) of the labeled amount of  $C_{16}H_{13}ClN_2O$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

### Assay—

**Mobile phase, System suitability solution, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Diazepam*.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of diazepam, to a 100-mL volumetric flask. Add about 50 mL of methanol, sonicate for 5 minutes, shake by mechanical means for 5 minutes, dilute with methanol to volume, mix, and filter, discarding the first few mL of the filtrate.

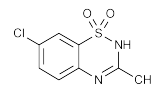
**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diazepam*. Calculate the quantity, in mg, of diazepam

( $C_{16}H_{13}ClN_2O$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazoxide



$C_8H_7ClN_2O_2S$  230.67

2*H*-1,2,4-Benzothiadiazine, 7-chloro-3-methyl-, 1,1-dioxide. 7-Chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide [364-98-7].

» Diazoxide contains not less than 97.0 percent and not more than 102.0 percent of  $C_8H_7ClN_2O_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Diazoxide RS

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Loss on drying (731)**—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition (281):** not more than 0.1%.

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.01 M sodium 1-pentanesulfonate, methanol, and glacial acetic acid (80:20:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 50 mg of hydrochlorothiazide to a 25-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diazoxide RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with a mixture of water and methanol (4:1) to volume, and mix to obtain a solution having a known concentration of about 50  $\mu$ g of USP Diazoxide RS per mL.

**Assay preparation**—Transfer about 50 mg of Diazoxide, accurately weighed, to a 50-mL volumetric flask, add methanol to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with a mixture of water and methanol (4:1) to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte

and internal standard peaks is not less than 5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.4 for hydrochlorothiazide and 1.0 for diazoxide. Calculate the quantity, in mg, of  $C_8H_7ClN_2O_2S$  in the portion of Diazoxide taken by the formula:

$$C(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Diazoxide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazoxide Capsules

» Diazoxide Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_8H_7ClN_2O_2S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Diazoxide RS

**Identification**—

**A:** Place a portion of the contents of Capsules, equivalent to about 50 mg of diazoxide, in a suitable centrifuge tube, add 25 mL of 0.1 N sodium hydroxide, shake for 15 minutes, and centrifuge: the supernatant so obtained responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (17:4:3) being used.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Dissolution** (711)—

**Medium:** pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_8H_7ClN_2O_2S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 268 nm of filtered portions of the solution under test in comparison with a solution of USP Diazoxide RS similarly prepared.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_8H_7ClN_2O_2S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Diazoxide*.

**Assay preparation**—Remove, as completely as possible, the contents of not less than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of diazoxide, to a 100-mL volumetric flask, add 15 mL of water, and shake by mechanical means for 5 minutes. Add 60 mL of methanol, shake by mechanical means for 15 minutes, dilute with methanol to volume, and mix.

Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with a mixture of water and methanol (4:1) to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diazoxide*. Calculate the quantity, in mg, of  $C_8H_7ClN_2O_2S$  in the portion of Capsules taken by the formula:

$$2C(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Diazoxide RS in the *Standard preparation*, and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazoxide Injection

» Diazoxide Injection is a sterile solution of Diazoxide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_8H_7ClN_2O_2S$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Diazoxide RS

USP Endotoxin RS

**Identification**—

**A:** A dilution of the Injection in methanol containing 1 mg of diazoxide per mL responds to the *Thin-layer Chromatographic Identification Test* (201), a solution of USP Diazoxide RS in methanol containing 1 mg per mL being used as the *Standard solution*. Develop the chromatogram with a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (17:4:3).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of diazoxide.

**pH** (791): between 11.2 and 11.9.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Diazoxide*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 45 mg of diazoxide, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with a mixture of water and methanol (4:1) to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diazoxide*. Calculate the quantity, in mg, of  $C_8H_7ClN_2O_2S$  in each mL of the Injection taken by the formula:

$$C / V(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Diazoxide RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diazoxide Oral Suspension

» Diazoxide Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diazoxide ( $C_8H_7ClN_2O_2S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Diazoxide RS

### Identification—

**A:** Place a portion of Oral Suspension, equivalent to about 50 mg of diazoxide, in a 50-mL volumetric flask, add 30 mL of 0.1 N sodium hydroxide, shake for 30 minutes, dilute with 0.1 N sodium hydroxide to volume, and mix: the solution so obtained responds to the *Thin-Layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (17:4:3) being used.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

### Uniformity of dosage units (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

### Assay—

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Diazoxide*.

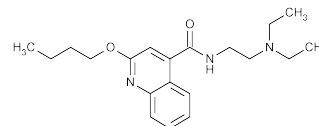
*Assay preparation*—Transfer an accurately measured volume of freshly mixed Oral Suspension, equivalent to about 100 mg of diazoxide, to a 50-mL centrifuge tube, add 2 mL of water and 35 mL of methanol, shake for 15 minutes, and centrifuge for 5 minutes. Transfer the supernatant to a 200-mL volumetric flask. Repeat the extraction process two times, beginning with the addition of 2 mL of water, combine the extracts in the 200-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with a mixture of water and methanol (4:1) to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Diazoxide*. Calculate the quantity, in mg, of diazoxide ( $C_8H_7ClN_2O_2S$ ) in each mL of the Oral Suspension taken by the formula:

$$2(C/V)(R_U / R_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Diazoxide RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dibucaine



$C_{20}H_{29}N_3O_2$  343.46

4-Quinolinecarboxamide, 2-butoxy-N-[2-(diethylamino)ethyl]-

2-Butoxy-N-[2-(diethylamino)ethyl]cinchoninamide [85-79-0].

» Dibucaine contains not less than 97.0 percent and not more than 102.5 percent of  $C_{20}H_{29}N_3O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Dibucaine Hydrochloride RS

### Identification—

**A:** The IR absorption spectrum of a mineral oil dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar dispersion of the residue prepared by dissolving 30 mg of USP Dibucaine Hydrochloride RS in 5 mL of 0.5 N sodium hydroxide, extracting the resulting solution with 5 mL of ether, evaporating the ether, and drying the residue over phosphorus pentoxide.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 62.5° and 66.0°, determined after drying.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Chromatographic purity**—Proceed as directed for *Chromatographic purity* under *Dibucaine Hydrochloride*, except to use a *Test solution* containing 36.2 mg of Dibucaine per mL: the principal spot obtained from the *Test solution* corresponds in  $R_f$  value, color, and intensity to that obtained from the *Standard solution*; the sum of the intensities of any secondary spots, if present in the chromatogram of the *Test solution*, corresponds to not more than 2.0% of that of the principal spot in the chromatogram of the *Standard solution* on the basis of comparison with the spots obtained from the *Comparison solutions*.

### Assay—

*Mobile phase*—Dissolve 1.20 g of sodium lauryl sulfate, 0.20 g of sodium acetate, and 2.0 mL of triethylamine in 300 mL of water. Adjust with glacial acetic acid to a pH of 5.6, add 700 mL of methanol, mix, and pass through a suitable filter having a 0.5- $\mu\text{m}$  or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Solvent mixture*—Prepare a mixture of methanol and water (70:30).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dibucaine Hydrochloride RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL. Pass through a suitable filter having a 0.5- $\mu\text{m}$  or finer porosity.

*Assay preparation*—Transfer about 90 mg of Dibucaine, accurately weighed, to a 100-mL volumetric flask, add *Solvent mixture* to volume, and mix. Pass through a suitable filter having a 0.5- $\mu\text{m}$  or finer porosity.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, determined from the analyte peak, is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{29}N_3O_2$  in the portion of Dibucaine taken by the formula:

$$(343.46/379.93)(100C)(r_U / r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dibucaine Cream

» Dibucaine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dibucaine ( $C_{20}H_{29}N_3O_2$ ) in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

**USP Reference standards** <11>—  
USP Dibucaine Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

**Assay**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Dibucaine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dibucaine Hydrochloride RS in an amount of 0.1 N hydrochloric acid equivalent to 20% of the flask's volume, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Weigh accurately a portion of Cream, equivalent to about 22 mg of dibucaine, transfer to a separator containing 25 mL of ether, and mix to dissolve. Extract successively with two 9-mL portions of 0.1 N hydrochloric acid, combining the extracts in a 100-mL volumetric flask. Extract the ether phase in the separator with 2 mL of water, collecting the aqueous extract in the 100-mL volumetric flask. Dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the

quantity, in mg, of dibucaine ( $C_{20}H_{29}N_3O_2$ ) in the portion of Cream taken by the formula:

$$(343.46/379.93)(100C)(r_U / r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dibucaine Ointment

» Dibucaine Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dibucaine ( $C_{20}H_{29}N_3O_2$ ) in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

**USP Reference standards** <11>—  
USP Dibucaine Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

**Assay**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Dibucaine*.

**Standard preparation**—Transfer about 55 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 24.0 mL of 0.1 N hydrochloric acid, and swirl to dissolve. Dilute with a mixture of methanol and 1.0 N hydrochloric acid (13:12) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

**Assay preparation**—Weigh accurately a portion of Ointment, equivalent to about 50 mg of dibucaine, transfer to a separator containing 50 mL of ether, and mix to dissolve. Extract successively with 50-mL, 40-mL, and 30-mL portions of 0.1 N hydrochloric acid, combining the extracts in a 250-mL volumetric flask. Dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of dibucaine ( $C_{20}H_{29}N_3O_2$ ) in the portion of Ointment taken by the formula:

$$(343.46/379.93)(250C)(r_U / r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dibucaine Hydrochloride

$C_{20}H_{29}N_3O_2 \cdot HCl$  379.92

4-Quinolinescarboxamide, 2-butoxy-*N*-[2-(diethylamino)ethyl]-, monohydrochloride.

2-Butoxy-*N*-[2-(diethylamino)ethyl]cinchoninamide monohydrochloride [61-12-1].

» Dibucaine Hydrochloride contains not less than 97.0 percent and not more than 100.5 percent of  $C_{20}H_{29}N_3O_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Dibucaine Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** A solution of it responds to the tests for *Chloride* (191) when tested as specified for alkaloidal hydrochlorides.

**Loss on drying** (731)—Dry it at 80° for 5 hours: it loses not more than 2.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—Dissolve a suitable quantity of Dibucaine Hydrochloride, accurately weighed, in chloroform to obtain a *Test solution* having a concentration of 40.0 mg per mL. Dissolve a suitable quantity of USP Dibucaine Hydrochloride RS, accurately weighed, in chloroform to obtain a *Standard solution* having a known concentration of about 40 mg per mL. Dilute portions of this solution quantitatively and stepwise with chloroform to obtain three *Comparison solutions* having concentrations of 40, 120, and 200 µg per mL (0.1%, 0.3%, and 0.5%) of the *Standard solution*, respectively. Apply separate 5-µL portions of the five solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, methanol, and ammonium hydroxide (50:30:5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and air-dry. Spray the plate heavily with a 1 in 200 solution of potassium dichromate in dilute sulfuric acid (1 in 5). Place the plate in an oven at 140° for 10 minutes, and view under short-wave-length UV light: the principal spot obtained from the *Test solution* corresponds in  $R_f$  value, color, and intensity to that obtained from the *Standard solution*; the sum of the intensities of any secondary spots, if present in the chromatogram of the *Test solution*, corresponds to not more than 1.0%, and the intensity of any secondary spot does not exceed 0.5% of that of the principal spot in the chromatogram of the *Standard solution* on the basis of comparison with the spots obtained from the *Comparison solutions*.

**Assay**—

*Mobile phase, Solvent mixture, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay under Dibucaine*.

*Assay preparation*—Transfer about 100 mg of Dibucaine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, add *Solvent mixture* to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Dibucaine*. Calculate the quantity, in mg, of

$C_{20}H_{29}N_3O_2 \cdot HCl$  in the portion of Dibucaine Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dibucaine Hydrochloride Injection

» Dibucaine Hydrochloride Injection is a sterile solution of Dibucaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dibucaine hydrochloride ( $C_{20}H_{29}N_3O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and protect from light.

**USP Reference standards** (11)—

USP Dibucaine Hydrochloride RS

USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Place a volume of Injection, equivalent to about 30 mg of dibucaine hydrochloride, in a suitable evaporating dish, and concentrate on a steam bath to a volume of about 10 mL. Transfer the solution to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with four 20-mL portions of ether. Wash the combined ether extracts with 5 mL of water, discarding the washing. Evaporate the ether extracts with the aid of a current of air to dryness, and dry the residue over phosphorus pentoxide for 3 hours: the dibucaine so obtained melts between 62° and 65°.

**Bacterial endotoxins** (85)—It contains not more than 35.7 USP Endotoxin Units per mg of dibucaine hydrochloride.

**pH** (791): between 4.5 and 7.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay under Dibucaine*.

*Standard preparation*—Transfer about 50 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add an accurately measured volume of water, equivalent to the volume of Injection taken to prepare the *Assay preparation*, dilute with methanol to volume, and mix. Where the *Assay preparation* is prepared in a 50-mL volumetric flask, transfer about 25 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add an accurately measured volume of water, equivalent to twice the volume of Injection taken to prepare the *Assay preparation*, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

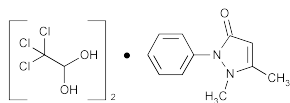
**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of dibucaine, to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. Where the Injection is labeled to contain 1 mg or less of dibucaine hydrochloride per mL, transfer an accurately measured volume of Injection, equivalent to about 13 mg of dibucaine hydrochloride, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5- $\mu$ m or finer porosity.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L, or 20  $\mu$ L where the concentration of dibucaine hydrochloride is about 0.25 mg per mL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of dibucaine hydrochloride ( $C_{20}H_{29}N_3O_2 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$C(v/V)(r_u/r_s)$$

in which C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation*; v is the volume, in mL, of the *Assay preparation*; V is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dichloralphenazone



$C_{15}H_{18}Cl_6N_2O_5$  519.03

1,2-Dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one, compound with 2,2,2-trichloro-1,1-ethanediol (1:2).

Antipyrine, compound with chloral hydrate (1:2) [480-30-8].

» Dichloralphenazone contains not less than 97.0 percent and not more than 100.5 percent of  $C_{15}H_{18}Cl_6N_2O_5$ , determined by both *Assay procedures*.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dichloralphenazone RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** Dissolve 0.1 g of it in 10 mL of water containing 0.1 g of sodium nitrite, and add 1 mL of 2 N sulfuric acid: a green color is produced.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

**Assay**—Perform the following determinations.

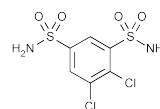
**Assay procedure 1**—Transfer about 1500 mg of Dichloralphenazone, accurately weighed, to a beaker, add 10 mL of water and 20.0 mL of 0.5 N sodium hydroxide VS. Allow to stand for 2 minutes, and titrate with 0.5 N sulfuric acid VS to a colorless phenolphthalein endpoint. Titrate this neutralized solution with 0.1 N silver nitrate VS, using potassium chromate TS as the indicator. To the volume of 0.5 N sulfuric acid used in the first titration add four-fifteenths of the volume of 0.1 N silver nitrate used in the second titration, and subtract the sum obtained from the volume of 0.5 N sodium hydroxide added. Each mL of 0.5 N sodium hydrox-

ide represented by the difference is equivalent to 129.8 mg of  $C_{15}H_{18}Cl_6N_2O_5$ .

**Assay procedure 2**—Transfer about 400 mg of Dichloralphenazone, accurately weighed, to a 125-mL conical flask, add 20 mL of a 1 in 10 solution of sodium acetate, and swirl to dissolve. Add 25.0 mL of 0.1 N iodine VS, and allow to stand in the dark for 20 minutes, gently swirling occasionally. Add 10 mL of chloroform, rinsing the walls of the flask, and swirl until the precipitate is dissolved. Titrate the excess iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank titration (see *Residual Titrations* under *Titrimetry* (541)). The difference between the titrations represents the amount of iodine consumed. Each mL of 0.1 N iodine consumed is equivalent to 25.95 mg of  $C_{15}H_{18}Cl_6N_2O_5$ .

**Calculation**—The difference between the percentages calculated from *Assay procedure 1* and *Assay procedure 2* is not more than 1.5%.

## Dichlorphenamide



$C_6H_6Cl_2N_2O_4S_2$  305.16

1,3-Benzenedisulfonamide, 4,5-dichloro-

4,5-Dichloro-m-benzenedisulfonamide [120-97-8].

» Dichlorphenamide contains not less than 98.0 percent and not more than 101.0 percent of  $C_6H_6Cl_2N_2O_4S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dichlorphenamide RS

**Identification**—

**A:** To 10 mL of a 1 in 10,000 solution in sodium hydroxide solution (1 in 2500) add 0.1 mL of hydrochloric acid: it exhibits absorption maxima at  $295 \pm 2$  nm and at  $286 \pm 2$  nm. The ratio  $A_{295} / A_{286}$  is between 0.90 and 1.00.

**B:** *Infrared Absorption* (197M).

**Melting range** (741): between  $236.5^\circ$  and  $240^\circ$ .

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at  $100^\circ$  to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Chloride** (221)—To 100 mg add 25 mL of 8 N nitric acid, and warm on a steam bath to dissolve. Cool to room temperature: the solution shows no more chloride than corresponds to 0.28 mL of 0.020 N hydrochloric acid (0.20%).

**Selenium** (291): 0.003%, a 100-mg test specimen, mixed with 100 mg of magnesium oxide, being used.

**Heavy metals, Method II** (231): 0.001%.

**Assay**—

**Mobile solvent**—Prepare a suitable solution of 0.02 M monobasic sodium phosphate and 0.02 M dibasic sodium phosphate in acetonitrile and water (approximately 1:1) such that the retention time of Dichlorphenamide is approximately 6 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dichlorphenamide RS in *Mobile solvent* to obtain a solution containing about 1 mg per mL.



**Assay preparation**—Accurately weigh about 50 mg of Dichlorphenamide, and prepare as directed for *Standard preparation*.

**Procedure**—Introduce separately 20- $\mu$ L portions of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* <621>) operated at 25°, by means of a suitable microsyringe or sampling valve, adjusting the operating parameters such that the peak obtained with the *Standard preparation* is full-scale. Typically, the apparatus is fitted with a 4-mm  $\times$  30-cm column, packed with packing L1, is equipped with an UV detector capable of monitoring absorption at 280 nm and a suitable recorder, and is capable of operating at a column pressure of up to 3500 psi. Five replicate injections of the *Standard preparation* show a relative standard deviation of not more than 1.5%. Calculate the quantity, in mg, of  $C_6H_6Cl_2N_2O_4S_2$  in the portion of Dichlorphenamide taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Dichlorphenamide RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the area responses obtained at equivalent retention times from the *Assay preparation* and the *Standard preparation*, respectively.

## Dichlorphenamide Tablets

» Dichlorphenamide Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_6H_6Cl_2N_2O_4S_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Dichlorphenamide RS

### Identification—

**A:** The polarogram of the solution employed for measurement in the *Assay* exhibits a half-wave potential ( $E_{1/2}$ ) within 1% of that of USP Dichlorphenamide RS, similarly measured ( $E_{1/2}$  is about  $-1.42$  volts against a saturated calomel electrode).

**B:** Fuse a quantity of powdered Tablets, equivalent to 200 mg of dichlorphenamide, with 1 pellet of sodium hydroxide: the ammonia fumes produced cause moistened red litmus paper to turn blue. The fusion mixture responds to the tests for *Sulfite* <191>.

### Dissolution <711>—

**Medium:** 0.1 M pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_6H_6Cl_2N_2O_4S_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 285 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium* in comparison with a Standard solution having a known concentration of USP Dichlorphenamide RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_6H_6Cl_2N_2O_4S_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

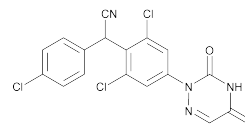
**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of dichlorphenamide, to a 50-mL

volumetric flask. Add about 40 mL of dimethylformamide, shake for 5 minutes to dissolve the dichlorphenamide, and add dimethylformamide to volume. Pipet 10 mL of this suspension into a 100-mL volumetric flask, add 25.0 mL of 0.1 N tetramethylammonium bromide, and dilute with water to volume. Transfer a portion of this solution to a polarographic cell that is immersed in a water bath regulated at 24.5° to 25.5°. Deaerate by bubbling purified nitrogen through the solution for 5 minutes. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from  $-0.80$  volt to  $-1.80$  volts, using a saturated calomel electrode as the reference electrode, and a galvanometer having a sensitivity of about 5 microamperes full scale. Determine the height of the diffusion current at  $-1.65$  volts. Calculate the quantity, in mg, of  $C_6H_6Cl_2N_2O_4S_2$  in the portion of Tablets taken by the formula:

$$500C[(i_d)_U / (i_d)_S]$$

in which  $(i_d)_U$  is the observed diffusion current of the assay solution and  $(i_d)_S$  is that determined similarly in a solution of USP Dichlorphenamide RS the concentration of which is C mg per mL (about 100  $\mu$ g per mL).

## Diclazuril



$C_{17}H_9Cl_3N_4O_2$  407.64  
Benzeneacetonitrile, 2,6-dichloro- $\alpha$ -(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)-;  
(p-Chlorophenyl)[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-*as*-triazin-2(3H)-yl)phenyl]acetonitrile [101831-37-2].

### DEFINITION

Diclazuril contains NLT 97.0% and NMT 101.0% of  $C_{17}H_9Cl_3N_4O_2$ , calculated on the dried basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** <197K>

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 6.3 g of ammonium formate in 800 mL of water, adjust with anhydrous formic acid to a pH of 4.0, and add 200 mL of water.

**Solution A:** Acetonitrile, water, and *Buffer* (3:15:2)

**Solution B:** Acetonitrile, water, and *Buffer* (85:5:10)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	0	100
25	0	100
26	100	0
36	100	0

**Standard solution:** 0.5 mg/mL of USP Diclazuril RS in dimethylformamide

**System suitability solution:** 0.5 mg/mL of USP Diclazuril System Suitability Mixture RS in dimethylformamide

**Sample solution:** 0.5 mg/mL of Diclazuril in dimethylformamide

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC  
 Detector: UV 230 nm  
 Column: 4.6-mm × 10-cm; base-deactivated 3-μm packing L1  
 Flow rate: 1 mL/min  
 Column temperature: 35°  
 Injection size: 5 μL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 1.9 between diclazuril and diclazuril ketone peaks, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of C<sub>17</sub>H<sub>9</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>2</sub> in the portion of Diclazuril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Diclazuril RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Diclazuril in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–101.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

**Organic Impurities**

• **PROCEDURE 1: RESIDUAL SOLVENTS** (467)

Acceptance criteria: NMT 4000 ppm of *N,N*-dimethylformamide

• **PROCEDURE 2**

Buffer, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the area percentage of each impurity, relative to diclazuril, in the portion of Diclazuril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of diclazuril from the *Standard solution*  
 $C_S$  = concentration of USP Diclazuril RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Diclazuril in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria**

[NOTE—Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.5%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Carboxylic acid <sup>a</sup>	0.62	0.85	0.50
6-Carboxamide <sup>b</sup>	0.80	0.92	0.50
Diclazuril	1.00	—	—
Ketone <sup>c</sup>	1.03	0.52	0.10
4-Amino derivative <sup>d</sup>	1.09	0.81	0.50
Des-cyano derivative <sup>e</sup>	1.16	1.1	0.50
Trichlorodiphenyl acetonitrile <sup>f</sup>	1.24	0.71	0.50
Any other individual impurity	—	1.0	0.20

<sup>a</sup> (RS)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxylic acid.

<sup>b</sup> (RS)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamide.

<sup>c</sup> 2-[3,5-Dichloro-4-(4-chlorobenzoyl)phenyl]-1,2,4-triazine-3,5-(2*H*,4*H*)-dione.

<sup>d</sup> (RS)-2-(4-Amino-2,6-dichlorophenyl)-2-(4-chlorophenyl)acetonitrile.

<sup>e</sup> 2-[3,5-Dichloro-4-(4-chlorobenzoyl)phenyl]-1,2,4-triazine-3,5-(2*H*,4*H*)-dione.

<sup>f</sup> (RS)-2-(4-Chlorophenyl)-2-(2,6-dichlorophenyl)acetonitrile.

**SPECIFIC TESTS**

• **LOSS ON DRYING** (731): Dry a sample at between 100° and 105° under a vacuum for 4 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

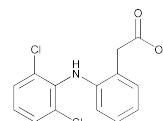
• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** (11)

USP Diclazuril RS

USP Diclazuril System Suitability Mixture RS

Contains diclazuril and specified impurities.

**Diclofenac Potassium**

C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub> 334.24

Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monopotassium salt.

Potassium [o-(2,6-dichloroanilino)phenyl]acetate [15307-81-0].

» Diclofenac Potassium contains not less than 99.0 percent and not more than 101.0 percent of C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Diclofenac Potassium RS

USP Diclofenac Related Compound A RS

N-(2,6-Dichlorophenyl)indolin-2-one.

C<sub>14</sub>H<sub>9</sub>Cl<sub>2</sub>NO 278.14**Identification**—**A:** Infrared Absorption (197K).**B:** Ultraviolet Absorption (197U)—

Solution: 0.01 mg per mL.

Medium: methanol.

**C:** It meets the requirements of the flame test for Potassium (191).**pH** (791): between 7.0 and 8.5, in a 1% aqueous solution.**Loss on drying** (731)—Dry it at 105° under vacuum for 3 hours: it loses not more than 0.5% of its weight.**Heavy metals, Method II** (231): not more than 10 ppm.**Related compounds**—**pH 2.5 Phosphate buffer**—Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate components to a pH of 2.5 ± 0.2.**Mobile phase**—Prepare a filtered and degassed mixture of methanol and pH 2.5 Phosphate buffer (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).**Diluent**—Prepare a mixture of methanol and water (70:30).**Standard solution**—Prepare a solution of USP Diclofenac Related Compound A RS in methanol having a known concentration of about 0.25 mg per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Diluent* to obtain a solution having a known concentration of about 1.5 µg per mL.**Resolution solution**—Prepare a solution in *Diluent* containing 40 µg per mL of diethyl phthalate, 0.5 mg per mL of USP Diclofenac Potassium RS, and 22.5 µg per mL of USP Diclofenac Related Compound A RS.**Test solution**—Transfer about 50 mg of Diclofenac Potassium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for diethyl phthalate, 0.7 for diclofenac related compound A, and 1.0 for diclofenac potassium; and the resolution, *R*, between diethyl phthalate and diclofenac related compound A is not less than 4.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.**Procedure**—Separately inject equal volumes (about 30 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Potassium taken by the formula:

$$10(C/W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Diclofenac Related Compound A RS in the *Standard solution*; *W* is the quantity, in mg, of Diclofenac Potassium taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the diclofenac related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% of diclofenac related compound A is found. Cal-

culate the percentage of each other impurity in the portion of Diclofenac Potassium taken by the formula:

$$10(C/W)(r_i / r_S)$$

in which *r<sub>i</sub>* is the individual peak response of each impurity obtained from the *Test solution*; and the other terms are as defined above: not more than 0.1% of each individual impurity is found, and not more than 0.3% of total impurities is found.

**Assay**—Dissolve about 300 mg of Diclofenac Potassium, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 33.424 mg of C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub>.

**Diclofenac Potassium Tablets**

» Diclofenac Potassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diclofenac potassium (C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub>).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Diclofenac Potassium RS

USP Diclofenac Related Compound A RS

N-(2,6-Dichlorophenyl)indolin-2-one.

C<sub>14</sub>H<sub>9</sub>Cl<sub>2</sub>NO 278.14**Identification**—

**A:** The retention time of the diclofenac peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

**B:** It meets the requirements of the flame test for Potassium (191).

**Dissolution** (711)—

**Medium:** simulated intestinal fluid (without enzyme); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on portions of the solution under test passed through a 0.45-µm filter, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Diclofenac Potassium RS in the same *Medium*. Calculate the percentage of diclofenac potassium (C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub>) dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times LC}$$

in which *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances obtained from the solution under test and the *Standard solution*, respectively; *C<sub>S</sub>* is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg, of diclofenac potassium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>KNO<sub>2</sub> is dissolved in 60 minutes.

**Uniformity of dosage units:** meets the requirements.

**Related compounds—**

*pH 2.5 Phosphate buffer, Mobile phase, Diluent, Resolution solution, and Chromatographic system*—Prepare as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Diclofenac Related Compound A RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.5 µg per mL.

**Test solution**—Use the Assay preparation.

**Procedure**—Separately inject equal volumes (about 30 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of diclofenac related compound A relative to the diclofenac potassium labeled content in the portion of Tablets taken by the formula:

$$100 \times 0.001 (C_A / C_T) (r_U / r_S)$$

in which 0.001 is a conversion coefficient from µg per mL to mg per mL;  $C_A$  is the concentration, in µg per mL, of diclofenac related compound A in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of diclofenac potassium in the *Test solution*, based on the label claim; and  $r_U$  and  $r_S$  are the diclofenac related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% of diclofenac related compound A is found. Calculate the percentage of each of the other impurities, other than diethyl phthalate, if present, relative to the diclofenac potassium labeled content in the portion of Tablets taken by the formula:

$$100 \times 0.001 (C_A / C_T) (r_i / r_S)$$

in which  $r_i$  is the response of an individual impurity peak obtained from the *Test solution*, and the other terms are as defined above: not more than 0.5% of each individual impurity is found, and not more than 1.5% of total impurities is found.

**Assay—**

*pH 2.5 Phosphate buffer*—Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate components to a pH of  $2.5 \pm 0.2$ .

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *pH 2.5 Phosphate buffer* (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Diluent*—Prepare a mixture of methanol and water (70:30).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diclofenac Potassium RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of diclofenac potassium, to a 100-mL volumetric flask. Add about 70 mL of *Diluent*, stir for 60 minutes, dilute with *Diluent* to volume, mix, and centrifuge.

**Resolution solution**—Prepare a solution in *Diluent* containing 40 µg per mL of diethyl phthalate, 0.5 mg per mL of USP Diclofenac Potassium RS, and 37.5 µg per mL of USP Diclofenac Potassium Related Compound A RS.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses

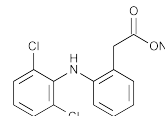
as directed for *Procedure*: the resolution,  $R$ , between diethyl phthalate and diclofenac related compound A is not less than 2.5, and the resolution,  $R$ , between diclofenac related compound A and diclofenac is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of diclofenac potassium ( $C_{14}H_{10}Cl_2KNO_2$ ) in the portion of the Tablets taken by the formula:

$$100 (C_S / C_U) (r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of diclofenac potassium in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of diclofenac potassium in the *Assay preparation*, based on the label claim; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diclofenac Sodium



$C_{14}H_{10}Cl_2NNaO_2$  318.13

Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monosodium salt.

Sodium [o-(2,6-dichloroanilino)phenyl]acetate  
[15307-79-6].

» Diclofenac Sodium contains not less than 99.0 percent and not more than 101.0 percent of  $C_{14}H_{10}Cl_2NNaO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Diclofenac Sodium RS

USP Diclofenac Related Compound A RS

N-(2,6-Dichlorophenyl)indolin-2-one.

$C_{14}H_9Cl_2NO$  278.14

**Identification—**

**A:** *Infrared Absorption* <197K>.

**B:** The retention time of the diclofenac peak in the chromatogram of the *Test solution* corresponds to that of the *Resolution solution* as obtained in the test for *Chromatographic purity*.

**C:** The residue obtained by igniting it responds to the flame test for *Sodium* <191>.

**Color of solution**—A 1 in 20 solution of it in methanol is colorless to faintly yellow, and the absorbance of the solution, determined in a 1-cm cell at 440 nm, is not more than 0.050, methanol being used as the blank.

**Clarity of solution**—The solution prepared as directed under *Color of solution* is not significantly less clear than an equal volume of methanol contained in a similar vessel and examined similarly.

**pH** <791>: between 7.0 and 8.5, in a solution (1 in 100).

**Loss on drying** <731>—Dry it at  $105^\circ$  to  $110^\circ$  for 3 hours: it loses not more than 0.5% of its weight.

**Heavy metals, Method II** (231)—To prepare the *Test Preparation*, use a 100-mL borosilicate glass beaker or a quartz crucible. If the residue is not completely white after the ignition at 500° to 600°, add enough hydrogen peroxide to dissolve it, heat gently until dry, and ignite for 1 hour. Repeat the hydrogen peroxide treatment and ignition until the residue is completely white. Proceed as directed in *Test Preparation*, beginning with "Cool, add 4 mL of 6 N hydrochloric acid." The limit is 0.001%.

#### Chromatographic purity—

**pH 2.5 Phosphate buffer**—Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate component to a pH of  $2.5 \pm 0.2$ .

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and pH 2.5 Phosphate buffer (700:300). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Increasing the proportion of buffer increases resolution.]

**Diluent**—Prepare a mixture of methanol and water (70:30).

**Standard solution**—Prepare a solution of USP Diclofenac Related Compound A RS in methanol having a known concentration of about 0.75 mg per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Diluent* to obtain a solution having a known concentration of about 1.5 µg per mL.

**Resolution solution**—Prepare a solution in *Diluent* containing 20 µg of diethyl phthalate, 7.5 µg of USP Diclofenac Related Compound A RS, and 0.75 mg of USP Diclofenac Sodium RS per mL.

**Test solution**—Transfer about 75 mg of Diclofenac Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column containing packing L7 (end-capped). The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for diethyl phthalate, 0.6 for diclofenac related compound A, and 1.0 for diclofenac; and the resolution,  $R$ , between diethyl phthalate and diclofenac related compound A is not less than 2.2, and that between diclofenac related compound A and diclofenac is not less than 6.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses over a period of 2.5 times the retention time of diclofenac. Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Sodium taken by the formula:

$$10(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Diclofenac Related Compound A RS in the *Standard solution*;  $W$  is the quantity, in mg, of Diclofenac Sodium taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the diclofenac related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.2% is found. Calculate the percentage of each other impurity in the portion of Diclofenac Sodium taken by the formula:

$$10(C/W)(r_i/r_S)$$

in which  $r_i$  is the response of an individual impurity peak obtained from the *Test solution*, and the other terms are as

defined above: not more than 0.2% of any individual impurity is found. The sum of all of the impurities found is not more than 0.5%.

**Assay**—Dissolve about 450 mg of Diclofenac Sodium, accurately weighed, in 25 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.81 mg of  $C_{14}H_{10}Cl_2NNaO_2$ .

## Diclofenac Sodium Delayed-Release Tablets

### DEFINITION

Diclofenac Sodium Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ( $C_{14}H_{10}Cl_2NNaO_2$ ).

### IDENTIFICATION

- **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Sodium (191):** It meets the requirements of the flame test.

### ASSAY

#### • PROCEDURE

**Solution A:** Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate component to a pH of  $2.5 \pm 0.2$ .

**Mobile phase:** Methanol and *Solution A* (7:3)

[NOTE—Increasing the proportion of buffer increases resolution.]

**Diluent:** Methanol and water (7:3)

**System suitability solution:** 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

**Standard solution:** 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

**Sample solution:** Transfer 20 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of 0.75 mg/mL of diclofenac sodium is obtained. Add *Diluent* to about 70% of the capacity of the flask, and shake by mechanical means for NLT 30 min to disintegrate the Tablets. Cool to room temperature, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 0.5-µm or finer pore size, and use the filtrate as the *Sample solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L7 (end-capped)

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks; NLT 6.5 between the diclofenac related compound A and diclofenac peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{10}Cl_2NNaO_2$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of diclofenac from the *Sample solution*  
 $r_S$  = peak response of diclofenac from the *Standard solution*  
 $C_S$  = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION** (711): Proceed as directed for *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B* to determine the amount of  $C_{14}H_{10}Cl_2NNaO_2$  dissolved.

**Acid stage**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm, paddles constructed of (or coated with) polytetrafluoroethylene being used

**Time:** 2 h

**Detector:** UV maxima at about 276 nm

**Standard solution:** Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with a mixture of 0.1 N hydrochloric acid and 5 N sodium hydroxide (900:20) to volume, and mix. This *Standard solution* contains 13.6 µg/mL of USP Diclofenac Sodium RS.

**Sample solution:** At the end of 2 h, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessels, and subject them to the test under *Buffer stage*. To the 0.1 N hydrochloric acid remaining in each vessel, add 20.0 mL of 5 N sodium hydroxide, and stir for 5 min.

**Buffer stage**

**Medium:** pH 6.8 phosphate buffer; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Detector:** UV maxima at about 276 nm

**Solution A:** 76 mg/mL of tribasic sodium phosphate

**pH 6.8 phosphate buffer:** *Solution A* and 0.1 N hydrochloric acid (1:3), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ , if necessary

**Standard solution:** Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask. Add 10.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Buffer stage Medium* to volume, and mix. The final concentration is about 0.0204 mg/mL of diclofenac sodium.

**Sample solution:** Sample per *Dissolution* (711). Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

**Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{14}H_{10}Cl_2NNaO_2$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE**

*Solution A, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system,*

and **System suitability:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.8 mg/mL of USP Diclofenac Related Compound A RS in methanol

**Standard solution:** 4 µg/mL of USP Diclofenac Related Compound A RS from the *Standard stock solution* in *Diluent*

**Analysis:** Measure the peak responses over a period of 40 min.

Calculate the percentage of diclofenac related compound A in relation to the quantity of diclofenac sodium in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of diclofenac related compound A from the *Sample solution*  
 $r_S$  = peak response of diclofenac related compound A from the *Standard solution*  
 $C_S$  = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity other than diethyl phthalate, if present, in relation to the diclofenac sodium in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for each impurity from the *Sample solution*  
 $r_S$  = peak response for each impurity from the *Standard solution*  
 $C_S$  = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 0.5% of diclofenac related compound A; NMT 1.0% of any other individual impurity

**Total impurities:** NMT 1.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Diclofenac Sodium RS  
 USP Diclofenac Related Compound A RS  
 N-(2,6-Dichlorophenyl)indolin-2-one.  
 $C_{14}H_9Cl_2NO$  278.14

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**Diclofenac Sodium Extended-Release Tablets**


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**DEFINITION**

Diclofenac Sodium Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ( $C_{14}H_{10}Cl_2NNaO_2$ ).

**IDENTIFICATION**

- A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 2.0 mg/mL of USP Diclofenac Sodium RS in methanol. [NOTE—Shake by mechanical means for 10 min before makeup to final volume.]

**Sample solution:** Equivalent to 2.0 mg/mL of diclofenac sodium from a portion of the powder (NLT 10 Tablets) in methanol. [NOTE—Sonicate for 10 min, and shake by mechanical means for 10 min before makeup to final volume. Centrifuge this solution, and use the clear supernatant.]

**Developing solvent system:** Methanol, toluene, and glacial acetic acid (8:12:0.1)

**ASSAY**

- PROCEDURE**

[NOTE—Protect the *Standard solution*, *System suitability solution*, and *Sample solution* from light.]

**Diluent:** Methanol and water (7:3)

**Buffer:** 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. Adjust with appropriate component to a pH of 2.5.

**Mobile phase:** Methanol and *Buffer* (7:3)

**Standard solution:** 0.5 mg/mL of USP Diclofenac Sodium RS in *Diluent*

**Resolution solution:** 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

**Sample solution:** Powder NLT 20 Tablets, and transfer a weighed portion of the powder, equivalent to 100 mg of diclofenac sodium, to a 200-mL volumetric flask, and add 150 mL of *Diluent*. Heat on a steam bath for 3–5 min, and sonicate for 20 min. Cool to room temperature, and dilute with *Diluent* to volume. Place the flask in an ice bath for 45 min, shaking occasionally to precipitate out any undissolved waxy material. Pass a portion of the chilled solution through a filter of 0.45-µm or finer pore size. Allow the filtrate to reach room temperature before using.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *Standard solution* and *Resolution solution*

[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks, and NLT 3.8 between the diclofenac related compound A and diclofenac peaks, *Resolution solution*

**Relative standard deviation:** NMT 2.0% for diclofenac, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{10}Cl_2NNaO_2$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of diclofenac from the *Sample solution*

$r_S$  = peak response of diclofenac from the *Standard solution*

$C_S$  = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- DISSOLUTION (711)**

**Test 1**

**Medium:** 0.05 M phosphate buffer, pH 7.5; 900 mL

**Apparatus 2:** 50 rpm; use wire sinkers.

**Times:** 1, 5, 10, 16, and 24 h

**Detector:** UV 276 nm

**Standard solution:** USP Diclofenac Sodium RS in *Medium*

**Analysis:** Pass portions of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

**Tolerances:** The percentages of the labeled amount of  $C_{14}H_{10}Cl_2NNaO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	15%–35%
5	45%–65%
10	65%–85%
16	75%–95%
24	NLT 80%

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus, and Analysis:** Proceed as directed for *Dissolution Test 1*.

**Times:** 1, 2, 4, 6, and 10 h

**Tolerances:** The percentages of the labeled amount of  $C_{14}H_{10}Cl_2NNaO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	NMT 28%
2	20%–40%
4	35%–60%
6	50%–80%
10	NLT 65%

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium and Analysis:** Proceed as directed for *Dissolution Test 1*.

**Apparatus 1:** 100 rpm

**Times:** 2, 4, 8, and 16 h

**Tolerances:** The percentages of the labeled amount of  $C_{14}H_{10}Cl_2NNaO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	22%–42%
4	34%–61%
8	52%–82%
16	NLT 73%

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium and Analysis:** Proceed as directed for *Test 1*.

**Apparatus 1:** 100 rpm

**Times:** 2, 4, 8, and 16 h

**Tolerances:** The percentages of the labeled amount of  $C_{14}H_{10}Cl_2NNaO_2$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	20%–40%
4	35%–55%
8	60%–85%
16	NLT 85%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

**Diluent, Buffer, Mobile phase, Resolution solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard stock solution:** 0.8 mg/mL of USP Diclofenac Related Compound A RS in *Diluent*

**Standard solution:** 4 µg/mL of USP Diclofenac Related Compound A RS, made by diluting a measured volume of *Standard stock solution* with *Diluent*

**System suitability solution:** 0.5 mg/mL of USP Diclofenac Sodium RS in *Diluent*

**System suitability**

**Samples:** *Resolution solution* and *System suitability solution*

[NOTE— The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks; and NLT 3.8 between the diclofenac related compound A and the diclofenac peaks, *Resolution solution*

**Standard deviation:** NMT 2.0% for the diclofenac peak, *System suitability solution*

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for any impurity from the *Sample solution*

$r_S$  = peak response for USP Diclofenac Related Compound A RS from the *Standard solution*

$C_S$  = concentration (mg/mL) of USP Diclofenac Related Compound A RS in the *Standard solution*

$C_U$  = concentration (mg/mL) of diclofenac sodium in the *Sample solution*

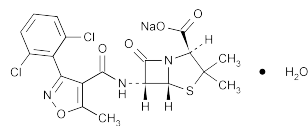
#### Acceptance criteria

Total impurities: NMT 1.5%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature, and protect from light.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
  - USP Diclofenac Sodium RS
  - USP Diclofenac Related Compound A RS
  - N*-(2,6-Dichlorophenyl)indolin-2-one.
  - $C_{14}H_9Cl_2NO$  278.14

## Dicloxacillin Sodium



$C_{19}H_{16}Cl_2N_3NaO_5S \cdot H_2O$  510.32

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2*S*-(2*α*,5*α*,6*β*)]-. Monosodium (2*S*,5*R*,6*R*)-6-[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [13412-64-1].

Anhydrous 492.32 [343-55-5].

» Dicloxacillin Sodium contains the equivalent of not less than 850 µg of dicloxacillin ( $C_{19}H_{17}Cl_2N_3O_5S$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Dicloxacillin Sodium RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** Ignite about 100 mg; a 1 in 20 solution of the residue in acetic acid responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.5 and 7.5, in a solution containing 10 mg per mL.

**Water, Method I** (921): between 3.0% and 5.0%.

**Dimethylaniline** (223): meets the requirement.

**Assay**—

**Diluent**—Dissolve 5.44 g of monobasic potassium phosphate in water to make 2000 mL of solution, and adjust with 8 N potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase**—Prepare a suitable filtered mixture of *Diluent* and acetonitrile (1500:500). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Increasing the acetonitrile concentration decreases the retention time of dicloxacillin.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dicloxacillin Sodium RS in *Diluent* to obtain a solution having a known concentration of about 1.1 mg per mL. [NOTE—Use this *Standard preparation* promptly, or refrigerate and use on the day prepared.]

**Assay preparation**—Transfer about 230 mg of Dicloxacillin Sodium, accurately weighed, to a 200-mL volumetric flask, add *Diluent* to volume, and mix. Stir with the aid of a magnetic stirrer for 5 minutes to ensure dissolution of the specimen. [NOTE—Use this *Assay preparation* promptly, or refrigerate and use on the day prepared.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm  $\times$  25-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the capacity factor, *k'*, for dicloxacillin is between 4 and 11; the column efficiency is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the



quantity, in  $\mu\text{g}$ , of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ) in each mg of the Dicloxacillin Sodium taken by the formula:

$$200(CE / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dicloxacillin Sodium RS in the *Standard preparation*;  $E$  is the dicloxacillin equivalent, in  $\mu\text{g}$  per mg, of USP Dicloxacillin Sodium RS;  $W$  is the weight, in mg, of the portion of Dicloxacillin Sodium taken; and  $r_U$  and  $r_S$  are the dicloxacillin peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dicloxacillin Sodium Capsules

» Dicloxacillin Sodium Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dicloxacillin Sodium RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for dicloxacillin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ) by a suitable validated spectrophotometric analysis of a filtered portion of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Dicloxacillin Sodium RS in the same *Medium*.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.0%.

**Assay**—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Dicloxacillin Sodium*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 10 Capsules, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 200 mg of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ), to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix for 10 minutes with the aid of a magnetic stirrer. Filter about 25 mL of the resulting solution, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*. [NOTE—Use this *Assay preparation* promptly, or refrigerate and use on the day prepared.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Dicloxacillin Sodium*. Calculate the quantity, in mg, of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ) in the portion of Capsule contents taken by the formula:

$$0.2CE(r_U / r_S)$$

in which the terms are as defined therein.

## Dicloxacillin Sodium for Oral Suspension

» Dicloxacillin Sodium for Oral Suspension is a dry mixture of Dicloxacillin Sodium and one or more suitable buffers, colors, flavors, and preservatives. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dicloxacillin Sodium RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for dicloxacillin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 4.5 and 7.5, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 2.0%.

**Assay**—

*Diluent, Mobile phase, and Chromatographic system*—Proceed as directed in the *Assay* under *Dicloxacillin Sodium*.

*Buffer*—Use *Buffer No. 1* (see *Phosphate Buffers and Other Solutions* under *Antibiotics—Microbial Assays* (81)).

**Standard preparation**—Transfer about 100 mg of USP Dicloxacillin Sodium RS, accurately weighed, to a 100-mL volumetric flask; add 20.0 mL of dimethylformamide, 5.0 mL of alcohol, and 20 mL of *Buffer*; and stir for 5 minutes with the aid of a magnetic stirrer. Dilute with *Buffer* to volume, and mix. [NOTE—Use this *Standard preparation* promptly, or refrigerate and use on the day prepared.]

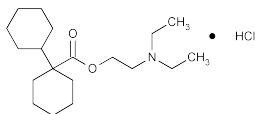
**Assay preparation**—Constitute Dicloxacillin Sodium for Oral Suspension as directed in the labeling. Transfer an accurately measured volume of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 125 mg of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ), to a 200-mL flask. Add 20.0 mL of dimethylformamide and 5.0 mL of alcohol, and stir for 15 minutes. Add 50.0 mL of *Buffer*, and stir for an additional 15 minutes. Add another 50.0 mL of *Buffer*, and stir for a third period of 15 minutes. Centrifuge this mixture for 15 minutes, and filter about 30 mL of the supernatant, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*. [NOTE—Use this *Assay preparation* promptly, or refrigerate and use on the day prepared.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Dicloxacillin Sodium*. Calculate the quantity, in mg, of dicloxacillin ( $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$ ) in each mL of the constituted Dicloxacillin for Oral Suspension taken by the formula:

$$(125 + V)(CE / 1000V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of constituted Dicloxacillin for Oral Suspension taken to prepare the *Assay preparation*; and the other terms are as defined therein.

## Dicyclomine Hydrochloride



$C_{19}H_{35}NO_2 \cdot HCl$  345.95

[Bicyclohexyl]-1-carboxylic acid, 2-(diethylamino)ethyl ester, hydrochloride.  
2-(Diethylamino)ethyl[bicyclohexyl]-1-carboxylate hydrochloride [67-92-5].

» Dicyclomine Hydrochloride contains not less than 99.0 percent and not more than 102.0 percent of  $C_{19}H_{35}NO_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Dicyclomine Hydrochloride RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Mix about 5 mL of a 1 in 500 solution of it with about 2 mL of 2 N nitric acid, and add about 2 mL of silver nitrate TS: a white precipitate is formed which is insoluble in nitric acid but soluble in a slight excess of 6 N ammonium hydroxide.

**Melting range**, Class I (741): between 169° and 174°.  
**pH** (791): between 5.0 and 5.5, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Readily carbonizable substances** (271)—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than Matching Fluid D.

**Assay**—Dissolve about 600 mg of Dicyclomine Hydrochloride, accurately weighed, in 70 mL of glacial acetic acid, add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.60 mg of  $C_{19}H_{35}NO_2 \cdot HCl$ .

## Dicyclomine Hydrochloride Capsules

» Dicyclomine Hydrochloride Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Dicyclomine Hydrochloride RS

### Identification—

**A:** Transfer a portion of the contents of the Capsules, equivalent to about 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Extract the aqueous acid solution with two 30-mL portions of chloroform, transfer the chloroform extracts to a second separator containing 20 mL of water and 1 mL of sodium hydroxide solution (1 in 10), and shake. Filter the chloroform layer through anhydrous sodium sul-

fate into a suitable container, and add 3 mL of a freshly prepared 1 in 20 solution of acetyl chloride in anhydrous methanol, prepared by cautiously adding acetyl chloride dropwise to anhydrous methanol with stirring. Evaporate under reduced pressure at room temperature until the residue has been thoroughly dried: the IR absorption spectrum of a potassium bromide dispersion of the dicyclomine hydrochloride so obtained exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Dicyclomine Hydrochloride RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

Determine the amount of  $C_{19}H_{35}NO_2 \cdot HCl$  dissolved by employing the following method.

*Mobile phase*—Prepare as directed in the *Assay*.

*0.04 M Phosphate buffer, pH 7.5*—Dissolve 2.72 g of monobasic potassium phosphate in 450 mL of water, adjust with 10% sodium hydroxide to a pH of  $7.5 \pm 0.1$ , dilute with water to 500 mL, and mix.

*Buffer-acetonitrile mixture*—Prepare a mixture of 0.04 M Phosphate buffer, pH 7.5 and acetonitrile (1:1).

*Standard solution*—Prepare a solution in *Medium* having a known concentration of about 20 µg per mL of USP Dicyclomine Hydrochloride RS. Transfer 25.0 mL of this solution to a suitable flask, add 25.0 mL of the *Buffer-acetonitrile mixture*, and mix.

*Test solution*—Pass a portion of the solution under test through a 0.7-µm glass microfiber filter. Transfer 5.0 mL of the filtrate to a suitable flask, add 5.0 mL of the *Buffer-acetonitrile mixture*, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column containing 3.5-µm packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 250 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in mg, of  $C_{19}H_{35}NO_2 \cdot HCl$  dissolved.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{19}H_{35}NO_2 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*0.02 M Phosphate buffer, pH 7.5*—Dissolve 2.72 g of monobasic potassium phosphate in 900 mL of water, adjust with 10% sodium hydroxide to a pH of  $7.5 \pm 0.1$ , dilute with water to 1000 mL, and mix.

*Mobile phase*—Prepare a mixture of acetonitrile and 0.02 M Phosphate buffer, pH 7.5 (70:30), filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of acetonitrile and water (70:30).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dicyclomine Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.4 mg per mL. [NOTE—This solution is stable for 2 days.]

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix the

contents. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of dicyclomine hydrochloride, to a 50-mL volumetric flask. Add 2.0 mL of water, and sonicate for at least 2 minutes to disperse the sample. Add 35 mL of acetonitrile, sonicate for at least 5 minutes, and shake by mechanical means for at least 30 minutes. Add 10 mL of water, allow the preparation to equilibrate to room temperature, then dilute with water to volume, and mix. Centrifuge, for at least 5 minutes, a portion of this solution in a 15-mL glass centrifuge tube. Use the clear supernatant.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column containing 3.5-μm packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ) in the portion of Capsules taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dicyclomine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the areas of the dicyclomine peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dicyclomine Hydrochloride Injection

» Dicyclomine Hydrochloride Injection is a sterile, isotonic solution of Dicyclomine Hydrochloride in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** <11>—

USP Dicyclomine Hydrochloride RS

USP Endotoxin RS

**Identification**—

**A:** Transfer a portion of Injection, equivalent to about 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Shake with 25 mL of ether, and discard the ether layer. Proceed as directed in *Identification test A* under *Dicyclomine Hydrochloride Capsules*, beginning with "Extract the aqueous acid solution."

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 17.2 USP Endotoxin Units per mg of dicyclomine hydrochloride.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

0.02 M Phosphate buffer, pH 7.5, *Mobile phase*, *Diluent*, *Standard preparation*, and *Chromatographic system*—Prepare

as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*.

**Assay preparation**—Prepare a composite sample of at least 5 ampules or 2 vials. Transfer an accurately measured volume of the composite sample, equivalent to 20.0 mg of dicyclomine hydrochloride, into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

**Procedure**—Proceed as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*. Calculate the quantity, in mg, of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ) in the portion of Injection taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dicyclomine Hydrochloride RS in the *Standard preparation*; and the other terms are as defined therein.

## Dicyclomine Hydrochloride Oral Solution

» Dicyclomine Hydrochloride Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Dicyclomine Hydrochloride RS

**Identification**—

**A:** Transfer a portion of the Oral Solution, equivalent to about 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Extract with two 30-mL portions of ether, and discard the ether. Proceed as directed in *Identification test A* under *Dicyclomine Hydrochloride Capsules*, beginning with "Extract the aqueous acid solution."

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Assay**—

0.02 M Phosphate buffer, pH 7.5, *Mobile phase*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*.

**Diluent**—Prepare a mixture of 0.02 M Phosphate buffer, pH 7.5 and acetonitrile (65:35).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dicyclomine Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL. [NOTE—This solution is stable for 2 days.]

**Assay preparation**—Using a "to contain" pipet, transfer an accurately measured volume of Oral Solution, equivalent to 10.0 mg of dicyclomine hydrochloride, to a 100-mL volumetric flask. Rinse the pipet with several small portions of *Diluent*, adding the rinsings to the volumetric flask. Dilute with *Diluent* to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*. Calculate the quantity, in mg, of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dicyclomine Hydrochloride RS in the *Standard preparation*; and the other terms are as defined therein.

## Dicyclomine Hydrochloride Tablets

» Dicyclomine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Dicyclomine Hydrochloride RS

### Identification—

**A:** Transfer a portion of finely powdered Tablets, equivalent to about 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Proceed as directed in *Identification* test A under *Dicyclomine Hydrochloride Capsules*, beginning with "Extract the aqueous acid solution."

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

*Mobile phase, 0.04 M Phosphate buffer, pH 7.5, Buffer-acetonitrile mixture, Test solution, and Chromatographic system*—Proceed as directed for *Dissolution* under *Dicyclomine Hydrochloride Capsules*.

*Standard solution*—Prepare a solution in *Medium* having a known concentration of about 40 µg per mL of USP Dicyclomine Hydrochloride RS. Transfer 25.0 mL of this solution to a suitable flask, add 25.0 mL of the *Buffer-acetonitrile mixture*, and mix.

*Procedure*—Proceed as directed for *Dissolution* under *Dicyclomine Hydrochloride Capsules*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{19}H_{35}NO_2 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*0.02 M Phosphate buffer, pH 7.5, Mobile phase, Diluent, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*.

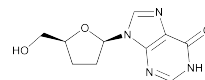
*Assay preparation*—Transfer not fewer than 20 Tablets to a tared container, and determine the average Tablet weight. Grind the Tablets to a fine powder using a glass mortar and pestle. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of dicyclomine hydrochloride, to a 50-mL volumetric flask. Proceed as directed under *Dicyclomine Hydrochloride Capsules* beginning with "Add 2.0 mL of water."

*Procedure*—Proceed as directed in the *Assay* under *Dicyclomine Hydrochloride Capsules*. Calculate the quantity, in mg, of dicyclomine hydrochloride ( $C_{19}H_{35}NO_2 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dicyclomine Hydrochloride RS in the *Standard preparation*; and the other terms are as defined therein.

## Didanosine



$C_{10}H_{12}N_4O_3$

236.23

Inosine, 2',3'-dideoxy-;

2',3'-Dideoxyinosine [69655-05-6].

### DEFINITION

Didanosine contains NLT 98.0% and NMT 102.0% of  $C_{10}H_{12}N_4O_3$ , calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 0.77 g/L of ammonium acetate in water

**Mobile phase:** Acetonitrile and *Buffer* (1:21)

**Standard solution:** 0.1 mg/mL of USP Didanosine RS in water

**Sample solution:** 0.1 mg/mL of Didanosine in water [NOTE—Mix for 1 h to dissolve completely before use.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of didanosine is between 7 and 11 min.]

#### Suitability requirements

**Column efficiency:** NLT 6000 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine ( $C_{10}H_{12}N_4O_3$ ) in the portion of Didanosine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.2%

• **HEAVY METALS, Method II** (231): NMT 20 ppm

#### • RELATED COMPOUNDS

**Buffer:** Prepare as directed in the *Assay*.

**Diluent:** Adjust the pH of the *Buffer* with sodium hydroxide to 9. Prepare a mixture of acetonitrile and *Buffer* (1:19).

**Solution A:** Acetonitrile and *Buffer* (1:19)

**Solution B:** Acetonitrile and *Buffer* (1:3)

**System suitability solution:** 0.5 mg/mL of didanosine from USP Didanosine System Suitability Mixture RS in *Diluent*

**Standard stock solution A:** 0.05 mg/mL of USP Didanosine Related Compound A RS in *Diluent*

**Standard stock solution B:** 0.025 mg/mL of USP Didanosine RS in *Diluent*

**Standard stock solution C:** 0.025 mg/mL of USP Didanosine Related Compound B RS in *Diluent*

**Standard solution:** 5 µg/mL of USP Didanosine Related Compound A RS, 1.5 µg/mL of USP Didanosine RS, and 1.5 µg/mL of USP Didanosine Related Compound B RS from a mixture of *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, respectively, diluted with *Diluent*

**Sample solution:** 0.5 mg/mL of Didanosine in *Diluent*

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
30	0	100
35	100	0
45	100	0

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—Didanosine elutes between 6 and 7.5 min; the relative retention times for didanosine, didanosine related compound A, and didanosine related compound B are 1.0, 0.28, and 2.11, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between didanosine and dideoxydideohydroinosine, *System suitability solution*

**Column efficiency:** NLT 6000 theoretical plates for dideoxydideohydroinosine, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for didanosine related compound A, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine related compound A in the portion of Didanosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of didanosine related compound A from the *Sample solution*

$r_S$  = peak response of didanosine related compound A from the *Standard solution*

$C_S$  = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of all other impurities in the portion of Didanosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity in the *Sample solution*

$r_S$  = peak response for didanosine from the *Standard solution*

$C_S$  = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Didanosine related compound A	0.28	0.5
Inosine	0.39	0.2
2'-Deoxyinosine	0.45	0.3
3'-Deoxyinosine	0.51	0.2
2',3'-Anhydroinosine	0.59	0.2
Dideoxydideohydroinosine	0.81	0.2
Didanosine	1.0	—
Didanosine related compound B	2.1	0.2
5'-Deoxydideoxyadenosine	3.1	0.2
Any other individual, unidentified impurity	—	0.1
Total impurities	—	1.0

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 10 mg/mL in water

**Acceptance criteria:** −28° to −24°, anhydrous

##### • WATER DETERMINATION, *Method I* <921>: NMT 2.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in well-closed containers, and store at controlled room temperature.

##### • USP REFERENCE STANDARDS <11>

USP Didanosine RS

USP Didanosine Related Compound A RS

Hypoxanthine.

USP Didanosine Related Compound B RS

2',3'-Dideoxyadenosine.

USP Didanosine System Suitability Mixture RS

**Add the following:**

### ▲Didanosine Delayed-Release Capsules

#### DEFINITION

Didanosine Delayed-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ).

#### IDENTIFICATION

##### • A. The retention time of major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

##### • B. INFRARED ABSORPTION <197>

Use either methods described under *Infrared Absorption* <197A> or <197K>.

**Sample:** Empty the contents of a suitable number of Capsules, and grind to a fine powder.

#### ASSAY

##### • PROCEDURE

**Buffer:** 0.77 g/L of ammonium acetate in water and pass through a suitable membrane filter of 0.45-µm pore size

**Mobile phase:** Acetonitrile and *Buffer* (35:965)

**Diluent:** 34.8 g/L of dibasic potassium phosphate and adjust with phosphoric acid to a pH of 7.5. Use within two weeks of preparation.

**Standard solution:** 0.1 mg/mL of USP Didanosine RS in water

**Sample stock solution:** Nominally prepare a solution at 0.6 mg/mL to 2.0 mg/mL of didanosine as follows. Mix a composite of the Capsule contents from NLT 20 Capsules in a suitable container, weigh 1 Capsule fill weight, transfer to a suitable size volumetric flask, and add 50% of the final volume of *Diluent*. Stir, shake or sonicate to dissolve. Cool to room temperature, and dilute with *Diluent* to final volume.

**Sample solution:** Nominally 0.1 mg/mL of didanosine in *Diluent* from the *Sample stock solution* and use within 24 h

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of didanosine from the *Sample solution*

$r_S$  = peak response of didanosine from the *Standard solution*

$C_S$  = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of didanosine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **DISSOLUTION** <711>: Proceed as directed for *Method B* under *Procedure*, *Apparatus 1*, *Apparatus 2*, and *Delayed-Release Dosage Forms*.

**Acid stage medium:** 0.1 N hydrochloric acid; 1000 mL

**Buffer 1:** 76 g/L of tribasic sodium phosphate in water

**Buffer stage medium:** 0.1 N hydrochloric acid and 0.2 M tribasic sodium phosphate (3:1). Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 6.8; 1000 mL

**Buffer 2:** 1.36 g/L of monobasic potassium phosphate in water

**Apparatus 1:** 100 rpm

#### Times

**Acid stage:** 2 h

**Buffer stage:** 45 min

**Mobile phase:** Acetonitrile and *Buffer 2* (2:98)

**Sample solution:** Run the *Acid stage*. After the time specified, withdraw a portion of the solution under test, and pass it through a suitable filter. To the filtrate, add a volume of 10 N sodium hydroxide equivalent to 1% of the filtrate volume. Raise the basket. Discard the *Acid stage medium* from the vessels. Rinse the vessel with water. Add the *Buffer stage medium* pre-warmed to the vessel. After the time specified, withdraw a portion of the solution under test and pass it through a suitable filter. Store the acid stage and buffer stage filtrates at 5°.

**Standard stock solution:** 0.8 mg/mL of USP Didanosine RS in water

**Didanosine related compound A standard stock solution:** 0.1 mg/mL of USP Didanosine Related Compound A RS in water

**Standard solution:** Accurately transfer portions of the *Standard stock solution* and the *Didanosine related compound A standard stock solution* to a volumetric flask, and dilute with water to volume to obtain a didanosine final concentration of  $(L/1000)$  mg/mL where  $L$  is the Capsule label claim in mg and 0.01 mg/mL of didanosine related compound A.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 3.9-mm × 5-cm; 5-μm packing L7

**Flow rate:** 1 mL/min

**Sample temperature:** 5°

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both the didanosine and didanosine related compound A peaks

**Relative standard deviation:** NMT 2.0% for the didanosine peak and NMT 5.0% for the didanosine related compound A peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine as calculated based on response of didanosine related compound A released in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (M_{r1}/M_{r2}) \times V \times 100$$

Calculate the percentage of didanosine released in the *Buffer stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

$r_U$  = peak response from the *Sample solution*.

[NOTE—During the *Acid stage*, didanosine is converted to didanosine related compound A so the peak response is the hypoxanthine peak.]

$r_S$  = peak response from the *Standard solution*.

[NOTE—During the *Acid stage*, didanosine is converted to didanosine related compound A so the peak response is the hypoxanthine peak.]

$C_S$  = concentration of didanosine in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$D$  = dilution factor of the *Sample solution*, if applicable

$M_{r1}$  = molecular weight of didanosine, 236.2

$M_{r2}$  = molecular weight of didanosine related compound A, 136.11

$V$  = volume of medium, 1000 mL

#### Tolerances

**Acid stage:** NMT 10% (Q) of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ) is dissolved in 2 h.

**Buffer stage:** NLT 80% (Q) of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ) is dissolved in 45 min.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, Diluent, Sample stock solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 0.1 mg/mL of USP Didanosine RS and 5 μg/mL of USP Didanosine Related Compound A RS in water

**Standard solution:** 5 μg/mL of USP Didanosine Related Compound A RS in water

**System suitability****Sample:** *System suitability solution***Suitability requirements****Tailing factor:** NMT 1.5 for the didanosine peak**Relative standard deviation:** NMT 2.0% for the didanosine peak and NMT 5.0% for the didanosine related compound A peak**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of didanosine related compound A from the *Sample solution* $r_S$  = peak response of didanosine related compound A from the *Standard solution* $C_S$  = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurities in the portion of Capsules taken:

$$\text{Result} = (r_X/r_{SUM}) \times 100$$

 $r_X$  = peak response of each impurity from the *Sample solution* $r_{SUM}$  = peak response of all peaks from the *Sample solution***Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Didanosine related compound A <sup>a</sup>	0.21	2
Didanosine	1.0	—
Any unspecified impurities	—	0.2
Total unspecified impurities	—	0.5
Total impurities	—	2.5

<sup>a</sup> Hypoxanthine.**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is not more than 10<sup>2</sup> cfu/g. The total yeasts and molds count is not more than 10<sup>2</sup> cfu/g.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS** (11)
  - USP Didanosine RS
  - USP Didanosine Related Compound A RS
  - 6-Hydroxypurine (Hypoxanthine), C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O 136.11 ▲USP36

**Didanosine for Oral Solution****DEFINITION**

Didanosine for Oral Solution, when reconstituted as directed in the labeling, yields a 10 mg/mL solution that contains

NLT 90.0% and NMT 110.0% of the labeled amount of didanosine (C<sub>10</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>).**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE****Solution A:** 0.77 mg/mL of ammonium acetate in water**Mobile phase:** Acetonitrile and *Solution A* (1:24)**Standard solution:** 0.1 mg/mL of USP Didanosine RS in water. [NOTE—Use this solution within 24 h of preparation.]**Sample solution:** 0.1 mg/mL obtained by diluting the contents of 1 bottle of Didanosine for Oral Solution in water. [NOTE—Use this solution within 24 h of preparation.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Analytical column:** 4-mm × 25-cm; packing L1**Guard column:** 4.6-mm × 20-cm; packing L1**Flow rate:** 2 mL/min**Injection size:** 20 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Retention time:** Between 7 and 11 min**Column efficiency:** NLT 6000 theoretical plates**Relative standard deviation:** NMT 1.5%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>10</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub> in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of the *Sample solution* $r_S$  = peak response of the *Standard solution* $C_S$  = concentration of USP Didanosine RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of didanosine in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- DELIVERABLE VOLUME** (698): Meets the requirements

**IMPURITIES****Organic Impurities****PROCEDURE****Solution A, Mobile phase, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.**Standard solution:** 5 µg/mL of USP Didanosine Related Compound A RS in water

[NOTE—Use this solution within 48 h of preparation.]

**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine related compound A in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of didanosine related compound A from the *Sample solution* $r_S$  = peak response of didanosine related compound A from the *Standard solution* $C_S$  = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of didanosine in the  
Sample solution (mg/mL)

Acceptance criteria: NMT 1%

### SPECIFIC TESTS

- **WATER DETERMINATION**, Method 1a (921): NMT 3%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 15° and 30°.
- **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of  $C_{10}H_{12}N_4O_3$  in a given volume of Oral Solution obtained after constitution.
- **USP REFERENCE STANDARDS** (11)  
USP Didanosine RS  
USP Didanosine Related Compound A RS  
Hypoxanthine.

## Didanosine Tablets for Oral Suspension

### DEFINITION

Didanosine Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak in the Sample solution corresponds to that of the major peak in the Standard solution, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Buffer:** 0.77 g/L of ammonium acetate in water

**Mobile phase:** Methanol and Buffer (5:95)

**Standard solution:** 0.1 mg/mL of USP Didanosine RS in water. [NOTE—Use this solution within 24 h of preparation.]

**Sample stock solution:** Transfer NLT 5 crushed Tablets for Oral Suspension to a 500-mL volumetric flask. Dissolve in 250 mL of water, dilute with water to volume, and shake for about 10 min.

**Sample solution:** Nominally 0.1 mg/mL of didanosine from the Sample stock solution diluted with water. [NOTE—Use this solution within 72 h of preparation. The Sample solution is stable for a longer time than the Standard solution because of the buffering agents in the Tablets for Oral Suspension.]

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 275 nm

**Columns**

**Guard:** Matching analytical column

**Analytical:** 4-mm × 12.5-cm; 5-μm packing L7

**Flow rate:** 2 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** Standard solution

[NOTE—The retention time of didanosine is more than 3.0 min.]

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Didanosine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of didanosine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Buffer and Mobile phase:** Proceed as directed in the Assay.

**Standard stock solution:** 0.8 mg/mL of USP Didanosine RS in Medium. [NOTE—This solution is stable for 48 h at 5°.]

**Standard solution:** Dilute the Standard stock solution in Medium to obtain a concentration of approximately (L/900), where L is the Tablet label claim.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

**Chromatographic system:** Proceed as directed in the Assay, except disregard the Injection volume requirement, and use equal volumes of the Standard solution and Sample solution, equivalent to 2 μg of didanosine, as the injection volume, and no guard column is used.

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 2.0%.

[NOTE—Didanosine elutes at about 4.8 min.]

#### Analysis

**Samples:** Standard solution and Sample solution

Record the chromatograms for at least 7 min.

Calculate the percentage of didanosine ( $C_{10}H_{12}N_4O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of the Sample solution

$r_S$  = peak response of the Standard solution

$C_S$  = concentration of USP Didanosine RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of didanosine ( $C_{10}H_{12}N_4O_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

### IMPURITIES

#### ORGANIC IMPURITIES

**Buffer:** Proceed as directed in the Assay.

**Mobile phase:** Methanol and Buffer (1:99)

**Standard stock solution:** 0.125 mg/mL of USP Didanosine Related Compound A RS in water. [NOTE—Use this solution within 48 h of preparation.]

**Standard solution:** 1.5 μg/mL of USP Didanosine Related Compound A RS in water from Standard stock solution. [NOTE—Use this solution within 48 h of preparation of the Standard stock solution.]

**Sample stock solution:** Proceed as directed in the Assay.

**Sample solution:** Nominally 0.1 mg/mL from the Sample stock solution diluted with Mobile phase

**Chromatographic system:** Proceed as directed in the Assay, except use an Injection volume of 100 μL.

**Run time:** 30 min

#### System suitability

**Sample:** Standard solution

[NOTE—The retention time of didanosine related compound A is 1.5–2.5 min.]



**Suitability requirements****Column efficiency:** NLT 1000 theoretical plates**Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of didanosine related compound A in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from didanosine related compound A in the *Sample solution* $r_S$  = peak response from didanosine related compound A in the *Standard solution* $C_S$  = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity as follows:

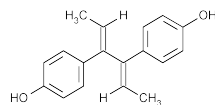
$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response from any other individual impurity in the *Sample solution* $r_T$  = sum of the responses from all the peaks in the *Sample solution*, including those of didanosine and didanosine related compound A**Acceptance criteria****Individual impurities:** NMT 0.7% of didanosine related compound A; NMT 0.2% of any other individual impurity**Total impurities:** NMT 1.2%, excluding didanosine related compound A**SPECIFIC TESTS**• **LOSS ON DRYING** (731)**Sample:** 4 Tablets for Oral Suspension**Analysis:** Dry the *Sample* at 130° for 16 h.**Acceptance criteria:** The Tablets for Oral Suspension lose NMT 6% of their weight.• **ACID-NEUTRALIZING CAPACITY** (301): NLT 17 mEq/Tablet**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 15° and 30°.• **LABELING:** The label states that the Tablets are not to be swallowed whole, and that they may be chewed or dispersed in water before administration.• **USP REFERENCE STANDARDS** (11)

USP Didanosine RS

USP Didanosine Related Compound A RS

Hypoxanthine.

**Dienestrol** $C_{18}H_{18}O_2$  266.33Phenol, 4,4'-(1,2-diethylidene-1,2-ethanediyl)bis-, (E,E)-  
(E,E)-4,4'-(Diethylideneethylene)diphenol [84-17-3;  
13029-44-2].» Dienestrol contains not less than 98.0 percent and not more than 100.5 percent of  $C_{18}H_{18}O_2$ , calculated on the dried basis.**Packaging and storage**—Preserve in well-closed containers.**USP Reference standards** (11)—

USP Dienestrol RS

**Identification**—**A:** *Infrared Absorption* (197K).**B:** *Ultraviolet Absorption* (197U)—*Solution:* 5 µg per mL.*Medium:* alcohol.

Absorptivities at 228 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** To a solution of about 10 mg in 0.5 mL of alcohol add 1 mL of hydrochloric acid and about 50 mg of vanillin: a blue color is produced immediately, and it persists on dilution with water but disappears on the addition of alkali (*distinction from diethylstilbestrol, which produces no color*).**Melting range** (741): between 227° and 234°, but the range between beginning and end of melting does not exceed 3°.**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.**Residue on ignition** (281): not more than 0.2%.**Assay**—*Mobile phase, Internal standard solution, and Standard preparation*—Prepare as directed in the *Assay* under *Dienestrol Cream*.*Assay preparation*—Transfer about 25 mg of Dienestrol, accurately weighed, to a 100-mL volumetric flask. Add methanol to volume, and mix. Pipet 2 mL of this solution, 5 mL of *Internal standard solution*, and 5 mL of water into a 50-mL volumetric flask. Dilute with methanol to volume, and mix.*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dienestrol Cream*. Calculate the quantity, in mg, of  $C_{18}H_{18}O_2$  in the portion of Dienestrol taken by the formula:

$$2.5C(R_U / R_S).$$

**Dienestrol Cream**» Dienestrol Cream is Dienestrol in a suitable water-miscible base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{18}H_{18}O_2$ .**Packaging and storage**—Preserve in collapsible tubes or tight containers.**USP Reference standards** (11)—

USP Dienestrol RS

**Identification**—The chromatogram of the *Assay preparation* employed in the *Assay* exhibits two peaks, for dienestrol and the internal standard, whose retention times are identical to those exhibited by the *Standard preparation*.**Minimum fill** (755): meets the requirements.**Assay**—*Mobile phase*—Prepare a suitable degassed solution of methanol (about 3 in 5) such that the retention time of dienestrol is about 8 to 10 minutes and that of methyltestosterone is about 11 to 14 minutes. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve methyltestosterone in methanol to obtain a solution having a concentration of about 125 µg per mL.

**Standard preparation**—Accurately weigh a suitable quantity of USP Dienestrol RS, dissolve in methanol, and dilute quantitatively and stepwise with methanol to obtain a concentration of about 50 µg per mL. Pipet 10 mL of this solution, 5 mL of *Internal standard solution*, and 5 mL of water to a 50-mL volumetric flask, dilute with methanol to volume, and mix. The concentration of dienestrol in the *Standard preparation* is about 10 µg per mL.

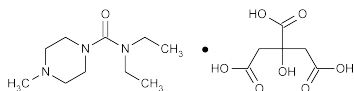
**Assay preparation**—Pipet 20 mL of methanol and 5 mL of *Internal standard solution* into a 50-mL screw-capped tube. Using a 5-mL plastic syringe, transfer an accurately weighed quantity of Cream, equivalent to about 0.5 mg of dienestrol, to the tube. Cap the tube, and disperse the mixture with a suitable vibrating mixer at high speed for 3 minutes, then in an ultrasonic bath for 5 minutes. Add 20 mL of methanol, continue shaking by mechanical means for another 10 minutes, then chill in an ice bath for 5 minutes. Filter the mixture through paper, discarding the first 5 mL of the filtrate.

**Procedure**—Introduce equal volumes (about 50 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph operated at room temperature, by means of a suitable microsyringe or sampling valve. Typically, the apparatus is fitted with a 25-cm × 4.6-mm column containing packing L1 and equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. The *Mobile phase* is maintained at a flow rate of about 2 mL per minute. In a suitable chromatographic system, six replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0% and a resolution factor of not less than 2.0 between the peaks for dienestrol and the internal standard. Calculate the quantity, in mg, of dienestrol (C<sub>18</sub>H<sub>18</sub>O<sub>2</sub>) in the portion of Cream taken by the formula:

$$0.05C(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Dienestrol RS in the *Standard preparation*, and  $R_U$  and  $R_S$  are the peak area ratios of dienestrol to methyltestosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diethylcarbamazine Citrate



C<sub>10</sub>H<sub>21</sub>N<sub>3</sub>O · C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> 391.42  
1-Piperazinecarboxamide, *N,N*-diethyl-4-methyl-, 2-hydroxy-1,2,3-propanetricarboxylate.  
*N,N*-diethyl-4-methyl-1-piperazinecarboxamide citrate (1:1).  
[1642-54-2].

» Diethylcarbamazine Citrate contains not less than 98.0 percent and not more than 102.0 percent of C<sub>10</sub>H<sub>21</sub>N<sub>3</sub>O · C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diethylcarbamazine Citrate RS

**Identification**—

A: It meets the requirements under *Identification—Organic Nitrogenous Bases* (181).

B: It meets the requirements of the test for *Citrate* (191).

**Water**, *Method I* (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals** (231)—Dissolve 1.0 g in 20 mL of water. Add 1 mL of 0.1 N hydrochloric acid, dilute with water to 25 mL, and mix: the limit is 0.002%.

**Ordinary impurities** (466)—

*Test solution*: methanol.

*Standard solution*: methanol.

*Eluant*: a mixture of methanol and ammonium hydroxide (100:1.5).

*Visualization*: 16.

**Chromatographic purity**—

*Phosphate buffer*, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Standard solution**—Prepare a solution of USP Diethylcarbamazine Citrate RS in *Phosphate buffer* having a known concentration of about 0.003 mg per mL.

**Test solution**—Transfer about 300 mg of Diethylcarbamazine Citrate, accurately weighed, to a 100-mL volumetric flask, add 100 mL of *Phosphate buffer*, and mix. Filter or centrifuge, and use the clear filtrate or supernatant.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion of Diethylcarbamazine Citrate taken by the formula:

$$10,000(C / W)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Diethylcarbamazine Citrate RS in the *Standard solution*; W is the weight, in mg, of Diethylcarbamazine Citrate taken to prepare the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for diethylcarbamazine citrate obtained from the *Standard solution*. Not more than 0.1% of any individual impurity is found.

**Assay**—

**Phosphate buffer**—Dissolve 31.24 g of monobasic potassium phosphate in 1000 mL of water.

**Mobile phase**—Dissolve 10 g of monobasic potassium phosphate in 1000 mL of water. Prepare a filtered and degassed mixture of 900 mL of this solution and 100 mL of methanol.

**Standard preparation**—Transfer about 5 mg of USP Diethylcarbamazine Citrate RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix.

**Assay preparation**—Transfer about 5 mg of Diethylcarbamazine Citrate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of  $C_{10}H_{21}N_3O \cdot C_6H_8O_7$  in the portion of Diethylcarbamazine Citrate taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Diethylcarbamazine Citrate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diethylcarbamazine Citrate Tablets

» Diethylcarbamazine Citrate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diethylcarbamazine citrate ( $C_{10}H_{21}N_3O \cdot C_6H_8O_7$ ).  
NOTE—Diethylcarbamazine Citrate Tablets labeled solely for veterinary use are exempt from the requirements of the test for *Dissolution*.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diethylcarbamazine Citrate RS

**Identification**—Tablets meet the requirements under *Identification*—Organic Nitrogenous Bases (181).

**Disintegration** (701)—

FOR TABLETS LABELED SOLELY FOR VETERINARY USE: 30 minutes.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{10}H_{21}N_3O \cdot C_6H_8O_7$  dissolved as directed in the *Assay*, preparing test solutions by quantitatively diluting filtered portions of the solution under test with phosphate buffer (1:1) containing 62.48 g of monobasic potassium phosphate in 1000 mL of water.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{10}H_{21}N_3O \cdot C_6H_8O_7$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Chromatographic purity**—

*Phosphate buffer*, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diethylcarbamazine Citrate*.

*Citric acid solution*—Prepare a solution of citric acid in *Phosphate buffer* containing 2 mg per mL.

*Standard solution*—Prepare a solution of USP Diethylcarbamazine Citrate RS in *Phosphate buffer* having a known concentration of about 0.003 mg per mL.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 300 mg of diethylcarbamazine citrate, to a 100-mL volumetric flask, dilute with *Phosphate buffer* to volume, and mix. Filter or centrifuge, and use the clear filtrate or supernatant.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution*, the *Test solution*, and the *Citric acid solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion Tablets taken by the formula:

$$100(C/3)(r_i / r_S)$$

in which C is the concentration, in mg per mL, of USP Diethylcarbamazine Citrate RS in the *Standard solution*;  $r_i$  is the

peak response for each impurity obtained from the *Test solution*, disregarding any peak having a retention time corresponding to that of the main peak in the chromatogram obtained from the *Citric acid solution*; and  $r_S$  is the peak response obtained from the *Standard solution*. Not more than 0.1% of any individual impurity is found.

**Assay**—

*Phosphate buffer*, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diethylcarbamazine Citrate*.

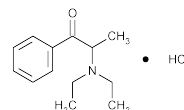
*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of diethylcarbamazine citrate, to a 50-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix.

*Procedure*—Proceed as directed in the *Assay* under *Diethylcarbamazine Citrate*. Calculate the quantity, in mg, of diethylcarbamazine citrate ( $C_{10}H_{21}N_3O \cdot C_6H_8O_7$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which the terms are as defined therein.

## Diethylpropion Hydrochloride



$C_{13}H_{19}NO \cdot HCl$  241.76

1-Propanone, 2-(diethylamino)-1-phenyl-, hydrochloride.

2-(Diethylamino)propiophenone hydrochloride [134-80-5].

» Diethylpropion Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of  $C_{13}H_{19}NO \cdot HCl$ , calculated on the anhydrous basis. It may contain tartaric acid as a stabilizer.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—The label indicates whether it contains tartaric acid as a stabilizer.

**USP Reference standards** (11)—

USP Diethylpropion Hydrochloride RS

**Identification**—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

C: A solution (1 in 100) responds to the tests for *Chloride* (191).

**Water**, *Method I* (921): not more than 0.5%.

**Secondary amines**—Dissolve 100 mg in 2 mL of methylene chloride in a centrifuge tube. Transfer to a second tube 2 mL of a *Standard solution* of diethylamine hydrochloride (dried at 105° for 2 hours before being used) in methylene chloride having a known concentration of 250  $\mu$ g per mL. Treat each solution as follows. Extract with 2 mL of a buffer solution containing 5.7 g of sodium carbonate and 3.0 g of sodium bicarbonate per 100 mL of water. Centrifuge, if necessary, to clarify the upper phase, and immediately transfer 0.5 mL of it to a spot plate. Immediately add 2 drops of acetaldehyde TS, and then, in rapid succession, add 1 drop of sodium nitroferrocyanide solution (1 in 100) to each spot.

Immediately and simultaneously stir both spots to mix the reagents: any blue color produced within 3 minutes by the test solution is not more intense than that of the Standard solution (not more than 0.5% of secondary amines as diethylamine hydrochloride).

**Free bromine**—One drop of a solution (1 in 10) produces no discoloration when placed upon starch iodide paper.

**Limit of hydrobromic acid and bromide**—To 10 mL of a solution (1 in 10) add 1 mL of sodium hydroxide solution (1 in 10), extract with about 25 mL of chloroform, and discard the chloroform extract. Add 1 mL of 6 N hydrochloric acid, 0.5 mL of chloroform, and 0.5 mL of freshly prepared chloramine T solution (1 in 10), and shake vigorously: no yellow or brown-red color is produced in the chloroform layer.

#### Chromatographic purity—

**Phosphate buffer**—Dissolve 136.1 g of monobasic potassium phosphate in 900 mL of water, add 3.2 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

**Diluent**—Prepare a mixture of water, Phosphate buffer, and acetonitrile (8:1:1).

**Mobile phase**—Mix 100 mL of acetonitrile, 100 mL of Phosphate buffer, 7.0 mL of diethylamine, and sufficient water to make 1 L. Filter, and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Test preparation**—Transfer 100 mg of Diethylpropion Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in about 40 mL of Diluent, add Diluent to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in Diluent, and dilute quantitatively, and stepwise if necessary, with Diluent to obtain a solution having a known concentration of about 0.01 mg per mL.

**System suitability solution**—Prepare a solution in Diluent containing about 25 µg of 2-ethylaminopropiophenone hydrochloride and 50 µg of USP Diethylpropion Hydrochloride RS per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for 2-ethylaminopropiophenone and 1.0 for diethylpropion, and the resolution, *R*, between the 2-ethylaminopropiophenone and diethylpropion peaks is not less than 6.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the peak responses. The sum of all of the peak responses, excluding the solvent peak responses and the diethylpropion response, from the *Test preparation* is not greater than the diethylpropion response from the *Standard preparation* (0.5%).

#### Assay—

**Phosphate buffer**—Dissolve 136.1 g of monobasic potassium phosphate in 900 mL of water, add 4.3 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, Phosphate buffer, and 1.0 M sodium nitrate (730:200:50:20), filter through a membrane filter (0.7-µm or finer porosity), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in *Mobile*

*phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.

**Assay preparation**—Transfer about 100 mg of Diethylpropion Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability preparation**—Prepare a solution in *Mobile phase* containing about 200 µg of benzoic acid and 40 µg of USP Diethylpropion Hydrochloride RS per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for diethylpropion hydrochloride and 1.0 for benzoic acid, and the resolution, *R*, between the diethylpropion hydrochloride and benzoic acid peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>13</sub>H<sub>19</sub>NO · HCl in the portion of Diethylpropion Hydrochloride taken by the formula:

$$2.5C(r_u / r_s)$$

in which *C* is the concentration, in µg per mL, of USP Diethylpropion Hydrochloride RS in the *Standard preparation*, and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diethylpropion Hydrochloride Tablets

» Diethylpropion Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>13</sub>H<sub>19</sub>NO · HCl.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Diethylpropion Hydrochloride RS

#### Identification—

**A:** The Tablets meet the requirements under *Identification*—*Organic Nitrogenous Bases* <181>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

#### Dissolution <711>—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of C<sub>13</sub>H<sub>19</sub>NO · HCl dissolved from UV absorbances at the wavelength of maximum absorbance at about 253 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Diethylpropion Hydrochloride RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{13}H_{19}NO \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Phosphate buffer, Mobile phase, and Chromatographic system*—Prepare as directed in the Assay under *Diethylpropion Hydrochloride*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a stock solution having a known concentration of about 160  $\mu g$  per mL. Transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 8  $\mu g$  per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of diethylpropion hydrochloride, to a 250-mL volumetric flask. Add 200 mL of 0.1 N hydrochloric acid, and stir with the aid of a stir bar for 45 minutes. Remove the stir bar, dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 25 mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. If necessary, filter the solution through a 0.7- $\mu m$  porosity membrane filter.

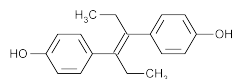
*System suitability preparation*—Dissolve benzoic acid in 0.1 N hydrochloric acid to obtain a solution having a concentration of about 1 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of the stock solution prepared as directed for the *Standard preparation*, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Diethylpropion Hydrochloride*. Calculate the quantity, in mg, of  $C_{13}H_{19}NO \cdot HCl$  in the portion of Tablets taken by the formula:

$$5C(r_U / r_S)$$

in which C is the concentration, in  $\mu g$  per mL, of USP Diethylpropion Hydrochloride RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diethylstilbestrol



$C_{18}H_{20}O_2$  268.35

Phenol 4,4'-(1,2-diethyl-1,2-ethenediyl)bis-, (E)-,  $\alpha, \alpha'$ -Diethyl-(E)-4,4'-stilbenediol [56-53-1].

» Diethylstilbestrol contains not less than 97.0 percent and not more than 100.5 percent of  $C_{18}H_{20}O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**USP Reference standards** (11)—

USP Diethylstilbestrol RS

**Identification**—

**A: Ultraviolet Absorption** (197U)—

*Solution*: 10  $\mu g$  per mL.

*Medium*: alcohol.

Absorptivities at 230 to 350 nm do not differ by more than 3.0%.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 169° and 175°, but the range between beginning and end of melting does not exceed 4°.

**Acidity or alkalinity**—A solution of 100 mg in 5 mL of neutralized 70% alcohol is neutral to litmus.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.05%.

**Assay**—

*Diluent*—Prepare a mixture of alcohol and water (1:1).

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Diethylstilbestrol RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 20  $\mu g$  per mL.

*System suitability solution*—Dissolve 10 mg of USP Diethylstilbestrol RS in 50 mL of chloroform, and allow the solution to stand in the dark for not less than 5 hours. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, and evaporate to dryness under a current of air. Dissolve the residue (the *cis*- and *trans*-isomers of diethylstilbestrol) in *Diluent*, sonicating if necessary. Dilute with *Diluent* to volume, and mix.

*Assay preparation*—Dissolve an accurately weighed quantity of Diethylstilbestrol in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a concentration of about 20  $\mu g$  per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.00 for *trans*-diethylstilbestrol and 1.33 for *cis*-diethylstilbestrol; and the resolution,  $R$ , between *trans*-diethylstilbestrol and *cis*-diethylstilbestrol is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses for the *trans*-isomer as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu L$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the peak responses for the *cis*- and *trans*-isomers of diethylstilbestrol. Calculate the quantity, in  $\mu g$ , of  $C_{18}H_{20}O_2$  in the portion of Diethylstilbestrol taken by the formula:

$$C(r_{t,U} + 1.26r_{c,U}) / (r_{t,S} + 1.26r_{c,S})$$

in which C is the concentration, in  $\mu g$  per mL, of USP Diethylstilbestrol RS in the *Standard preparation*; and  $r_{t,U}$  and  $r_{t,S}$  are the peak responses for the *trans*-isomer obtained from the *Assay preparation* and the *Standard preparation*, respectively; and  $r_{c,U}$  and  $r_{c,S}$  are the peak responses for the *cis*-isomer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diethylstilbestrol Injection

» Diethylstilbestrol Injection is a sterile solution of Diethylstilbestrol in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{18}H_{20}O_2$ .

**Packaging and storage**—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Diethylstilbestrol RS  
USP Endotoxin RS

**Identification**—The absorption spectrum, in the range of 250 to 450 nm, of the yellow solution obtained in the *Assay* after irradiation of the *Assay preparation* exhibits inflections only at the same wavelengths as that of the solution obtained after irradiation of the *Standard preparation*.

**Bacterial endotoxins** (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of diethylstilbestrol.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diethylstilbestrol RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution having a known concentration of about 20 µg per mL. Mix 25.0 mL of this solution with an equal volume of dibasic potassium phosphate solution (1 in 55).

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to not less than 1 mg and not more than 50 mg of diethylstilbestrol, to a 125-mL separator containing 75 mL of isooctane. Extract the solution with one 20-mL and two 10-mL portions of 1 N sodium hydroxide. Wash the combined alkaline extracts with two 10-mL portions of chloroform. Transfer the alkaline solution to a 150-mL beaker, and with the careful addition of 2.5 M phosphoric acid adjust the pH of the solution to 9.5. Transfer the adjusted solution, with the aid of a small volume of water, to a 125-mL separator, and extract with four 20-mL portions of chloroform. Filter the extracts into a 100-mL volumetric flask through a chloroform-wetted pledget of cotton, washing the filter with several small portions of chloroform to adjust the solution to volume. If necessary, dilute the solution quantitatively and stepwise with chloroform so that it contains about 10 µg of diethylstilbestrol per mL. Transfer 20 mL of this solution to a 50-mL conical flask, and evaporate with the aid of gentle heating in a current of air to about 5 mL. Complete the evaporation of the solvent in the air current without further application of heat. Dissolve the residue in 10.0 mL of alcohol, add 10.0 mL of dibasic potassium phosphate solution (1 in 55), and mix.

**Procedure**—[Caution—Protect the eyes from direct rays of UV light throughout this procedure.]

Transfer 4 mL of the *Standard preparation* to a stoppered, 1-cm quartz cell, place about 5 cm from a low-pressure, short-wave mercury lamp rated at from 2 to 20 watts, and irradiate for about 5 minutes. Place the cell in the sample compartment of a spectrophotometer, and measure the absorbance at the wavelength of maximum absorbance at about 418 nm, using water as the blank. Continue irradiation for successive 1- to 3-minute intervals, measuring at 418 nm until the maximum absorbance (about 0.7) has been obtained. If necessary, adjust the geometry of the irradiation apparatus so as to obtain maximum, reproducible absorbance at 418 nm. Similarly irradiate a 4-mL portion of the *Assay preparation*, recording the absorbance at 418 nm, at successive short intervals until maximum absorbance is obtained. Concomitantly determine the absorbances of the

*Assay preparation* and the *Standard preparation* in 1-cm cells at 418 nm, using water as the blank, and subtract these values from those for the respective irradiated solutions to obtain the corrected maximum absorbances. Calculate the quantity, in µg, of diethylstilbestrol ( $C_{18}H_{20}O_2$ ) in each mL of the *Assay preparation* taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Diethylstilbestrol RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the corrected maximum absorbances of the irradiated *Assay preparation* and *Standard preparation*, respectively.

## Diethylstilbestrol Tablets

» Diethylstilbestrol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{18}H_{20}O_2$ .

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Diethylstilbestrol RS

**Identification**—The Tablets respond to *Identification* test B under *Diethylstilbestrol*.

**Disintegration** (701): 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Diluent, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Diethylstilbestrol*.

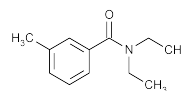
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of diethylstilbestrol, to a 50-mL volumetric flask. Add about 35 mL of *Diluent*, and sonicate until the powder is dissolved (about 2 hours). Dilute with *Diluent* to volume, and mix. Allow the mixture to stand until a clear supernatant is obtained. Pipet 10.0 mL of the supernatant into a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diethylstilbestrol*. Calculate the quantity, in mg, of diethylstilbestrol ( $C_{18}H_{20}O_2$ ) in the portion of Tablets taken by the formula:

$$250C(r_{t,u} + 1.26r_{c,u}) / (r_{t,s} + 1.26r_{c,s})$$

in which the terms are as defined therein.

## Diethyltoluamide



$C_{12}H_{17}NO$  191.27

Benzamide, *N,N*-diethyl-3-methyl-,  
*N,N*-Diethyl-*m*-toluamide [134-62-3].

» Diethyltoluamide contains not less than 95.0 percent and not more than 103.0 percent of

the *meta*-isomer of  $C_{12}H_{17}NO$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diethyltoluamide RS

**Identification**, *Infrared Absorption* (197S): solution; *Assay preparation* versus *Standard preparation* in *Assay*, in spectral region between 8  $\mu m$  and 15  $\mu m$ .

**Specific gravity** (841): between 0.996 and 1.002.

**Refractive index** (831): between 1.520 and 1.524.

**Acidity**—Dissolve 10.0 g in 50 mL of neutralized alcohol, add phenolphthalein TS, and titrate with 0.010 N sodium hydroxide: not more than 4.0 mL of 0.010 N sodium hydroxide is required.

**Water**, *Method I* (921): not more than 0.5%.

**Assay**—Transfer about 200 mg of Diethyltoluamide, accurately weighed, to a 10-mL volumetric flask, dilute with carbon disulfide to volume, and mix to obtain the *Assay preparation*. Concomitantly determine the absorbances of the *Assay preparation* and of a *Standard preparation* of USP Diethyltoluamide RS in carbon disulfide having a known concentration of about 20 mg per mL in 1-mm cells at the wavelength of maximum absorbance at about 14.1  $\mu m$  and at the wavelength of minimum absorbance at about 14.4  $\mu m$ , with a suitable IR spectrophotometer, using carbon disulfide as the blank. Calculate the quantity, in mg, of the *meta*-isomer of  $C_{12}H_{17}NO$  in the Diethyltoluamide taken by the formula:

$$10C(A_{U14.1} - A_{U14.4}) / (A_{S14.1} - A_{S14.4})$$

in which C is the concentration, in mg per mL, of USP Diethyltoluamide RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively, at the wavelengths indicated by the subscripts.

## Diethyltoluamide Topical Solution

» Diethyltoluamide Topical Solution is a solution of Diethyltoluamide in Alcohol or Isopropyl Alcohol. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of the *meta*-isomer of  $C_{12}H_{17}NO$ .

If it contains Alcohol, not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_2H_5OH$  is present.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diethyltoluamide RS

**Identification**—It responds to the *Identification* test under *Diethyltoluamide*.

**Alcohol content** (if present) (611): between 29.0% and 89.0% of  $C_2H_5OH$ .

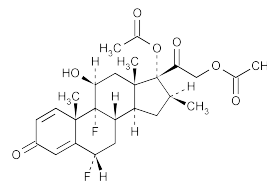
**Assay**—Transfer an accurately weighed quantity of Topical Solution, equivalent to about 200 mg of diethyltoluamide, to a beaker. Place the beaker in a vacuum oven containing silica gel and calcium chloride, and adjusted to a pressure of about 380 mm of mercury, and heat at 35° for 6 hours. Transfer the residue with the aid of carbon disulfide to a 10-mL volumetric flask, and carbon disulfide to volume, and mix. Transfer to a 10-mL volumetric flask about 200 mg of USP Diethyltoluamide RS, accurately weighed, add carbon disulfide to volume, and mix, to obtain the *Standard solu-*

*tion*. Concomitantly determine the absorbances of both solutions in 1-mm cells at the wavelength of maximum absorbance at about 14.1  $\mu m$  and at the wavelength of minimum absorbance at about 14.4  $\mu m$ , with a suitable IR spectrophotometer, using carbon disulfide as the blank. Calculate the quantity, in mg, of the *meta*-isomer of diethyltoluamide ( $C_{12}H_{17}NO$ ) in the portion of Topical Solution taken by the formula:

$$10C(A_{U14.1} - A_{U14.4}) / (A_{S14.1} - A_{S14.4})$$

in which C is the concentration, in mg per mL, of USP Diethyltoluamide RS in the *Standard solution*, and  $A_U$  and  $A_S$  are the absorbances of the solution from Diethyltoluamide Solution and the *Standard solution*, respectively, at the wavelengths indicated by the subscripts.

## Diflorasone Diacetate



$C_{26}H_{32}F_2O_7$  494.52

Pregna-1,4-diene-3,20-dione,17,21-bis(acetoxy)-6,9-difluoro-11-hydroxy-16-methyl-, (6 $\alpha$ ,11 $\beta$ ,16 $\beta$ )-.

6 $\alpha$ ,9-Difluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17,21-diacetate [33564-31-7].

» Diflorasone Diacetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{26}H_{32}F_2O_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diflorasone Diacetate RS

**Identification**, *Infrared Absorption* (197M).

**Specific rotation** (781S): between +58° and +68°.

*Test solution*: 20 mg, undried, per mL, in chloroform.

**Loss on drying** (731)—Dry it in vacuum at 60° and at a pressure not exceeding 5 mm of mercury for 16 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Chromatographic purity**—

*Mobile phase and Chromatographic system*—Prepare as directed in the *Assay*.

*Test solution*—Dissolve an accurately weighed quantity of Diflorasone Diacetate in water-saturated chloroform to obtain a solution having a concentration of about 6 mg per mL.

*Procedure*—Inject a volume (about 10  $\mu L$ ) of the *Test solution* into the chromatograph, record the chromatogram for a period equal to 5 times the retention time of the major peak, and measure the areas of all the peaks. Calculate the percentage of each impurity in the portion of Diflorasone Diacetate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area for each impurity; and  $r_s$  is the sum of the areas of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

**Assay—**

**Mobile phase**—Prepare a solution containing a mixture of water-saturated *n*-butyl chloride, water-saturated methylene chloride, glacial acetic acid, and tetrahydrofuran (350:125:15:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Using water-saturated chloroform, prepare a solution of isoflupredone acetate containing about 0.04 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diflorasone Diacetate RS in *Internal standard solution* to obtain a solution having a known concentration of about 33 µg per mL.

**Assay preparation**—Transfer about 15 mg of Diflorasone Diacetate, accurately weighed, to a 500-mL volumetric flask. Add *Internal standard solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L3. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the resolution, *R*, between the analyte and internal standard peaks is not less than 12; and the relative standard deviation for not less than four replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The relative retention times are about 1.0 for diflorasone diacetate and 2.4 for the internal standard. Calculate the quantity, in mg, of C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub> in the portion of Diflorasone Diacetate taken by the formula:

$$0.5C(R_U / R_S)$$

in which *C* is the concentration, in µg per mL, of USP Diflorasone Diacetate RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak areas for diflorasone diacetate and the internal standard areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diflorasone Diacetate Cream

» Diflorasone Diacetate Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub>.

**Packaging and storage**—Preserve in collapsible tubes, preferably at controlled room temperature.

**USP Reference standards** <11>—

USP Diflorasone Diacetate RS

**Identification**—The chromatogram of the *Assay preparation* exhibits a major peak for diflorasone diacetate at a retention time that corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained in the *Assay*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>—meets the requirements.

**Assay—**

**Mobile phase**, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diflorasone Diacetate*.

**Assay preparation**—Transfer an accurately weighed amount of Cream, equivalent to about 1 mg of diflorasone

diacetate, to a suitable container. Add 30.0 mL of *Internal standard solution*, and shake for about 30 minutes. Centrifuge the solution, and remove and discard the top (excipient) layer. Use the lower, clear chloroform layer.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diflorasone Diacetate*. Calculate the quantity, in mg, of diflorasone diacetate (C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub>) in the portion of Cream taken by the formula:

$$0.03C(R_U / R_S)$$

in which the terms are as defined therein.

## Diflorasone Diacetate Ointment

» Diflorasone Diacetate Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub>.

**Packaging and storage**—Preserve in collapsible tubes, preferably at controlled room temperature.

**USP Reference standards** <11>—

USP Diflorasone Diacetate RS

**Identification**—It responds to the *Identification* test under *Diflorasone Diacetate Cream*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—Proceed with Ointment as directed for *Microbial limits* under *Diflorasone Diacetate Cream*.

**Minimum fill** <755>: meets the requirements.

**Assay—**

**Mobile phase**, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diflorasone Diacetate*.

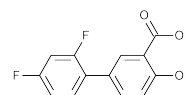
**Assay preparation**—Using Ointment instead of the Cream, proceed as directed for *Assay preparation* in the *Assay* under *Diflorasone Diacetate Cream*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diflorasone Diacetate*. Calculate the quantity, in mg, of diflorasone diacetate (C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub>) in the portion of Ointment taken by the formula:

$$0.03C(R_U / R_S)$$

in which the terms are as defined therein.

## Diflunisal



C<sub>13</sub>H<sub>8</sub>F<sub>2</sub>O<sub>3</sub> 250.20

[1,1'-Biphenyl]-3-carboxylic acid, 2',4'-difluoro-4-hydroxy-, 2',4'-Difluoro-4-hydroxy-3-biphenylcarboxylic acid [22494-42-4].

» Diflunisal contains not less than 98.0 percent and not more than 101.5 percent of C<sub>13</sub>H<sub>8</sub>F<sub>2</sub>O<sub>3</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.



**USP Reference standards** (11)—

USP Diflunisal RS

**Identification**—**A:** *Infrared Absorption* (197M).**B:** *Ultraviolet Absorption* (197U)—*Solution:* 40 µg per mL.*Medium:* hydrochloric acid in methanol (1 in 120).

Absorptivities at 315 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 4 hours: it loses not more than 0.3% of its weight.**Residue on ignition** (281): not more than 0.1%.**Heavy metals, Method II** (231): 0.001%.

**Chromatographic purity**—Prepare a solution of it in methanol containing about 10 mg per mL. Prepare solutions of USP Diflunisal RS in methanol having concentrations of 10, 0.05, and 0.02 mg per mL, respectively (*Standard solutions A, B, and C*). Apply 5-µL portions of all four solutions to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Allow the spots to dry, and develop the chromatogram in a freshly prepared solvent system consisting of a mixture of *n*-hexane, dioxane, and glacial acetic acid (85:10:5) in a paper-lined, equilibrated tank, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under short-wavelength UV light: the chromatograms show principal spots at about the same  $R_f$  value. Estimate the concentration of any spot observed in the chromatogram of the test solution, other than the principal spot, by comparison with the spots in the chromatograms of *Standard solutions B* and *C*: the intensity of any individual spot is not greater than that of the principal spot obtained from *Standard solution C* (0.2%), and the sum of all additional spots is not greater than that of the principal spot obtained from *Standard solution B* (0.5%).

**Assay**—

**Mobile phase**—Prepare a suitable mixture of water, methanol, acetonitrile, and glacial acetic acid (55:23:10:2) such that the retention time of diflunisal is about 18 minutes. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diflunisal RS in a mixture of acetonitrile and water (4:1) to obtain a solution having a known concentration of about 1 mg per mL. Dilute an accurately measured volume of this solution with a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer about 50 mg of Diflunisal, accurately weighed, to a 50-mL volumetric flask. Dilute with a mixture of acetonitrile and water (4:1) to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1 and is maintained at a temperature of 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2500 theoretical plates, the tailing factor is not more than 2.0, the capacity factor is not less than 7.2, and the relative standard deviation for replicate injections is not more than 1%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{13}H_8F_2O_3$  in the portion of Diflunisal taken by the formula:

$$250C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Diflunisal RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses of the major peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Diflunisal Tablets**


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» Diflunisal Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{13}H_8F_2O_3$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Diflunisal RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay*.

**B:** Transfer a quantity of finely ground Tablets, equivalent to about 100 mg of diflunisal, to a 10-mL volumetric flask, add 2 mL of water, and sonicate for 5 minutes. Dilute with methanol to volume, sonicate for an additional 5 minutes, mix, and filter. Separately apply 10 µL each of the filtrate and a *Standard solution* of USP Diflunisal RS in methanol solution (4 in 5) containing 10 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of *n*-hexane, glacial acetic acid, and chloroform (17:3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under long-wavelength UV light: the  $R_f$  value of the principal spot in the chromatogram of the test solution corresponds to that obtained from the *Standard solution*.

**Dissolution** (711)—

**pH 7.20, 0.1 M Tris buffer**—Dissolve 121 g of tris(hydroxymethyl)aminomethane (THAM) in 9 liters of water. Adjust the solution with a 7 in 100 solution of anhydrous citric acid in water to a pH of 7.45, at 25°. Dilute with water to 10.0 liters, equilibrate to 37°, and adjust to a pH of 7.20, if necessary.

**Medium:** pH 7.20, 0.1 M Tris buffer; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{13}H_8F_2O_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 306 nm of filtered portions of the solution under test, suitably diluted with pH 7.20, 0.1 M Tris buffer, in comparison with a *Standard solution* having a known concentration of USP Diflunisal RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{13}H_8F_2O_3$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 200-mL volumetric flask, add 50 mL of water, shake by mechanical means for 30 minutes, and sonicate for 2 minutes. Add 100 mL of alcohol to the flask,

shake by mechanical means for 15 minutes, and sonicate for 2 minutes. Dilute with alcohol to volume, mix, and centrifuge a portion of the solution. Quantitatively dilute an accurately measured volume of the resultant clear supernatant with alcohol, if necessary, to obtain a test solution containing about 1.25 mg per mL. Transfer about 125 mg of USP Diflunisal RS, accurately weighed, to a 100-mL volumetric flask, add 75 mL of alcohol to dissolve, dilute with water to volume, and mix to obtain the Standard solution. Transfer 3.0 mL each of the Standard solution and the test solution to separate 50-mL volumetric flasks. To each flask add 5.0 mL of a solution containing 1 g of ferric nitrate in 100 mL of 0.08 N nitric acid, dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{13}H_8F_2O_3$  in the Tablet by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of diflunisal in the Tablet;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Diflunisal RS in the Standard solution;  $D$  is the concentration, in  $\mu\text{g}$  per mL, of diflunisal in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the test solution and the Standard solution, respectively.

#### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of water, methanol, acetonitrile, and glacial acetic acid (45:40:17:6) such that the retention time of diflunisal is about 8 minutes.

**Standard preparation**—Dissolve a suitable quantity of USP Diflunisal RS in a mixture of acetonitrile and water (60:40) to obtain a solution having a known concentration of about 1.0 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of diflunisal, to a 100-mL volumetric flask containing about 5 mL of water. Sonicate for 5 minutes, add 60.0 mL of acetonitrile, sonicate for an additional 5 minutes, dilute with water to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diflunisal ( $C_{13}H_8F_2O_3$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Diflunisal RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digitalis

» Digitalis is the dried leaf of *Digitalis purpurea* Linné (Fam. Scrophulariaceae). The potency of Digitalis is such that, when assayed as directed, 100 mg is equivalent to not less than 1 USP Digitalis Unit.\*

NOTE—When Digitalis is prescribed, Powdered Digitalis is to be dispensed.

**Packaging, storage, and labeling**—Preserve in containers that protect it from absorbing moisture. Digitalis labeled to indicate that it is to be used only in the manufacture of glycosides is exempt from the moisture and storage requirements.

#### USP Reference standards <11>—

USP Digitalis RS

#### Botanic characteristics—

**Unground Digitalis**—This occurs as more or less crumpled or broken leaves. The leaf blades are ovate, oblong-ovate to ovate-lanceolate, mostly 10 to 35 cm in length and 4 cm to 11 cm in width and contracted into a winged petiole. The apex is obtuse; the margin irregularly crenate or serrate; the lower surface densely pubescent, the upper surface wrinkled and finely hairy. The venation is conspicuously reticulate, the mid-rib and principal veins broad and flat, and the lower veins are continued into the wings of the petiole. The color of the upper surface is dark green, of the lower surface grayish from the dense pubescence, the larger veins often purplish. The odor is slight when dry, peculiar and characteristic when moistened.

**Histology**—Digitalis shows an upper epidermis whose cells possess slightly wavy anticlinal walls, numerous hairs, and no stomata; a lower epidermis with wavy anticlinal walls, numerous oval stomata, and many hairs, and frequently not attached over irregular areas to the cell layer within, especially near the veins; a broad chlorenchyma of a single layer of short palisade cells and several layers of spongy parenchyma; and numerous vascular bundles in the larger veins and petioles, separated by vascular rays one cell in width. On the apex of each marginal tooth one or two water stomata occur.

**Ground Digitalis**—This is dark green in color. Present are chiefly numerous irregular fragments of epidermis and chlorenchyma; nonglandular hairs that are frequently curved or crooked, up to 500  $\mu\text{m}$  in length, uniseriate, two- to eight-celled, some of the cells collapsed so that the planes of adjoining cells may be at right angles, the terminal cell pointed or rounded; few, small glandular hairs, usually with a one- or two-celled stalk, and a one- or two-celled head; fragments of veins and petioles with annular, reticulate, spiral and simple pitted vessels and tracheids. Calcium oxalate is absent.

**Acid-insoluble ash** (561): not more than 5.0%.

**Foreign organic matter** (561)—The proportion of stems, browned leaves, flowers, and other foreign organic matter does not exceed 2.0%.

**Water**, Method III, *Procedure for Articles of Botanical Origin* <921>: not more than 6.0%.

#### Assay—

**Standard preparation**—Weigh the contents of 1 container of USP Digitalis RS to the nearest mg, either in the original container or in a weighing bottle, and transfer to a dry, hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Complete the weighing within 5 minutes after opening the ampul. Add a menstruum consisting of 4 volumes of alcohol and 1 volume of water so

\* One USP Digitalis Unit represents the potency of 100 mg of USP Digitalis RS.

that the total volume of menstruum added corresponds to 10 mL for each g of powder. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture for  $24 \pm 2$  hours at  $25 \pm 5^\circ$  by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter transfer, if necessary, to a centrifuge tube, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle having a tight closure. Preserve under refrigeration, and use within 30 days.

**Assay preparation**—Transfer about 5 g of Digitalis, reduced to a fine powder and accurately weighed, to a hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Proceed as directed under *Standard preparation*, beginning with "Add a menstruum." Preserve under refrigeration, and use within 30 days.

**Pigeons**—Employ adult pigeons free from gross evidence of disease or emaciation, and of such weight that the heaviest weighs less than twice the weight of the lightest. Divide the pigeons into groups as nearly alike as practicable with respect to breed and weight so that the average weight of the group assigned by random choice to the *Standard preparation* shall not differ by more than 30% from the average weight of the group assigned to the preparation to be assayed. Withhold food but not water during the period 16 to 28 hours prior to use. Preparatory to injection, lightly anesthetize the pigeon with ether, and immobilize it; expose an alar vein, and cannulate with a suitable cannula. Maintain the anesthesia during cannulation and throughout the subsequent injection period at such a level that pain is absent, the pupillary and corneal reflexes are present, and the voluntary musculature is not relaxed beyond permitting the pigeon to make some voluntary movement occasionally.

**Preparation of test dilutions**—On the day of the assay, dilute portions of the *Standard preparation* and of the preparation to be assayed (*Assay preparation*) with isotonic sodium chloride solution in such a way that the estimated fatal dose of each dilution will be 15 mL per kg of body weight.

**Injection of test dilutions**—Arrange to inject the appropriate test dilution by suitable means such as a small-bore buret calibrated to 0.05 mL. Start the injection after ensuring the absence of air bubbles from the injection apparatus, by infusing, within a few seconds, a volume of the test dilution equivalent to 1 mL per kg of body weight. Repeat this dose at 5-minute intervals thereafter until the pigeon dies of cardiac arrest.

Use a total of not less than 6 pigeons for the *Standard preparation* and not less than 6 pigeons for the preparation to be assayed. If the average number of doses for any given dilution required to produce death is less than 13 or greater than 19, or if the larger exceeds the smaller in the same assay by more than 4 doses, regard these data as preliminary. Use them as a guide, and repeat with a fresh, higher or lower dilution. Complete the assay within the period of 30 days for preservation of the *Standard preparation* and *Assay preparation*.

**Calculation of potency**—Tabulate and average the number of doses of the *Standard preparation*, designating the average  $\bar{z}_s$ , and likewise obtain the corresponding average,  $\bar{z}_u$ , for the *Assay preparation*. Compute the potency in USP Digitalis Units per mL (i.e., per 100 mg) of the *Assay preparation* as:

$$\text{Potency} = \bar{z}_s R / \bar{z}_u$$

where  $R$  equals  $v_s / v_u$ , in which  $v_s$  is the number of USP Digitalis Units per mL of *Standard preparation* dilution, and  $v_u$  is the volume, in mL, of *Assay preparation* per mL of dilution. Compute the confidence interval,  $L$  (see Equation (31) under *Confidence Intervals for Individual Assays in Design and Analysis of Biological Assays* (111)). If  $L$  exceeds 0.30, repeat the assay or inject more pigeons with one or both preparations until the confidence interval is 0.30 or less.

The potency of Digitalis, calculated from that of the *Assay preparation*, is satisfactory if the result is not less than 0.85 USP Digitalis Unit per 100 mg.

## Powdered Digitalis

» Powdered Digitalis is Digitalis dried at a temperature not exceeding  $60^\circ$ , reduced to a fine or a very fine powder, and adjusted, if necessary, to conform to the official potency by admixture with sufficient Lactose, Starch, or exhausted marc of digitalis, or with Powdered Digitalis having either a lower or a higher potency.

The potency of Powdered Digitalis is such that, when assayed as directed, 100 mg is equivalent to 1 USP Digitalis Unit.\*

NOTE—When Digitalis is prescribed, Powdered Digitalis is to be dispensed.

**Packaging and storage**—Preserve in tight, light-resistant containers. A package of a suitable desiccant may be enclosed in the container.

### USP Reference standards (11)—

USP Digitalis RS  
USP Digitoxin RS  
USP Gitoxin RS  
 $C_{41}H_{64}O_{14}$  780.96

### Identification—

**A:** It conforms to the description for *Ground Digitalis* in the section *Botanic characteristics* under *Digitalis*.

**B:** Transfer 100 mg to a 15-mL centrifuge tube containing 2.0 mL of diluted alcohol and 1.0 mL of lead acetate TS, mix, shake, and boil for 2 minutes. Centrifuge, decant the supernatant into a second 15-mL centrifuge tube, add 2.0 mL of chloroform, and mix. Centrifuge, then remove the lower layer, and filter it through a chloroform-washed small column of anhydrous sodium sulfate (100 to 300 mg) into a 5-mL centrifuge tube. Evaporate the chloroform solution under a stream of nitrogen to dryness, and dissolve the residue in 100  $\mu$ L of a mixture of methanol and chloroform (1:1). Prepare a Standard solution in the same manner, using 100 mg of USP Digitalis RS (*Standard solution A*). Prepare a second Standard solution by dissolving USP Digitoxin RS and USP Gitoxin RS in a mixture of methanol and chloroform (1:1) such that the final concentration of each is approximately 0.2 mg per mL (*Standard solution B*). Apply 10  $\mu$ L of the test solution, 10  $\mu$ L of *Standard solution A*, and 10  $\mu$ L of *Standard solution B*, each as a narrow band about 15 mm long, to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the bands to dry. Develop the chromatogram in a saturated chamber, using a solvent system consisting of a mixture of ethyl acetate, methanol, and water (30:4:3) until the solvent front has moved about 15 cm from the origin. Mix 10 mL of chloramine T solution (3 in 100) with 40 mL of a 1 in 4 solution of trichloroacetic acid in alcohol (store the mixture in a cool place, and use it within 1 week), and spray the air-dried chromatographic plate with this mixture. Heat the plate at  $110^\circ$  for 15 to 20 minutes, and examine it under long-wavelength UV light. Locate the 2 prominent bands obtained from *Standard solution A* corresponding in  $R_f$  value to the 2 bands obtained from *Standard solution B*. The chromatogram obtained from the solution under test shows bands corresponding to them, and also shows bands corresponding to the 3 other bands most prominent in the chromato-

\* One USP Digitalis Unit represents the potency of 100 mg of USP Digitalis RS.

gram from *Standard solution A* but of lower  $R_F$  value. Relative  $R_F$  values for the 5 bands are: 1.0 (digitoxin); 0.8 to 0.9 (gitoxin); 0.6 to 0.7; 0.4 to 0.5; and 0.3 to 0.4.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Salmonella* species.

**Water**, *Method III, Procedure for Articles of Botanical Origin* (921): not more than 5.0%.

**Acid-insoluble ash** (561): not more than 5.0%.

**Assay**—Proceed with Powdered Digitalis as directed in the *Assay under Digitalis*.

The potency of Powdered Digitalis, calculated from that of the *Assay preparation*, is satisfactory if the result is not less than 0.85 USP Digitalis Unit and not more than 1.20 USP Digitalis Units per 100 mg.

## Digitalis Capsules

» Digitalis Capsules contain an amount of Powdered Digitalis equivalent to not less than 85.0 percent and not more than 120.0 percent of the labeled potency.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Digitalis RS

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Salmonella* species.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Standard preparation*—Prepare as directed in the *Assay under Digitalis*.

*Assay preparation*—Empty the contents of not less than 20 Capsules into a hard-glass, glass-stoppered container of not less than 50-mL capacity. Add a menstruum consisting of 4 volumes of alcohol and 1 volume of water so that the total volume of menstruum corresponds to 1 mL for each expected USP Digitalis Unit. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture at  $25 \pm 5^\circ$  for  $24 \pm 2$  hours by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter transfer to a centrifuge tube, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle having a tight closure. Preserve under refrigeration, and use within 30 days.

*Pigeons, Preparation of test dilutions, Injection of test dilutions, and Calculation of potency*—Proceed as directed in the *Assay under Digitalis*.

## Digitalis Tablets

» Digitalis Tablets contain an amount of Powdered Digitalis equivalent to not less than 85.0 percent and not more than 120.0 percent of the labeled potency.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Digitalis RS

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Salmonella* species.

**Disintegration** (701): 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

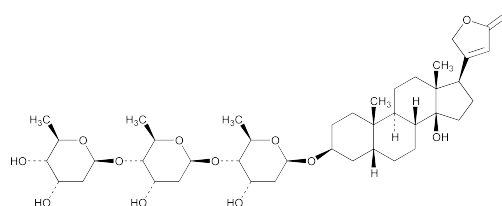
**Assay**—

*Standard preparation*—Prepare as directed in the *Assay under Digitalis*.

*Assay preparation*—Weigh and finely powder not fewer than 25 Tablets. Weigh accurately a portion of the powder, equivalent to not less than 20 Tablets. Transfer to a dry, hard-glass, glass-stoppered container of not less than 50-mL capacity. Proceed as directed for the *Assay preparation* in the *Assay under Digitalis Capsules*, beginning with "Add a menstruum."

*Pigeons, Preparation of test dilutions, Injection of test dilutions, and Calculation of potency*—Proceed as directed in the *Assay under Digitalis*.

## Digitoxin



$C_{41}H_{64}O_{13}$  764.94

Card-20(22)-enolide, 3-[(O-2,6-dideoxy- $\beta$ -D-ribohexopyranosyl-(1 $\rightarrow$ 4)-O-2,6-dideoxy- $\beta$ -D-ribohexopyranosyl-(1 $\rightarrow$ 4)-2,6-dideoxy- $\beta$ -D-ribohexopyranosyl)oxy]-14-hydroxy, (3 $\beta$ ,5 $\beta$ )-.

Digitoxin [71-63-6].

» Digitoxin is a cardiotonic glycoside obtained from *Digitalis purpurea* Linné, *Digitalis lanata* Ehrhart (Fam. Scrophulariaceae), and other suitable species of *Digitalis*. Digitoxin contains not less than 92.0 percent and not more than 103.0 percent of  $C_{41}H_{64}O_{13}$ , calculated on the dried basis.

**Caution**—Handle Digitoxin with exceptional care since it is highly potent.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Digitoxin RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** Prepare a test solution in methanol containing 1 mg per mL. Apply 1  $\mu$ L of the test solution and 1  $\mu$ L of a Standard solution of USP Digitoxin RS in methanol containing 1 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride and methanol (93:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at  $100^\circ$  to remove the solvent. Spray the plate with a 6 in 10 solution of sulfuric acid in methanol, heat at  $105^\circ$  for 10 minutes, and examine the chromatogram under long-wavelength UV light: the  $R_F$  value of the principal spot ob-

tained from the test solution corresponds to that obtained from the Standard solution.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it in vacuum at 105° for 1 hour: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): negligible, from 100 mg.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Digitoxin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.

**Assay preparation**—Transfer about 50 mg of Digitoxin, accurately weighed, to a 200-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 4 mL of this solution into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability preparation**—Prepare a solution in *Mobile phase* containing about 40 µg each of digitoxin and digoxin per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 218-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.35 for digoxin and 1.0 for digitoxin; the resolution, *R*, between the digoxin and digitoxin peaks is not less than 2.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub> in the portion of Digitoxin taken by the formula:

$$1.25C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digitoxin Injection

» Digitoxin Injection is a sterile solution of Digitoxin in 5 to 50 percent (v/v) of alcohol, and may contain Glycerin or other suitable solubilizing agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub>.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Digitoxin RS

USP Endotoxin RS

#### Identification—

**A:** To a portion of Injection, equivalent to about 1 mg of digitoxin, add 10 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform extract on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 2 mL of a solution prepared by mixing 0.3 mL of ferric chloride TS and 50 mL of glacial acetic acid, and underlay with 2 mL of sulfuric acid: at the zone of contact of the two liquids a brown color, which gradually changes to light green, then to blue, is produced, and finally the entire acetic acid layer acquires a blue color.

**B:** To a portion of Injection, equivalent to about 0.2 mg of digitoxin, add 10 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform extract on a steam bath with the aid of a current of air to dryness. Add 2 mL of a freshly prepared 1 in 100 solution of *m*-dinitrobenzene in alcohol, and allow to stand for 10 minutes with frequent shaking. Add 2 mL of a 1 in 200 solution of tetramethylammonium hydroxide in alcohol, and mix: a red-violet color develops slowly and then fades.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 111.0 USP Endotoxin Units per mg of digitoxin.

**Alcohol content** (611): between 90.0% and 110.0% of the labeled percentage of C<sub>2</sub>H<sub>5</sub>OH.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Digitoxin*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of digitoxin, to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Digitoxin*. Calculate the quantity, in µg, of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub> in each mL of the Injection taken by the formula:

$$25(C / V)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digitoxin Tablets

» Digitoxin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub>.

**NOTE**—Avoid the use of strongly adsorbing substances, such as bentonite, in the manufacture of Digitoxin Tablets.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Digitoxin RS

#### Identification—

**A:** Transfer a quantity of finely powdered Tablets, equivalent to not less than 1 mg of digitoxin, to a suitable flask, add 20 mL of chloroform, and sonicate. Filter, and evapo-

rate the filtrate on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 2 mL of a solution prepared by mixing 0.3 mL of ferric chloride TS and 50 mL of glacial acetic acid, and underlay with 2 mL of sulfuric acid: at the zone of contact of the two liquids a brown color, which gradually changes to light green, then to blue, is produced, and finally the entire acetic acid layer acquires a blue color.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Dissolution** (711)—[NOTE—Throughout this procedure, use scrupulously clean glassware, which previously has been rinsed successively with hydrochloric acid, water, and alcohol, and carefully dried. Take precautions to prevent contamination from fluorescent particles and from metal and rubber surfaces.]

*Medium:* dilute hydrochloric acid (3 in 500); 500 mL. [NOTE—Use the same batch of *Medium* throughout the test.]

*Apparatus 1:* 120 ± 5 rpm.

*Times:* 30 minutes; 60 minutes.

*Standard stock solution*—Weigh accurately about 30 mg of USP Digitoxin RS, dissolve in a minimum amount of alcohol in a 500-mL volumetric flask, add dilute alcohol (4 in 5) to volume, and mix.

*Standard solutions*—Just prior to use, dilute 5.0 mL of the *Standard stock solution* with *Medium* to 500.0 mL, and mix. Transfer aliquots (2.0 to 10.0 mL) of this solution to individual separators to prepare standards equivalent to 20, 40, 60, 80, and 100% of the labeled amount of digitoxin in 500 mL. Add *Medium* to make 10 mL, and proceed as directed for *Procedure*, beginning with "Extract with three 15-mL portions of chloroform."

*Procedure*—Proceed as directed for *Procedure* under *Disso-*  
*lution* (711). After 30 minutes, accurately timed, withdraw a suitable aliquot of the solution under test from a point midway between the stirring shaft and the wall of the vessel, and approximately midway in depth. Filter the solution promptly after withdrawal, using a suitable membrane filter of not greater than 0.8-μm porosity, discarding the first 10 mL of the filtrate. Without replacing the *Medium* withdrawn, continue to rotate the basket, and after an additional 30 minutes, accurately timed, similarly withdraw and filter another aliquot. Treat each of these solutions as follows: Assuming dissolution of 100% of the labeled amount of digitoxin, transfer aliquots, equivalent to 6 μg of digitoxin, to suitable separators. Extract with three 15-mL portions of chloroform, and combine the chloroform extracts in glass-stoppered flasks. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. In a similar manner, prepare a blank using a suitable volume of *Medium*.

*Measurement of fluorescence*—Begin with the *Standard solutions*, and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each set. Treat 1 flask at a time as follows: Add 10 mL of a solution freshly prepared by dissolving 35 mg of ascorbic acid in 25 mL of methanol and cautiously adding the solution to 100 mL of hydrochloric acid. Mix, and add 1 mL of a solution freshly prepared by diluting 1 mL of 30 percent hydrogen peroxide with water to 500 mL and diluting 1 volume of the resulting solution with 20 volumes of water. Mix, and insert the stopper in the flask. After 45 minutes, measure the fluorescence at about 575 nm, the excitation wavelength being about 395 nm. Correct each reading for the blank, and plot a standard curve of fluorescence versus percentage dissolution. By calculation from the standard curve, determine the percentage dissolution of digitoxin in each Tablet within 30 minutes and the total percentage dissolution of digitoxin within 60 minutes, taking into account the volume of the solution under test removed after the first 30 minutes of the test.

**Tolerances**—Not less than 60% of the labeled amount of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub> is dissolved within 30 minutes for each Tablet tested, and not less than 85% of the labeled amount is dissolved within 60 minutes for the average of the Tablets tested.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Place 1 Tablet in a suitable glass-stoppered conical flask. Add an accurately measured volume of *Mobile phase* (prepared as directed in the *Assay* under *Digitoxin*) sufficient to obtain a solution containing about 10 μg of digitoxin per mL, and shake by mechanical means until the Tablet has completely disintegrated (not less than 30 minutes). Centrifuge, and use the clear supernatant as the test solution. Dissolve an accurately weighed quantity of USP Digitoxin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a Standard solution having a known concentration of about 10 μg per mL. Proceed as directed in the *Assay*. Calculate the quantity, in mg, of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub> in the Tablet by the formula:

$$(LC / D)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of digitoxin in the Tablet, *C* is the concentration, in μg per mL, of USP Digitoxin RS in the Standard solution, *D* is the concentration, in μg per mL, of digitoxin in the test solution based on the labeled quantity in the Tablet and the extent of dilution, and *r<sub>U</sub>* and *r<sub>S</sub>* are the digitoxin peak responses obtained from the test solution and the Standard solution, respectively.

#### Assay—

*Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Digitoxin*.

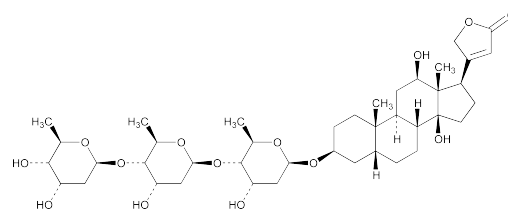
*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of powder, equivalent to about 1 mg of digitoxin, to a 25-mL volumetric flask. Add 15 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution, discarding the first few mL of the filtrate. The filtrate is the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Digitoxin*. Calculate the quantity, in μg, of C<sub>41</sub>H<sub>64</sub>O<sub>13</sub> in the portion of Tablets taken by the formula:

$$25C(r_U / r_S)$$

in which *C* is the concentration, in μg per mL, of USP Digitoxin RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digoxin



C<sub>41</sub>H<sub>64</sub>O<sub>14</sub> 780.94

Card-20(22)-enolide, 3-[(O-2,6-dideoxy-β-D-ribohexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribohexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribohexopyranosyl)oxy]-12,14-dihydroxy-, (3β,5β,12β)-.

Digoxin.

$3\beta$ -[(O-2,6-Dideoxy- $\beta$ -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4)-O-2,6-dideoxy- $\beta$ -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4))-2,6-dideoxy- $\beta$ -D-ribo-hexopyranosyl]oxy]-12 $\beta$ ,14-dihydroxy-5 $\beta$ -card-20(22)-enolide [20830-75-5].

» Digoxin is a cardiotonic glycoside obtained from the leaves of *Digitalis lanata* Ehrhart (Fam. Scrophulariaceae). It contains not less than 95.0 percent and not more than 101.0 percent of  $C_{41}H_{64}O_{14}$ , calculated on the dried basis.

**Caution**—Handle Digoxin with exceptional care, since it is extremely poisonous.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Digoxin RS

USP Gitoxin RS

$C_{41}H_{64}O_{14}$  780.96

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**C:** Examine in visible light the thin-layer chromatograph prepared as directed in the test for *Related glycosides*: the  $R_f$  value of the principal blue spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**Loss on drying** (731)—Dry it in vacuum at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%, a 100-mg specimen being used.

**Related glycosides**—

*Chloramine T-trichloroacetic acid reagent*—Mix 10 mL of a freshly prepared solution of chloramine T (3 in 100) and 40 mL of a 1 in 4 solution of trichloroacetic acid in dehydrated alcohol.

*Spotting solvent*—Prepare a mixture of chloroform and methanol (2:1).

*Standard solution*—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution containing 10 mg per mL.

*Gitoxin standard solution*—Dissolve an accurately weighed quantity of USP Gitoxin RS in *Spotting solvent* to obtain a solution containing 0.30 mg per mL.

*Test solution*—Transfer 250.0 mg of Digoxin to a 25-mL volumetric flask, dissolve in and dilute with *Spotting solvent* to volume, and mix.

*Procedure*—Apply 10  $\mu$ L of the *Test solution*, 10  $\mu$ L of the *Standard solution*, and 10  $\mu$ L of the *Gitoxin standard solution* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture to which is permanently bonded octadecylsilane (C18). Allow the spots to dry, and place the plates in a developing chamber containing a mixture of methanol and water (7:3). Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Chloramine T-trichloroacetic acid reagent*, freshly mixed, and heat in an oven at 110° for 10 minutes. Examine the plate under long-wavelength UV light: no spot from the *Test solution* except that due to digoxin is more intense than the spot from the *Gitoxin standard solution* (not more than 3% of any related glycoside as gitoxin).

**Residual solvents** (467): The limits for methylene chloride and chloroform are 2000  $\mu$ g per g.

**Assay**—

*Mobile phase*—Prepare a suitable degassed and filtered mixture of water and acetonitrile (37:13), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 250  $\mu$ g per mL. Use a sonic bath to aid dissolution.

*Assay preparation*—Transfer about 50 mg of Digoxin, accurately weighed, to a 200-mL volumetric flask. Dissolve in about 150 mL of diluted alcohol by sonication, dilute with diluted alcohol to volume, and mix.

*System suitability preparation*—Prepare a solution in diluted alcohol of USP Digoxin RS and digoxigenin having concentrations of about 40  $\mu$ g of each per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 218-nm detector and a 4.2-mm  $\times$  25-cm column that contains packing L1 and a 3.2-mm  $\times$  15-mm guard column that contains packing L1. The flow rate is about 3.0 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between digoxin and digoxigenin is not less than 4.0; the column efficiency determined from the digoxin peak is not less than 1200 theoretical plates; the tailing factor for the digoxin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{41}H_{64}O_{14}$  in the portion of Digoxin taken by the formula:

$$0.2C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Digoxin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses for the digoxin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digoxin Injection

» Digoxin Injection is a sterile solution of Digoxin in Water for Injection and Alcohol or other suitable solvents. It contains not less than 90.0 percent and not more than 105.0 percent of the labeled amount of  $C_{41}H_{64}O_{14}$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass. Avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Digoxin RS

USP Endotoxin RS

**Identification**—

**A:** Injection meets the requirements for *Identification test A* under *Digoxin Oral Solution*.

**B:** *Chloramine T-trichloroacetic acid reagent*, *Spotting solvent*, and *Standard solution*—Proceed as directed for *Identification test B* under *Digoxin Oral Solution*.

*Test solution*—Pipet a volume of Injection, equivalent to 0.5 mg of digoxin, into a separator, and add 5 mL of water. Extract with three 10-mL portions of chloroform, combining the extracts in a conical flask. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of

air to dryness. (If traces of water or propylene glycol remain, dry the flask in vacuum at 100° for 30 minutes.) Dissolve the residue in 2 mL of *Spotting solvent*.

**Procedure**—Proceed as directed for *Procedure* in the test for *Related glycosides* under *Digoxin*, except to omit the use of the *Gitoxin standard solution*. Examine the plate under long-wavelength UV light: the  $R_f$  value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution*.

**Bacterial endotoxins** (85)—It contains not more than 200.0 USP Endotoxin Units per mg of digoxin.

**Alcohol content** (611): between 9.0% and 11.0% of  $C_2H_5OH$ .

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Proceed as directed in the *Assay* under *Digoxin*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively with diluted alcohol to obtain a solution having a known concentration of about 250 µg per mL. Use a sonic bath to aid dissolution. If necessary, dilute quantitatively to match, approximately, the concentration of the *Injection*.

**Chromatographic system and System suitability preparation**—Proceed as directed in the *Assay* under *Digoxin*.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and *Injection* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of  $C_{41}H_{64}O_{14}$  in each mL of the *Injection* taken by the formula:

$$C(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Digoxin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses for the digoxin peaks obtained from the *Injection* and the *Standard preparation*, respectively.

## Digoxin Oral Solution

» Digoxin Oral Solution contains, in each 100 mL, not less than 4.50 mg and not more than 5.25 mg of digoxin ( $C_{41}H_{64}O_{14}$ ).

**Packaging and storage**—Preserve in tight containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Digoxin RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of Oral Solution corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** *Chloramine T-trichloroacetic acid reagent*—Mix 10 mL of a freshly prepared solution of chloramine T (3 in 100) and 40 mL of a 1 in 4 solution of trichloroacetic acid in dehydrated alcohol.

**Spotting solvent**—Prepare a mixture of chloroform and methanol (2:1).

**Standard solution**—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution containing 0.25 mg per mL.

**Test solution**—Pipet a volume of Oral Solution, equivalent to 0.5 mg of digoxin, into a separator. Add sufficient water to obtain a final volume of approximately 50 mL. Extract the

aqueous layer with three 30-mL portions of chloroform, combining the extracts in a conical flask. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of air to dryness. Add 2 mL of *Spotting solvent* to the residue, and shake for 2 minutes.

**Procedure**—Proceed as directed for *Procedure* in the test for *Related glycosides* under *Digoxin*, except to omit the use of the *Gitoxin standard solution*. Examine the plate under long-wavelength UV light: the  $R_f$  value of the principal spot in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*.

**Alcohol content** (611): between 90.0% and 115.0% of the labeled amount of  $C_2H_5OH$ .

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and isopropyl alcohol (70:27.5:2.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 20 µg per mL.

**Assay preparation**—Transfer an accurately measured volume of 10.0 mL of Oral Solution, equivalent to about 500 µg of digoxin, to a 25-mL volumetric flask, dilute with diluted alcohol to volume, and mix.

**System suitability preparation**—Prepare as directed in the *Assay* under *Digoxin*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 218-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 0.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between digoxin and digoxigenin bisdigitoxoside is not less than 2.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of digoxin ( $C_{41}H_{64}O_{14}$ ) in each mL of the Oral Solution taken by the formula:

$$2.5C(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Digoxin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the digoxin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Digoxin Tablets

» Digoxin Tablets contain not less than 90.0 percent and not more than 105.0 percent of the labeled amount of  $C_{41}H_{64}O_{14}$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Digoxin RS

#### Identification—

**A:** *Chloramine T-trichloroacetic acid reagent*—Prepare as directed for *Identification* test B under *Digoxin Oral Solution*.

**Spotting solvent**—Use dehydrated alcohol.

**Test solution**—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to 0.5 mg of digoxin, to



a 10-mL centrifuge tube. Add 2 mL of *Spotting solvent*, sonicate for 10 to 15 minutes, and centrifuge. Decant and use the supernatant.

**Standard solution**—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution having a known concentration of about 0.25 mg per mL.

**Procedure**—Proceed as directed for *Procedure* in the test for *Related glycosides* under *Digoxin*, except to omit the use of the *Gitoxin standard solution*. Examine the plate under long-wavelength UV light: the  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—[NOTE—Throughout this procedure, use scrupulously clean glassware, which previously has been rinsed successively with hydrochloric acid, water, and alcohol, and carefully dried. Take precautions to prevent contamination from fluorescent particles and from metal and rubber surfaces.]

**Medium:** 0.1 N hydrochloric acid; 500 mL. [NOTE—Use the same batch of *Dissolution Medium* throughout the test.]

**Apparatus 1:** 120 rpm.

**Time:** 60 minutes.

**Ascorbic acid-methanol solution**—Prepare a solution containing 2 mg of ascorbic acid per mL of methanol.

**Hydrogen peroxide-methanol solution**—On the day of use, dilute 2.0 mL of recently assayed 30 percent hydrogen peroxide with methanol to 100 mL. Store in a refrigerator. Just prior to use, dilute 2.0 mL of this solution with methanol to 100 mL.

**Standard solutions**—Transfer about 25 mg of USP Digoxin RS, accurately weighed, to a 500-mL volumetric flask. Dissolve in a minimum amount of alcohol, add dilute alcohol (4 in 5) to volume, and mix. Dilute 10.0 mL of this solution with dilute alcohol (4 in 5) to 100.0 mL, and mix. Just prior to use, dilute suitable aliquots of the resulting solution with *Dissolution Medium* to 50.0 mL to prepare *Standard solutions* equivalent to 20%, 40%, 60%, 80%, and 100%, respectively, of the labeled amount of digoxin in 500 mL.

**Test solution**—Promptly after withdrawal, pass a portion of the solution under test through a filter having a 0.8- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. This is the *Test solution*.

**Procedure**—Transfer to individual glass-stoppered flasks duplicate 1.0-mL portions of the *Test solution*, 1.0-mL portions of each of the *Standard solutions*, and 1.0 mL of the *Medium* to provide a blank. Begin with the *Standard solutions*, and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each flask in the set. Treating one flask at a time, add the following three reagents, in the order named, in as rapid a sequence as possible, swirling after each addition: 1.0 mL of *Ascorbic acid-methanol solution*, 5.0 mL of hydrochloric acid, and 1.0 mL of *Hydrogen peroxide-methanol solution*. Insert the stoppers in the flasks, and after 2 hours, measure the fluorescence at about 485 nm, the excitation wavelength being about 372 nm. To check the stability of the fluorometer, repeat the measurement of fluorescence on one or more treated *Standard solutions*. Correct each reading for the blank, and plot a standard curve of fluorescence versus percentage dissolution. Determine the percentage dissolution of digoxin in the *Test solution* by reading from the standard graph.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{41}H_{64}O_{14}$  is dissolved in 60 minutes. The requirement is met if the quantities dissolved from the Tablets tested con-

form to the accompanying acceptance table instead of the table shown under *Dissolution* <711>.

Acceptance Table

Stage	Number Tested	Acceptance Criteria
S <sub>1</sub>	6	Each unit is not less than Q + 5%.
S <sub>2</sub>	6	Average of 12 units (S <sub>1</sub> + S <sub>2</sub> ) is equal to or greater than Q, and no unit is less than Q – 5%.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

**Mobile phase, Chromatographic system, and System suitability preparation**—Proceed as directed in the *Assay* under *Digoxin*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 40  $\mu$ g per mL. Use a sonic bath to aid dissolution.

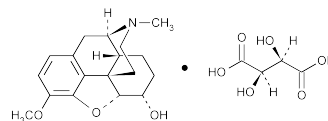
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of digoxin, to a glass-stoppered, 50-mL conical flask. Add 25.0 mL of diluted alcohol with swirling, sonicate for about 30 minutes, and cool. Filter a portion of this solution through a 0.8- $\mu$ m porosity membrane filter, discarding the first 10 mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{41}H_{64}O_{14}$  in the portion of Tablets taken by the formula:

$$25C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Digoxin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses for the digoxin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydrocodeine Bitartrate



$C_{18}H_{23}NO_3 \cdot C_4H_6O_6$  451.47  
Morphinan-6-ol, 4,5-epoxy-3-methoxy-17-methyl-, (5 $\alpha$ ,6 $\alpha$ )-2,3-dihydroxybutanedioate (1:1) (salt).  
4,5 $\alpha$ -Epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol (+)-tartrate (salt).

» Dihydrocodeine Bitartrate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Dihydrocodeine Bitartrate RS

**Identification—**

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for dihydrocodeine, the retention time of which corresponds to that in the chromatogram of the *Standard preparation* similarly determined.

**B:** It responds to the tests for *Tartrate* (191).

**C:** To a solution of 20 mg in 5 mL of sulfuric acid in a test tube add 1 drop of ferric chloride TS, and heat in a boiling water bath for 2 minutes: although the solution may darken, no blue color is produced (*distinction from codeine and morphine*).

**Melting range**, *Class I* (741): between 186° and 190°, but the range between beginning and end of melting does not exceed 2.5°.

**Specific rotation** (781S): between −72° and −75°.

*Test solution:* 10 mg per mL, in water.

**pH** (791): between 3.2 and 4.2, in a solution (1 in 10).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Ammonium salts**—To about 100 mg of it in a suitable test tube add 5 mL of 1 N sodium hydroxide, and heat on a steam bath: the odor of ammonia is not detected.

**Ordinary impurities** (466)—

*Test solution:* water.

*Standard solution:* water.

*Eluent:* a mixture of methylene chloride, methanol, and ammonium hydroxide (90:10:1).

*Visualization:* 17, and view under short-wavelength UV light.

**Assay—**

**Mobile phase**—Prepare a mixture of acetonitrile, water, and diethylamine (800:4:1). Prepare a suitable mixture of this solution and methanol (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Prepare a solution in methanol containing about 0.4 mg of USP Dihydrocodeine Bitartrate RS and 0.6 mg of hydrocodone bitartrate per mL. Prepare a mixture of this solution and water (1:1).

**Standard preparation**—Transfer about 20 mg of USP Dihydrocodeine Bitartrate RS, accurately weighed, to a 50-mL volumetric flask, add 25 mL of methanol, swirl to dissolve, dilute with water to volume, and mix.

**Assay preparation**—Transfer about 20 mg of Dihydrocodeine Bitartrate, accurately weighed, to a 50-mL volumetric flask, add 25 mL of methanol, swirl to dissolve, dilute with water to volume, and mix.

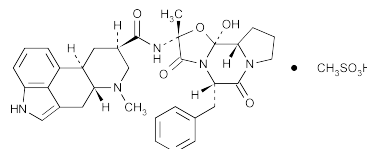
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed under *Procedure*: the relative retention times are about 0.8 for hydrocodone and 1.0 for dihydrocodeine, and the resolution, *R*, between the hydrocodone and dihydrocodeine peaks is not less than 1.8. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the column efficiency determined from the dihydrocodeine peak is not less than 900 theoretical plates, the tailing factor for the dihydrocodeine peak is not more than 1.7, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of  $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$  in the portion of Dihydrocodeine Bitartrate taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dihydrocodeine Bitartrate RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Dihydroergotamine Mesylate**

$C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$  679.78  
Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'α)-, monomethanesulfonate (salt).  
Dihydroergotamine monomethanesulfonate [6190-39-2].

» Dihydroergotamine Mesylate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—  
USP Dihydroergotamine Mesylate RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 50 μg per mL.

*Medium:* 70% alcohol.

Absorptivities at 280 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** The principal spot from the *Test preparation* found in the test for *Related alkaloids* corresponds in *R<sub>f</sub>* value to that obtained from the *Standard preparation*.

**Specific rotation** (781S): between −16.7° and −22.7°.

*Test solution:* 25 mg per mL, in a mixture of chloroform, alcohol, and ammonium hydroxide (10:10:1).

**pH** (791): between 4.4 and 5.4, in a solution (1 in 1000).

**Loss on drying** (731)—Dry it in vacuum at 100° to constant weight: it loses not more than 4.0% of its weight.

**Related alkaloids—**

**Solvent mixture**—Mix 10 volumes of chloroform, 10 volumes of methanol, and 1 volume of ammonium hydroxide.

**Test solution**—Prepare a solution of Dihydroergotamine Mesylate in *Solvent mixture* to contain 20 mg per mL.

**Standard solution and Standard dilutions**—Prepare a solution of USP Dihydroergotamine Mesylate RS in *Solvent mixture* to contain 20 mg per mL (*Standard solution*). Prepare a series of dilutions of the *Standard solution* in *Solvent mixture* to contain 0.40 mg, 0.20 mg, and 0.10 mg per mL (*Standard dilutions*).

**Procedure**—In a suitable chromatographic chamber arranged for thin-layer chromatography place a volume of a solvent system consisting of a mixture of chloroform and alcohol (9:1) sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 minutes. Apply 5-μL portions

of the *Test solution*, the *Standard solution*, and each of the three *Standard dilutions* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a solution prepared by dissolving 800 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 80 g of alcohol and 20 g of sulfuric acid. The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*. Estimate the concentration of any other spots observed in the lane for the *Test solution* by comparison with the *Standard dilutions*. The spots from the 0.40-, 0.20-, and 0.10-mg-per-mL dilutions are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is not greater than 2.0%.

#### Assay—

*Diluent 1*—Prepare a solution of 0.1 mL of phosphoric acid in 1000 mL of water.

*Diluent 2*—Prepare a mixture of *Diluent 1* and acetonitrile (60:40).

*Solution A*—Prepare a filtered and degassed mixture of water, 25 percent ammonia water, and 98% formic acid (1000:10:5). Adjust the pH to 8.50.

*Solution B*—Prepare a filtered and degassed mixture of acetonitrile and *Solution A* (80:20).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments to either solution as necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dihydroergotamine Mesylate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with *Diluent 1* to obtain a solution having a known concentration of about 0.6 mg per mL. [NOTE—The final ratio of acetonitrile and *Diluent 1* should be similar to the final ratio obtained in the *Assay preparation*.]

*Assay preparation*—Transfer about 30 mg of Dihydroergotamine Mesylate, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, dilute with *Diluent 1* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and 4.0-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	60	40	equilibration
0–12	60→50	40→50	linear gradient
12–20	50→15	50→85	linear gradient
20–24	15	85	isocratic
24–25	15→60	85→40	linear gradient
25–31	60	40	re-equilibration

Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is between 0.8 and 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the analyte peaks. Calculate the quantity,

in mg, of  $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$  in the portion of Dihydroergotamine Mesylate taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dihydroergotamine Mesylate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydroergotamine Mesylate Injection

» Dihydroergotamine Mesylate Injection is a sterile solution of Dihydroergotamine Mesylate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** <11>—  
USP Dihydroergotamine Mesylate RS  
USP Endotoxin RS

**Identification**—Dilute 2 mL of Injection with water to 25 mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Dihydroergotamine Mesylate RS, concomitantly measured.

**Bacterial endotoxins** <85>—It contains not more than 175.0 USP Endotoxin Units per mg of dihydroergotamine mesylate.

**pH** <791>: between 3.4 and 4.9.

**Other requirements**—It meets the requirements under *Injections* <1>.

#### Assay—

*Reagent preparation*—Dissolve 250 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 130 mL of sulfuric acid and 70 mL of water, and add 0.40 mL of ferric chloride solution (1 in 20).

*Standard preparation*—Dissolve in tartaric acid solution (1 in 100) a suitable quantity of USP Dihydroergotamine Mesylate RS, accurately weighed, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 50  $\mu$ g per mL.

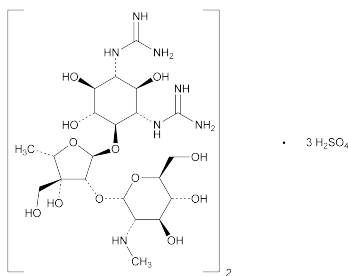
*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of dihydroergotamine mesylate, to a 100-mL volumetric flask, dilute with tartaric acid solution (1 in 100) to volume, and mix.

*Procedure*—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and tartaric acid solution (1 in 100) to provide the blank, to separate 50-mL conical flasks. Add 10.0 mL of the *Reagent preparation* to each, shake, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 585 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$  in each mL of the Injection taken by the formula:

$$(0.1 C / V)(A_U / A_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Dihydroergotamine Mesylate RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydrostreptomycin Sulfate



(C<sub>21</sub>H<sub>41</sub>N<sub>7</sub>O<sub>12</sub>)<sub>2</sub> · 3H<sub>2</sub>SO<sub>4</sub> 1461.42  
Dihydrostreptomycin sulfate (2:3) (salt) [5490-27-7].

» Dihydrostreptomycin Sulfate has a potency equivalent to not less than 650 µg of dihydrostreptomycin (C<sub>21</sub>H<sub>41</sub>N<sub>7</sub>O<sub>12</sub>) per mg, except that if it is labeled as being crystalline, it has a potency equivalent to not less than 725 µg of dihydrostreptomycin per mg, or if it is labeled as being solely for oral use, it has a potency equivalent to not less than 450 µg of dihydrostreptomycin per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only. If it is crystalline, it may be so labeled. If it is intended solely for oral use, it is so labeled. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—  
USP Dihydrostreptomycin Sulfate RS  
USP Endotoxin RS  
USP Streptomycin Sulfate RS

### Identification—

**A:** To a solution of 4 mg in 2 mL of water, add 0.5 mL of 1 N hydrochloric acid, and heat in a water bath for 20 minutes. Remove the tube from the bath, and add 1.0 mL of a 1 in 200 solution of 1-naphthol in 1 N sodium hydroxide. Heat again for 10 minutes, cool briefly in an ice bath, and add water to make 25 mL: a red color develops, intensifying during about 10 minutes.

**B:** A solution (1 in 50) responds to the tests for *Sulfate* (191).

**Crystallinity** (695) (where labeled as being crystalline): meets the requirements.

**pH** (791): between 4.5 and 7.0, in a solution containing 200 mg of dihydrostreptomycin per mL, except that if it is labeled as being solely for oral use, the pH is between 3.0 and 7.0.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight, except that if it is labeled as being solely for oral use, it loses not more than 14.0% of its weight.

### Streptomycin—

**Ferric chloride stock solution**—Dissolve 5 g of ferric chloride in 50 mL of 0.1 N hydrochloric acid.

**Ferric chloride solution**—Dilute 2.5 mL of *Ferric chloride stock solution* with sufficient 0.01 N hydrochloric acid to make 100 mL. Use this solution within 1 day.

**Standard solutions**—Dissolve an accurately weighed quantity of USP Streptomycin Sulfate RS in water to obtain a

stock solution containing 1.0 mg of streptomycin (C<sub>21</sub>H<sub>39</sub>N<sub>7</sub>O<sub>12</sub>) per mL. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of this stock solution to each of five 25-mL volumetric flasks. Transfer 9.0, 8.0, 7.0, 6.0, and 5.0 mL of water to the flasks, respectively.

**Test solution**—Transfer about 800 mg of Dihydrostreptomycin Sulfate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 25-mL volumetric flask.

**Procedure**—To each of the flasks containing the *Standard solutions* and the *Test solution*, and to a seventh 25-mL volumetric flask containing 10.0 mL of water to provide a blank, add 2.0 mL of 1 N sodium hydroxide, and heat in a water bath for 10 minutes. Cool the flasks in ice water for 3 minutes, and to each add 2.0 mL of 1.2 N hydrochloric acid and 5.0 mL of *Ferric chloride solution*. Dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard solutions* and the *Test solution* at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Plot the absorbance values of the solutions from the *Standard solutions* versus concentration, in µg per mL, of streptomycin, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of streptomycin in the solution from the *Test solution*. Calculate the percentage of streptomycin in the portion of Dihydrostreptomycin Sulfate taken by the formula:

$$6250C / WP$$

in which *W* is the weight, in mg, of Dihydrostreptomycin Sulfate taken, and *P* is the potency, in µg of dihydrostreptomycin per mg, of the Dihydrostreptomycin Sulfate taken as determined in the *Assay*: not more than 3.0% is found, except that if it is labeled as being crystalline, not more than 1.0% is found, or if it is labeled as being solely for oral use, not more than 5.0% is found.

**Other requirements**—Where the label states that Dihydrostreptomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Dihydrostreptomycin Injection*. Where the label states that Dihydrostreptomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Dihydrostreptomycin Injection*.

**Assay**—Proceed with Dihydrostreptomycin Sulfate as directed for the turbidimetric assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* (81).

## Dihydrostreptomycin Sulfate Boluses

» Dihydrostreptomycin Sulfate Boluses contain the equivalent of not less than 85.0 percent and not more than 120.0 percent of the labeled amount of dihydrostreptomycin (C<sub>21</sub>H<sub>41</sub>N<sub>7</sub>O<sub>12</sub>).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Boluses to indicate that they are intended for veterinary use only.

**USP Reference standards** (11)—  
USP Dihydrostreptomycin Sulfate RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, of finely ground Boluses in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 10.0% of its weight.

**Assay**—Proceed as directed for the cylinder-plate assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* (81), using 3 Boluses added to a mixture of 499.0 mL of *Buffer No. 3* and 1.0 mL of polysorbate 80 and blended for 5 minutes in a high-speed glass blender jar. Allow to stand for not less than 1 hour, and repeat the blending. While stirring this mixture, withdraw an accurately measured volume of it, and dilute quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Dihydrostreptomycin Injection

» Dihydrostreptomycin Injection contains an amount of Dihydrostreptomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of dihydrostreptomycin ( $C_{21}H_{41}N_7O_{12}$ ). It contains one or more suitable preservatives.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards** (11)—  
USP Dihydrostreptomycin Sulfate RS  
USP Endotoxin RS

**Identification**—It responds to the *Identification* tests under *Dihydrostreptomycin Sulfate*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of dihydrostreptomycin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 8.0.

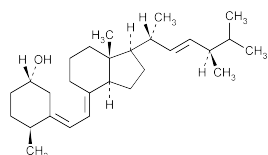
### Assay—

*Assay preparation 1* (where it is represented as being in a single-dose container)—Withdraw all of the withdrawable contents of Injection, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing a convenient quantity of dihydrostreptomycin in each mL.

*Assay preparation 2* (where the label states the quantity of dihydrostreptomycin in a given volume of solution)—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing a convenient quantity of dihydrostreptomycin in each mL.

*Procedure*—Proceed as directed for the turbidimetric assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Dihydrotachysterol



$C_{28}H_{46}O$  398.66

9,10-Secoergosta-5,7,22-trien-3-ol, (3 $\beta$ ,5E,7E,10 $\alpha$ ,22E)-.  
Dihydrotachysterol.  
9,10-Secoergosta-5,7,22-trien-3 $\beta$ -ol [67-96-9].

» Dihydrotachysterol contains not less than 97.0 percent and not more than 103.0 percent of  $C_{28}H_{46}O$ .

**Packaging and storage**—Preserve in light-resistant, hermetic glass containers from which air has been displaced by an inert gas.

**USP Reference standards** (11)—  
USP Dihydrotachysterol RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 8  $\mu$ g per mL.

*Medium:* alcohol.

**Specific rotation** (781S): between +100° and +103°.

*Test solution:* 20 mg per mL, in alcohol.

**Residue on ignition** (281): not more than 0.1%.

### Assay—

*Mobile phase*—Prepare a degassed and filtered solution of isooctane and isopropyl alcohol (100:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dihydrotachysterol RS in *Mobile phase*, and dilute quantitatively and stepwise, if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

*Assay preparation*—Transfer about 50 mg of Dihydrotachysterol, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*System suitability preparation*—Prepare a solution of ergocalciferol in *Mobile phase* having a concentration of about 0.7 mg per mL. Reflux under nitrogen for 20 minutes, and cool to ambient temperature (*Solution A*). Pipet 3 mL of *Solution A* and 2 mL of a solution in *Mobile phase* containing 0.1 mg of dihydrotachysterol per mL into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.5%. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for pre-ergocalciferol, 0.7 for dihydrotachysterol, and 1.0 for ergocalciferol; and the resolution,  $R$ , between the pre-ergocalciferol and dihydrotachysterol peaks is not less than 1.5.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{28}H_{46}O$  in the portion of Dihydrotachysterol taken by the formula:

$$5C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Dihydrotachysterol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses for dihydrotachysterol obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydrotachysterol Capsules

» Dihydrotachysterol Capsules contain a solution of Dihydrotachysterol in a suitable vegetable oil. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{28}H_{46}O$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Dihydrotachysterol RS

**Identification**—

**A:** Cut open 1 Capsule, and remove the contents: the Capsule contents respond to *Identification test A* under *Dihydrotachysterol Oral Solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dihydrotachysterol*.

*Assay preparation*—Combine the contents of not less than 20 Capsules. Transfer an accurately weighed quantity of Capsule contents, equivalent to about 500  $\mu\text{g}$  of dihydrotachysterol, to a 50-mL volumetric flask, dissolve in 25 mL of *Mobile phase*, and mix. Dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dihydrotachysterol*. Calculate the quantity, in  $\mu\text{g}$ , of  $C_{28}H_{46}O$  in the portion of Capsules taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Dihydrotachysterol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydrotachysterol Oral Solution

» Dihydrotachysterol Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dihydrotachysterol ( $C_{28}H_{46}O$ ).

**Packaging and storage**—Preserve in tight, light-resistant glass containers.

**USP Reference standards** (11)—

USP Dihydrotachysterol RS

**Identification**—

**A:** Place 1 drop of Oral Solution on a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Spray the plate with 3.5% phosphomolybdic acid solution (prepared by dissolving 3.5 g of phosphomolybdic acid in 100 mL of isopropyl alcohol), and immediately heat the plate over a hot plate: a dark blue spot appears on a yellow background.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay**—

*Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dihydrotachysterol*.

*Assay preparation* [FOR ORAL SOLUTION IN AN OIL MEDIUM]—Transfer an accurately measured volume of Oral Solution, equivalent to about 500  $\mu\text{g}$  of dihydrotachysterol, by means of a “to contain” pipet to a 50-mL volumetric flask. Rinse the pipet with *Mobile phase*, add the rinsing to the flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation* [FOR ORAL SOLUTION IN AN AQUEOUS MEDIUM]—Transfer an accurately measured volume of Oral Solution, equivalent to about 600  $\mu\text{g}$  of dihydrotachysterol, to a separator containing about 30 mL of water. Add about 1 g of sodium chloride, mix, and extract with three 15-mL portions of chloroform, filtering each portion through absorbent cotton into a suitable glass-stoppered conical flask. Wash the cotton with about 5 mL of chloroform, collecting the washing in the glass-stoppered conical flask. Evaporate the chloroform extracts, and wash with the aid of a current of air to dryness. Dissolve the residue in 50.0 mL of *Mobile phase*, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dihydrotachysterol*. Calculate the quantity, in  $\mu\text{g}$ , of dihydrotachysterol ( $C_{28}H_{46}O$ ) in the volume of Oral Solution taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Dihydrotachysterol RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydrotachysterol Tablets

» Dihydrotachysterol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{28}H_{46}O$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Dihydrotachysterol RS

**Identification**—

**A:** Transfer a portion of powdered Tablets, equivalent to about 2 mg of dihydrotachysterol, to a glass-stoppered flask, add about 25 mL of methylene chloride, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 0.4 mL of methylene chloride. Apply 10  $\mu\text{L}$  of this solution and 10  $\mu\text{L}$  of a Standard solution of USP Dihydrotachysterol RS in methylene chloride containing 5 mg per mL at separate points on a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram using a solvent system consisting of a mixture of ether and cyclohexane (1:1) until the solvent front has moved about three-fourths of the length of the plate. Air-dry, spray lightly with a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1), and heat at 80° until reddish brown spots appear (about 10 minutes): the  $R_f$  value

of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**Disintegration** (701): 10 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dihydrotachysterol*.

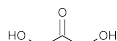
*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2.5 mg of dihydrotachysterol, to a suitable container, add 100.0 mL of dehydrated alcohol, shake by mechanical means for 10 minutes, sonicate, and centrifuge. Transfer 20.0 mL of the supernatant to a 50-mL volumetric flask, and evaporate with the aid of a stream of nitrogen to dryness, observing precautions to avoid exposure to moisture and light. Dissolve the residue in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Dihydrotachysterol*. Calculate the quantity, in  $\mu\text{g}$ , of  $\text{C}_{28}\text{H}_{46}\text{O}$  in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Dihydrotachysterol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dihydroxyacetone



$\text{C}_3\text{H}_6\text{O}_3$  90.08  
1,3-Dihydroxy-2-propanone. [96-26-4].

» Dihydroxyacetone contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_3\text{H}_6\text{O}_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers in a cool place.

**USP Reference standards** (11)—

USP Dihydroxyacetone RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The  $R_f$  value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard preparation* as obtained in the *Chromatographic purity* test.

**pH** (791): between 4.0 and 6.0, in a solution (1 in 20).

**Water, Method I** (921): not more than 0.2%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231): 0.001%.

**Limit of iron** (241): not more than 0.002%.

#### Chromatographic purity—

*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture.

*Glycerin solution*—Dilute an accurately measured volume of glycerin with methanol to obtain a solution having a concentration of about 0.25 mg per mL.

*Test solution*—Dissolve an accurately weighed quantity of Dihydroxyacetone in methanol to obtain a solution containing about 50 mg per mL.

*Standard solution*—Dissolve an accurately weighed quantity of USP Dihydroxyacetone RS in methanol, and mix to obtain a solution having a known concentration of about 50 mg per mL.

*Application volume:* 1  $\mu\text{L}$ .

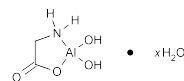
*Developing solvent system:* a mixture of acetone and water (19:1).

*Procedure*—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Spray the plate with a mixture of toluene, a saturated solution of lead tetraacetate in glacial acetic acid, and a 1% solution of dichlorofluorescein in alcohol (190:5:1), and dry the plate for 5 minutes at 105°. Examine the plate under short-wavelength UV light, and compare the intensities of the glycerin spot observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Glycerin solution*: the glycerin spot from the chromatogram of the *Test solution* is not larger or more intense than the principal spot obtained from the *Glycerin solution* (0.5%), and no other secondary spots are observed in the chromatogram of the *Test solution*.

**Limit of protein**—Dissolve 25 g of Dihydroxyacetone in 100 mL of water. Transfer 100  $\mu\text{L}$  of this solution to a 5-mL volumetric flask, dilute with brilliant blue G TS to volume, and mix. Determine the absorbance of this solution at about 595 nm with a suitable spectrophotometer, using 100  $\mu\text{L}$  of water and 5 mL of brilliant blue G TS as the blank: the absorbance of the test solution is not more than 0.400.

**Assay**—Dissolve an accurately weighed quantity of about 0.1 g of Dihydroxyacetone in 20 mL of water, add 20 mL of 0.1 M periodic acid, and allow to stand at room temperature in the dark for 20 minutes. Add about 3 g of sodium bicarbonate, 20 mL of 0.6 M potassium iodide, and 3 mL of starch TS, and titrate with 0.05 M sodium arsenite VS. Perform a blank titration, and make any necessary correction. Each mL of 0.05 M sodium arsenite is equivalent to 4.504 mg of  $\text{C}_3\text{H}_6\text{O}_3$ .

## Dihydroxyaluminum Aminoacetate



$\text{C}_2\text{H}_6\text{AlNO}_4 \cdot x\text{H}_2\text{O}$

Aluminum, (glycinato-*N,O*)dihydroxy-, hydrate.

(Glycinato)dihydroxyaluminum hydrate [41354-48-7].

Anhydrous 135.05 [13682-92-3].

» Dihydroxyaluminum Aminoacetate yields not less than 94.0 percent and not more than 102.0 percent of dihydroxyaluminum aminoacetate ( $\text{C}_2\text{H}_6\text{AlNO}_4$ ), calculated on the dried basis. It may contain small amounts of aluminum oxide and of Aminoacetic Acid.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Suspend 1 g in 25 mL of water, add hydrochloric acid, dropwise, until a clear solution is formed, and divide it into two equal parts for the following tests.

**A:** One portion of the solution responds to the tests for *Aluminum* (191).

**B:** To the other portion of the solution add 1 drop of liquefied phenol and 5 mL of sodium hypochlorite TS: a blue color is produced.

**pH** (791): between 6.5 and 7.5, in a suspension of 1 g of it, finely powdered, in 25 mL of water.

**Loss on drying** (731)—Dry it at 130° to constant weight: it loses not more than 14.5% of its weight.

**Mercury, Method IIa** (261)—Transfer 2.0 g to a 100-mL beaker, and add 35 mL of 1 N sulfuric acid: the limit is 1 ppm.

**Isopropyl alcohol**—Transfer about 5 g to a flask provided with a reflux condenser, and add 100 mL of potassium permanganate solution (1 in 300) and 10 mL of sulfuric acid. Reflux the mixture for 30 minutes, distill, and collect 10 mL of the distillate. To 1 mL of the distillate add 5 drops of sodium nitroferricyanide TS and 2 mL of 1 N sodium hydroxide, then add a slight excess of 6 N acetic acid: no red color is produced.

**Nitrogen**—Determine the nitrogen content as directed under *Nitrogen Determination, Method II* (461), using about 100 mg, previously dried and accurately weighed. Each mL of 0.1 N sulfuric acid is equivalent to 1.401 mg of nitrogen. Not less than 9.90% and not more than 10.60% of nitrogen is found.

#### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Transfer about 2.5 g of Dihydroxyaluminum Aminoacetate, accurately weighed, to a 150-mL beaker, add 15 mL of hydrochloric acid, and warm, if necessary, to dissolve the specimen completely. Transfer the solution with the aid of water to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 250-mL beaker, and add, with continuous stirring, 25.0 mL of *Edetate disodium titrant* and then 20 mL of acetic acid–ammonium acetate buffer TS. Heat the solution near the boiling point for 5 minutes, cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose pink. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 6.753 mg of  $C_2H_6AlNO_4$ .

## Dihydroxyaluminum Aminoacetate Magma

» Dihydroxyaluminum Aminoacetate Magma is a suspension that contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_2H_6AlNO_4$ .

**Packaging and storage**—Preserve in tight containers, and protect from freezing.

**Identification**—Dilute a volume of Magma, equivalent to about 1 g of dihydroxyaluminum aminoacetate, with water to 25 mL, add hydrochloric acid, dropwise, until a solution results, and then filter: the filtered solution responds to the *Identification* tests under *Dihydroxyaluminum Aminoacetate*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 100 cfu per mL, and the test for *Escherichia coli* is negative.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recom-

mended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0148D)$$

in which 0.0148 is the theoretical acid-neutralizing capacity, in mEq, of  $C_2H_6AlNO_4$ , and *D* is the quantity, in mg, of  $C_2H_6AlNO_4$  in the specimen tested, based on the labeled quantity.

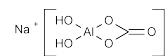
**pH** (791): between 6.5 and 7.5, in a dilution in water, equivalent to about 1 g of dihydroxyaluminum aminoacetate in 25 mL.

#### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—[NOTE—Shake the container by mechanical means for 1 hour before removing the specimen.] Weigh accurately in a tared beaker a quantity of Dihydroxyaluminum Aminoacetate Magma, equivalent to about 2.5 g of dihydroxyaluminum aminoacetate, add 15 mL of hydrochloric acid, and boil gently for about 5 minutes. Cool, transfer the solution with the aid of water to a 500-mL volumetric flask, dilute with water to volume, mix, and filter, if necessary, to obtain a clear solution. Proceed as directed in the Assay under *Dihydroxyaluminum Aminoacetate*, beginning with "Transfer 20.0 mL of this solution."

## Dihydroxyaluminum Sodium Carbonate



$NaAl(OH)_2CO_3$  143.99

Aluminum, [carbonato(1-)-O]dihydroxy-, monosodium salt. Sodium (7-4)-[carbonato(2-)-O,O']dihydroxyaluminate(1-). Sodium (carbonato)dihydroxyaluminate(1-) [539-68-4; 16482-55-6].

» Dihydroxyaluminum Sodium Carbonate contains not less than 98.3 percent and not more than 107.9 percent of dihydroxyaluminum sodium carbonate ( $CH_2AlNaO_5$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A 1-g portion, treated with 20 mL of 3 N hydrochloric acid, dissolves with effervescence, and the resulting solution responds to the tests for *Aluminum* (191) and to the flame test for *Sodium* (191).

**pH** (791): between 9.9 and 10.2 in a suspension (1 in 25).

**Acid-neutralizing capacity** (301): not less than 75.0% of the expected mEq value, determined on about 425 mg of the undried material, accurately weighed, and calculated in relation to the results of the Assay. Each mg of  $NaAl(OH)_2CO_3$  has an expected acid-neutralizing capacity of 0.0278 mEq.

**Loss on drying** (731)—Dry it at 130° to constant weight: it loses not more than 14.5% of its weight.

#### Isopropyl alcohol—

*Isopropyl alcohol-free dihydroxyaluminum sodium carbonate*—Use a portion of Dihydroxyaluminum Sodium Carbonate that has been previously tested as directed in this section and found to be free of isopropyl alcohol.

*Sodium chloride solution*—Prepare a solution (1 in 5) of sodium chloride in water.

*Standard stock solution*—Transfer about 1 g of isopropyl alcohol, accurately weighed, to a 50-mL volumetric flask



containing about 10 mL of *Sodium chloride solution*, dilute with *Sodium chloride solution* to volume, and mix. This *Standard stock solution* contains about 20 mg of isopropyl alcohol per mL.

**Standard solutions**—Transfer 2.0, 4.0, 5.0, and 6.0 mL of the *Standard stock solution* to four separate 100-mL volumetric flasks, dilute with *Sodium chloride solution* to volume, and mix. These solutions contain about 0.4, 0.8, 1.0, and 1.2 mg of isopropyl alcohol per mL, respectively.

**Headspace containers**—Use suitable 20-mL containers capable of being tightly closed with an inert septum and a metallic crimp cap.

**Standard preparations**—To four separate 20-mL *Headspace containers* add 1.0 g of *Isopropyl alcohol-free dihydroxyaluminum sodium carbonate*. To the containers add, respectively, 10.0 mL of the appropriate *Standard solution*. These containers contain about 4, 8, 10, and 12 mg of isopropyl alcohol, respectively. [NOTE—Keep the containers cool until sealed.] Seal the containers, place in a water bath maintained at 70°, and allow to stand for 1 hour.

**Test preparation**—Transfer 1.0 g of the *Dihydroxyaluminum Sodium Carbonate* under test to a *Headspace container*, and add 10.0 mL of *Sodium chloride solution*. [NOTE—Keep the container cool until sealed.] Seal the container, place in a water bath maintained at 70°, and allow to stand for 1 hour.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.9-m × 3-mm column packed with support S3. The injection port temperature is maintained at 200°, the detector temperature is maintained at 250°, and the column temperature is maintained at 180°. Inject 1 mL of the gaseous phase of the *Standard preparation* containing 10 mg per container as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 4%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Using a gas-tight syringe, separately inject equal volumes (about 1 mL) of the gaseous headspace of the *Standard preparations* and the *Test preparation* into the gas chromatograph. Record the chromatograms, and measure the peak responses. Determine, based on a retention time comparison, if isopropyl alcohol is detected in the *Test preparation*. Plot the responses of the *Standard preparations* versus the content, in mg, of isopropyl alcohol in each container, draw the straight line best fitting the plotted points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. From the graph, determine the total amount,  $T_U$ , in mg, of isopropyl alcohol in the *Test preparation*. Calculate the percentage of isopropyl alcohol in the *Dihydroxyaluminum Sodium Carbonate* taken by the formula:

$$0.1 T_U / W_U$$

in which  $W_U$  is the weight, in g, of the *Dihydroxyaluminum Sodium Carbonate* taken. The limit is 1.0%.

#### Sodium content—

**Potassium chloride solution**—Prepare a solution of potassium chloride in water containing 38 mg per mL.

**Sodium chloride stock solution**—Dissolve a suitable quantity of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution containing 25.42 µg per mL (10.0 µg of sodium per mL).

**Standard solutions**—On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume, and mix. These solutions contain about 0.5 and 1.0 µg of sodium per mL, respectively.

**Test solution**—Transfer about 250 mg of *Dihydroxyaluminum Sodium Carbonate*, previously dried and accurately weighed, to a 200-mL volumetric flask, add 40 mL of 1 N hydrochloric acid, and boil for 1 minute. Cool, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask containing 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using as a blank a solution prepared by pipeting 4 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* into a 100-mL volumetric flask, diluting with water to volume, and mixing. Plot the absorbances of the *Standard solutions* versus concentrations, in µg per mL of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration,  $C$ , in µg per mL of sodium, in the *Test solution*. Calculate the percentage of sodium in the portion of *Dihydroxyaluminum Sodium Carbonate* taken by the formula:

$$4000C / W$$

in which  $W$  is the quantity, in mg, of *Dihydroxyaluminum Sodium Carbonate* taken: between 15.2% and 16.8% is found.

**Mercury, Method IIa** <261>—Transfer 2.0 g to a 100-mL beaker, and add 35 mL of 1 N sulfuric acid: the limit is 1 ppm.

#### Assay—

**Edate disodium titrant**—Dissolve 18.6 g of edetate disodium in water to make 500 mL, and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Transfer about 300 mg of undried *Dihydroxyaluminum Sodium Carbonate*, accurately weighed, to a 250-mL beaker, add 10 mL of 2 N sulfuric acid, cover the beaker, heat to 80° for 5 minutes, and boil for 1 minute. Add 30.0 mL of 0.1 M edetate disodium, again boil for 1 minute, cool, and then add 10 mL of acetic acid–ammonium acetate buffer TS, 50 mL of acetone, and 2 mL of dithizone TS. Using a pH meter, adjust with the addition of ammonium hydroxide or dilute sulfuric acid to a pH of 4.5, and titrate with 0.05 M zinc sulfate VS, maintaining the pH at 4.5 by the addition of ammonium hydroxide as necessary, to an orange-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M *Edate disodium titrant* is equivalent to 14.40 mg of  $\text{CH}_2\text{AlNaO}_5$ .

## Dihydroxyaluminum Sodium Carbonate Chewable Tablets

*Former Title: Dihydroxyaluminum Sodium Carbonate Tablets*

» *Dihydroxyaluminum Sodium Carbonate Chewable Tablets* contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $\text{CH}_2\text{AlNaO}_5$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the *Chewable Tablets* to indicate that they are to be chewed before swallowing.

**Identification**—A 1 in 10 suspension of powdered Chewable Tablets in 3 N hydrochloric acid meets the requirements of the tests for *Aluminum* <191> and for *Sodium* <191>.

**Uniformity of dosage units** <905>: meet the requirements.

**Acid-neutralizing capacity** <301>—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0278D)$$

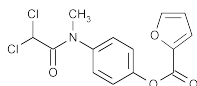
in which 0.0278 is the theoretical acid-neutralizing capacity, in mEq, of  $\text{CH}_2\text{AlNaO}_5$ , and  $D$  is the quantity, in mg, of  $\text{CH}_2\text{AlNaO}_5$  in the specimen tested, based on the labeled quantity.

**Assay**—

**Edate disodium titrant**—Dissolve 18.6 g of edetate disodium in water to make 500 mL, and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 300 mg of dihydroxyaluminum sodium carbonate, to a 250-mL beaker, and proceed as directed in the Assay under *Dihydroxyaluminum Sodium Carbonate*, beginning with “add 10 mL of 2 N sulfuric acid.” Each mL of 0.1 M *Edate disodium titrant* is equivalent to 14.40 mg of  $\text{CH}_2\text{AlNaO}_5$ .

## Diloxanide Furoate



$\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{NO}_4$  328.15

4-(*N*-methyl-2,2-dichloroacetamido)phenyl 2-furoate.  
2,2-Dichloroacetamido-4-*N*-methylphenyl 2-furoate  
[3736-81-0].

» Diloxanide Furoate contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{NO}_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers at controlled room temperature.

**USP Reference standards** <11>—

USP Diloxanide Furoate RS

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 14 µg per mL.

*Medium:* alcohol. The solution exhibits a maximum at 258 nm.

**C:** Burn 20 mg by employing the method under *Oxygen-Flask Combustion* <471>, using 10 mL of 1 N sodium hydroxide as the absorbing liquid. When the process is complete, acidify the liquid with nitric acid, and add a solution of silver nitrate (5 in 100). A white precipitate forms.

**Melting range** <741>: between 114° and 116°.

**Acidity**—Shake 3 g with 50 mL of water, filter, wash the residue with three 20-mL portions of water, and combine the filtrate and washings. Titrate with 0.1 N sodium hydroxide VS using phenolphthalein TS as the indicator: not more than 1.3 mL of 0.1 N sodium hydroxide is required for neutralization.

**Loss on drying** <731>—Dry it at 105° to constant weight: it loses not more than 0.5%.

**Residue on ignition** <281>: not more than 0.1%.

**Related compounds**—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution**—Dissolve an accurately weighed amount of Diloxanide Furoate in chloroform to obtain a solution having a concentration of about 100 mg per mL.

**Standard solution**—Dilute 0.5 mL of *Test solution* with chloroform to 200 mL.

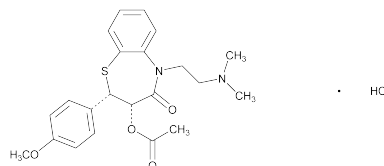
**Application volume:** 5 µL.

**Developing solvent system:** a mixture of dichloromethane and methanol (96:4).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* <621>. Examine the plate under short-wavelength UV light: any secondary spot obtained from the *Test solution* is not more intense than the spot obtained from the *Standard solution*.

**Assay**—Dissolve about 300 mg of Diloxanide Furoate, accurately weighed, in 50.0 mL of dried pyridine, and titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically using suitable electrodes (see *Titrimetry* <541>). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 32.82 mg of  $\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{NO}_4$ .

## Diltiazem Hydrochloride



$\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4\text{S} \cdot \text{HCl}$

450.98

1,5-Benzothiazepin-4(5*H*)-one, 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (+)-*cis*-;  
(+)-5-[2-(Dimethylamino)ethyl]-*cis*-2,3-dihydro-3-hydroxy-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one acetate (ester) monohydrochloride [33286-22-5].

**DEFINITION**

Diltiazem Hydrochloride contains NLT 98.0% and NMT 102.0% of diltiazem hydrochloride ( $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4\text{S} \cdot \text{HCl}$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** <197K>

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL Chloride** <191>: Meets the requirements

**ASSAY**

• **PROCEDURE**

**Buffer:** 1.16 g/L of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (25:25:50)

**System suitability solution:** 12 µg/mL each of USP Diltiazem Hydrochloride RS and USP Desacetyl Diltiazem Hydrochloride RS in methanol

**Standard solution:** 1.2 mg/mL of USP Diltiazem Hydrochloride RS in methanol

**Sample solution:** 1.2 mg/mL of Diltiazem Hydrochloride in methanol

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.6 mL/min

**Injection volume:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between desacetyl diltiazem and diltiazem, *System suitability solution*

**Column efficiency:** NLT 1200 theoretical plates for the diltiazem peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% determined from replicate injections, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS** (231): NMT 20 ppm

• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** Use the *System suitability solution* prepared as directed in the *Assay*.

**System suitability:** Proceed as directed in the *Assay*, except for the following:

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 10.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl diltiazem hydrochloride in the portion of Diltiazem Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of desacetyl diltiazem from the *Sample solution*

$r_S$  = peak response of desacetyl diltiazem from the *Standard solution*

$C_S$  = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity peak, other than the main peak and the desacetyl diltiazem peak:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of each impurity peak from the *Sample solution*

$r_S$  = response of each impurity peak from the *Standard solution*

$C_S$  = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.5% of desacetyl diltiazem hydrochloride; NMT 0.5% of each individual impurity; and NMT 1.0% total impurities, including desacetyl diltiazem hydrochloride

**SPECIFIC TESTS**

• **OPTICAL ROTATION** *Specific Rotation* (781)

**Sample solution:** 10 mg/mL in water

**Acceptance criteria:** Between +110° and +116°

• **LOSS ON DRYING** (731)

**Sample:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Desacetyl Diltiazem Hydrochloride RS

*d-cis*-3-Hydroxy-2,3-dihydro-5-[2-dimethylaminoethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(*5H*)-one hydrochloride.

$C_{20}H_{24}N_2O_3S \cdot HCl$  408.95

USP Diltiazem Hydrochloride RS

## Diltiazem Hydrochloride Extended-Release Capsules

**DEFINITION**

Diltiazem Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ).

**IDENTIFICATION**

• **A.**

**Indicator solution:** Transfer 17.4 g of ammonium thiocyanate and 2.8 g of cobalt chloride to a 100-mL volumetric flask, add 50 mL of water, and sonicate for 10 min. Dilute with water to volume.

**Analysis:** Grind the content of 1 Capsule, and transfer to a 15-mL screw-capped test tube. Add 10 mL of 0.1 N hydrochloric acid, shake, and filter. Add 2 mL of *Indicator solution* to 2 mL of the filtrate, and shake. Add 5 mL of chloroform, and shake.

**Acceptance criteria:** A blue color develops in the chloroform layer.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 6.9 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 0.1 N hydrochloric acid to a pH of 3.0, and add 0.50 mL of triethylamine.

**Mobile phase:** Acetonitrile and *Buffer* (50:50)

**Standard stock solution:** 1.2 mg/mL of USP Diltiazem Hydrochloride RS and 0.02 mg/mL of USP Desacetyl Diltiazem Hydrochloride RS in methanol

**Standard solution:** 24 µg/mL of USP Diltiazem Hydrochloride RS from *Standard stock solution*, in *Mobile phase*

**Sample stock solution:** 1.2 mg/mL of diltiazem hydrochloride prepared as follows. Transfer the equivalent to 120 mg of diltiazem hydrochloride from NLT 20 Capsules (mix and grind the contents thoroughly) to a 100-mL volumetric flask. Add approximately 60 mL of

methanol, and shake by mechanical means for 30 min. Sonicate the resulting solution for 10 min to complete the extraction. Dilute with methanol to volume.

**Sample solution:** 24 µg/mL of diltiazem hydrochloride in *Mobile phase* from *Sample stock solution*, in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for desacetyl diltiazem and diltiazem are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between desacetyl diltiazem and diltiazem

**Relative standard deviation:** NMT 2.0% for diltiazem

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**For products labeled for dosing every 12 h**

**Test 1:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** Water; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 3, 9, and 12 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*

**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved (%)
3	10–25
9	45–85
12	NLT 70

The percentages of the labeled amount of  $C_{22}H_{26}N_2O_4S \cdot HCl$  dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** Water; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 4, 8, 12, and 24 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*

**Sample solutions:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Amount Dissolved (%)
4	10–25
8	35–60
12	55–80
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** 0.05 M phosphate buffer, pH 7.2; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 3, and 8 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*

**Sample solutions:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See *Table 3*.

**Table 3**

Time (h)	Amount Dissolved (%)
1	NMT 15
3	45–70
8	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 10:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.

**Buffer:** Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 6.5.

**Medium:** *Buffer*; 900 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 6, 9, and 24 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*

**Sample solutions:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See *Table 4*.

**Table 4**

Time (h)	Amount Dissolved (%)
1	NMT 10
6	10–30
9	34–60
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**For products labeled for dosing every 24 h**

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL  
**Apparatus 2:** 100 rpm  
**Times:** 1, 4, 10, and 15 h  
**Detector:** UV 237 nm  
**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.  
**Tolerances:** See *Table 5*.

**Table 5**

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	70–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** 0.1 N hydrochloric acid; 900 mL  
**Apparatus 2:** 100 rpm  
**Times:** 6, 12, 18, 24, and 30 h  
**Detector:** UV 237 nm  
**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.  
**Tolerances:** See *Table 6*.

**Table 6**

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium:** Water; 900 mL  
**Apparatus 1:** 100 rpm  
**Times:** 2, 4, 8, 12, and 16 h  
**Detector:** UV 237 nm  
**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.  
**Tolerances:** See *Table 7*.

**Table 7**

Time (h)	Amount Dissolved (%)
2	NMT 25
4	25–50
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

**Buffer:** Transfer 115 mL of acetic acid to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution A*). Transfer 165.4 g of anhydrous sodium acetate to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution B*). Mix 4410 mL of *Solution A* with 1590 mL of *Solution B*. Adjust, if necessary, with the addition of *Solution A* or *Solution B* to a pH of  $4.2 \pm 0.05$ .

**Medium:** *Buffer*; 900 mL  
**Apparatus 2:** 100 rpm  
**Times:** 1, 4, 10, and 15 h  
**Detector:** UV 237 nm  
**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.  
**Tolerances:** See *Table 8*.

**Table 8**

Time (h)	Amount Dissolved (%)
1	NMT 10
4	15–35
10	65–85
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 8:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

**Medium:** Water; 900 mL  
**Apparatus 2:** 100 rpm  
**Times:** 1, 4, 10, and 15 h  
**Detector:** UV 237 nm  
**Standard solution:** USP Diltiazem Hydrochloride RS in *Medium*  
**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.  
**Tolerances:** See *Table 9*.

**Table 9**

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	60–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 9:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

[NOTE—Perform the test separately in each of the two media.]

**Medium 1:** 0.1 N hydrochloric acid; 900 mL  
**Medium 2:** Simulated intestinal fluid TS, prepared without enzyme and adjusted to a pH of  $7.5 \pm 0.1$ ; 900 mL

**Apparatus 2:** 75 rpm

**Time for Medium 1:** 2 h

**Times for Medium 2:** 2, 12, 18, and 24 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See Table 10.

**Table 10**

Time (h)	Amount Dissolved, Medium 1 (%)	Amount Dissolved, Medium 2 (%)
2	0–5	20–45
12	—	35–55
18	—	NLT 60
24	—	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 11:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 1, 6, 12, and 18 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See Table 11.

**Table 11**

Time (h)	Amount Dissolved (%)
1	NMT 10
6	30–40
12	36–58
18	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 12:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*. Proceed as directed for *Extended-Release Dosage Forms*.

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Times:** 2, 8, 14, and 24 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See Table 12.

**Table 12**

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	NLT 65
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 13:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*. Proceed as directed for *Extended-Release Dosage Forms*.

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Times:** 2, 8, 14, and 24 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See Table 13.

**Table 13**

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	60–80
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 14:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*. Proceed as directed for *Extended-Release Dosage Forms*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 6, 12, 18, 24, and 30 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** See Table 14.

**Table 14**

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 15:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 15*. Proceed as directed for *Extended-Release Dosage Forms*.

**Medium:** 0.05 M phosphate buffer, pH 7.5; 900 mL

**Apparatus 2:** 75 rpm

**Times:** 2, 4, 8, 12, and 16 h

**Detector:** UV 237 nm

**Standard solution:** USP Diltiazem Hydrochloride RS in Medium

**Sample solution:** Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to that of the *Standard solution*.

Tolerances: See Table 15.

Table 15

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in *Dissolution* <711>.

**Test 16:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 16*. **Medium, Apparatus, Times, Standard solution, and Sample solution:** Proceed as directed for *Test 3*. **Detector:** UV 238 nm  
**Tolerances:** See Table 16.

Table 16

Time (h)	Amount Dissolved (%)
6	20–45
12	30–55
18	40–75
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in *Dissolution* <711>.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.
- **USP REFERENCE STANDARDS** <11>
  - USP Desacetyl Diltiazem Hydrochloride RS
  - $C_{20}H_{24}N_2O_3S \cdot HCl$  408.95
  - USP Diltiazem Hydrochloride RS

## Diltiazem Hydrochloride Oral Solution

### DEFINITION

Diltiazem Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ).

Prepare Diltiazem Hydrochloride Oral Solution 12 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Diltiazem Hydrochloride powder	1.2 g
Vehicle for Oral Solution (regular or sugar-free), NF, a sufficient quantity to make	100 mL

Add *Diltiazem Hydrochloride powder* and 10 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Solution A:** 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (50:25:25)

**Standard solution:** 120 µg/mL of USP Diltiazem Hydrochloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for diltiazem is about 9.6 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 1.3% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of diltiazem hydrochloride in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

- **pH** <791>: 3.7–4.7

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded
- **USP REFERENCE STANDARDS** <11>
  - USP Diltiazem Hydrochloride RS

## Diltiazem Hydrochloride Oral Suspension

### DEFINITION

Diltiazem Hydrochloride Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ).

Prepare Diltiazem Hydrochloride Oral Suspension 12 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Diltiazem Hydrochloride	1.2 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

If using tablets, comminute the tablets to a fine powder in a suitable mortar, or add *Diltiazem Hydrochloride* powder to the mortar. Add 10 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* to the mortar in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume.

## ASSAY

### PROCEDURE

**Solution A:** 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (50:25:25). Filter and degas.

**Standard solution:** 120 µg/mL of USP Diltiazem Hydrochloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample solution into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for diltiazem is about 9.6 min.]

### Suitability requirements

**Relative standard deviation:** NMT 1.3% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of diltiazem hydrochloride in *Standard solution* (µg/mL)

$C_U$  = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

## SPECIFIC TESTS

- pH** <791>: 3.7–4.7

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.
- LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded

- USP REFERENCE STANDARDS** <11>  
USP Diltiazem Hydrochloride RS

## Diltiazem Hydrochloride Tablets

### DEFINITION

Diltiazem Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S \cdot HCl$ ).

### IDENTIFICATION

#### A. PROCEDURE

**Indicator solution:** Transfer 17.4 g of ammonium thiocyanate and 2.8 g of cobalt chloride to a 100-mL volumetric flask, add 50 mL of water, and sonicate for 10 min. Dilute with water to volume.

**Analysis:** Finely powder 1 Tablet, and transfer to a 15-mL screw-capped test tube. Add 10 mL of 0.1 N hydrochloric acid, shake, and filter. Add 2 mL of *Indicator solution* to 2 mL of the filtrate, and shake. Add 5 mL of chloroform, and shake.

**Acceptance criteria:** A blue color develops in the chloroform layer.

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

**Solution A:** Dissolve 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate, and adjust with 0.1 N sodium hydroxide to a pH of 6.2.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (1:1:2)

**Standard solution:** 1.2 mg/mL of USP Diltiazem Hydrochloride RS in methanol

**Sample solution:** Transfer an equivalent to 600 mg of diltiazem hydrochloride from finely powdered Tablets (NLT 20) to a 500-mL volumetric flask. Add 200 mL of methanol, and sonicate for 1 h. Cool, and dilute with methanol to volume. Centrifuge a 25-mL aliquot at 3500 rpm for 15 min, and use the clear supernatant.

**System suitability solution:** 12 µg/mL of USP Diltiazem Hydrochloride RS and 12 µg/mL of USP Desacetyl Diltiazem Hydrochloride RS, in methanol

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.6 mL/min

**Injection size:** 10 µL

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3 between desacetyl diltiazem and diltiazem, *System suitability solution*

**Column efficiency:** NLT 1200 theoretical plates for the diltiazem peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{26}N_2O_4S \cdot HCl$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min and 3 h

Detector: UV 237 nm

Standard solution: USP Diltiazem Hydrochloride RS in Medium

Sample solution: Sample per *Dissolution* <711>. Dilute with Medium to a concentration that is similar to the *Standard solution*.

Tolerances: Use the following acceptance criteria for the 30-min time point: at  $S_1$  no unit is more than Q; at  $S_2$  the average value is equal to or less than Q, and no unit is greater than  $Q + 10\%$ ; at  $S_3$  the average value is equal to or less than Q, and not more than 2 units are more than  $Q + 10\%$ , and no unit is more than  $Q + 25\%$ . Use the criteria in *Dissolution* <711>, *Acceptance Table 1* for the 3-h time point. NMT 60% (Q) of the labeled amount of  $C_{22}H_{26}N_2O_4S \cdot HCl$  is dissolved in 30 min, and NLT 75% (Q) is dissolved in 3 h.

### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers.

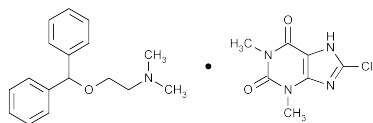
### • USP REFERENCE STANDARDS <11>

USP Desacetyl Diltiazem Hydrochloride RS

$C_{20}H_{24}N_2O_3S \cdot HCl$  408.95

USP Diltiazem Hydrochloride RS

## Dimenhydrinate



$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$  469.96

1*H*-Purine-2,6-dione, 8-chloro-3,7-dihydro-1,3-dimethyl-, compd. with 2-(diphenylmethoxy)-*N,N*-dimethylethanamine (1:1).

8-Chlorotheophylline, compound with 2-(diphenylmethoxy)-*N,N*-dimethylethanamine (1:1) [523-87-5].

» Dimenhydrinate contains not less than 53.0 percent and not more than 55.5 percent of diphenhydramine ( $C_{17}H_{21}NO$ ), and not less than 44.0 percent and not more than 47.0 percent of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards <11>—

USP Dimenhydrinate RS

**Identification**—Infrared Absorption <197K>.

**Melting range** <741>: between 102° and 107°.

**Loss on drying** <731>—Dry it in vacuum over phosphorus pentoxide for 24 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.3%.

**Bromide and iodide**—Mix in a test tube 100 mg of Dimenhydrinate, 50 mg of sodium nitrite, and 10 mL of chloroform. Add 10 mL of 3 N hydrochloric acid, insert the stopper in the tube, and shake: the chloroform remains colorless.

**Chloride**—When the ammoniacal filtrate from the precipitation of silver chlorotheophylline in the *Assay for 8-chlorotheophylline* is acidified preparatory to titration, the solution shows not more than a faint opalescence.

**Assay for diphenhydramine**—Dissolve about 150 mg of Dimenhydrinate, accurately weighed, in 75 mL of glacial acetic acid, and titrate with 0.05 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 12.77 mg of diphenhydramine ( $C_{17}H_{21}NO$ ).

**Assay for 8-chlorotheophylline**—Place about 800 mg of Dimenhydrinate, accurately weighed, in a 200-mL volumetric flask, add 50 mL of water, 3 mL of 6 N ammonium hydroxide, and 6 mL of ammonium nitrate solution (1 in 10), and warm the mixture on a steam bath for 5 minutes. Add 25.0 mL of 0.1 N silver nitrate VS, mix, and warm on a steam bath for 15 minutes with frequent shaking. Cool, dilute with water to volume, mix, and allow the precipitate to settle. Filter through a dry filter paper, discarding the first 20 mL of the filtrate. Pipet 100 mL of the filtrate into a 250-mL flask, acidify with nitric acid, and add an excess of 3 mL of the acid. Add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 21.46 mg of  $C_7H_7ClN_4O_2$ .

## Dimenhydrinate Injection

» Dimenhydrinate Injection is a solution of Dimenhydrinate in a mixture of Propylene Glycol and water. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.

### USP Reference standards <11>—

USP Dimenhydrinate RS

**Identification**—The relative retention times of the major peaks for 8-chlorotheophylline and diphenhydramine in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** <791>: between 6.4 and 7.2.

### Content of 8-chlorotheophylline—

**Mobile phase**—Dissolve 0.81 g of *dl*-10-camphorsulfonic acid and 0.70 g of sodium acetate in 700 mL of water. Add 300 mL of methanol, mix, and pass through a membrane filter having a 0.5-μm or finer porosity.

**Standard solution**—Dissolve an accurately weighed quantity of USP Dimenhydrinate RS in methanol to obtain a Standard stock solution having a known concentration of about 0.5 mg per mL. Retain a portion of this Standard stock solution for use in the *Assay*. Pipet 5 mL into a 50-mL

volumetric flask, dilute with methanol to volume, mix, and pass through a membrane filter having a 0.5- $\mu$ m or finer porosity.

**Test solution**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of dimenhydrinate, to a 100-mL volumetric flask, dilute with methanol to volume, and mix to obtain a stock solution. Retain a portion of this stock solution for use in the Assay. Pipet 5 mL of this stock solution into a 50-mL volumetric flask, dilute with methanol to volume, mix, and pass through a membrane filter having a 0.5- $\mu$ m or finer porosity.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector, a 2-mm  $\times$  12.5-cm guard column that contains packing L2, and a 4.6-mm  $\times$  25-cm analytical column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ) in each mL of the Injection taken by the formula:

$$(214.61/469.96)(1000C/V)(r_U / r_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; C is the concentration, in mg per mL, of USP Dimenhydrinate RS in the *Standard solution*; V is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the Assay is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

#### Assay—

**Solution A, Solution B, Mobile phase, Internal standard solution, and Chromatographic system**—Prepare as directed in the Assay under *Dimenhydrinate Tablets*.

**Standard preparation**—Use a portion of the Standard stock solution that was prepared for the *Standard solution* in the test for *Content of 8-chlorotheophylline*. Mix 5.0 mL of this Standard stock solution and 5.0 mL of *Internal standard solution*, and pass through a membrane filter having a 0.5- $\mu$ m or finer porosity.

**Assay preparation**—Use a portion of the stock solution that was prepared for the *Test solution* in the test for *Content of 8-chlorotheophylline*. Mix 5.0 mL of this stock solution and 5.0 mL of *Internal standard solution*, and pass through a membrane filter having a 0.5- $\mu$ m or finer porosity.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Dimenhydrinate Tablets*. Calculate the quantity, in mg, of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ) in each mL of the Injection taken by the formula:

$$(200C / V)(R_U / R_S)$$

in which C is the concentration of USP Dimenhydrinate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and the other terms are as defined therein.

### Dimenhydrinate Oral Solution

» Dimenhydrinate Oral Solution contains not less than 90.0 percent and not more than 110.0 per-

cent of the labeled amount of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Dimenhydrinate RS

**Identification**—The relative retention times of the major peaks for 8-chlorotheophylline and diphenhydramine in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the Assay.

#### Content of 8-chlorotheophylline—

**Ammonium bicarbonate solution, Diluent, Solution A, Solution B, Mobile phase, Internal standard solution, and Chromatographic system**—Proceed as directed in the Assay under *Dimenhydrinate Tablets*.

**Standard solution**—Prepare as directed for *Standard preparation* in the Assay under *Dimenhydrinate Tablets*.

**Test solution**—Prepare as directed for *Assay preparation* in the Assay.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg per mL, of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ) in the portion of Oral Solution taken by the formula:

$$(214.61/469.96)(0.05W)(R_U / R_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard solution*; and  $R_U$  and  $R_S$  are peak area ratios of 8-chlorotheophylline to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the Assay is found.

**Alcohol content** <611>: between 4.0% and 6.0% of  $C_2H_5OH$ .

#### Assay—

**Ammonium bicarbonate solution, Diluent, Solution A, Solution B, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay under *Dimenhydrinate Tablets*.

**Assay preparation**—Pipet 5.0 mL of Oral Solution into a suitable container, add 5.0 mL of *Internal standard solution*, and mix. Transfer about 1 mL of this solution to a suitable container, add about 5 mL of *Diluent*, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Dimenhydrinate Tablets*. Calculate the quantity, in mg per mL, of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ) in the portion of the Oral Solution taken by the formula:

$$0.05W(R_U / R_S)$$

in which W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak area ratios of diphenhydramine to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Dimenhydrinate Tablets

» Dimenhydrinate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dimenhydrinate RS

**Identification**—The relative retention times of the 8-chlorotheophylline and diphenhydramine peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Dimenhydrinate RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements, the following procedure being used where the test for *Content Uniformity* is required. Transfer 1 Tablet to a 50-mL volumetric flask, add about 5 mL of *Ammonium bicarbonate solution* obtained from the *Assay*, and shake gently to disperse, sonicating, if necessary. Add 20.0 mL of *Internal standard solution* obtained from the *Assay*, shake by mechanical means for 30 minutes, and centrifuge. To 1 mL of the clear supernatant add about 9 mL of *Diluent* obtained from the *Assay*, and mix. Continue as directed for *Procedure* in the *Assay*.

**Content of 8-chlorotheophylline**—

*Ammonium bicarbonate solution*, *Diluent*, *Solution A*, *Solution B*, *Mobile phase*, *Internal standard solution*, and *Chromatographic system*—Prepare as directed in the *Assay*.

**Standard solution**—Prepare as directed for *Standard preparation* in the *Assay*.

**Test solution**—Prepare as directed for *Assay preparation* in the *Assay*.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ) per Tablet taken by the formula:

$$(214.61/469.96)W(R_U / R_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; *W* is the weight, in mg, of USP Dimenhydrinate RS in the *Standard solution*; and  $R_U$  and  $R_S$  are peak area ratios of 8-chlorotheophylline to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the *Assay* is found.

**Assay**—

**Ammonium bicarbonate solution**—Dissolve 4 g of ammonium bicarbonate in 250 mL of water.

**Diluent**—Dissolve 4 g of ammonium bicarbonate in 200 mL of water. Add 50 mL of methanol, and mix.

**Solution A**—Dissolve 0.8 g of ammonium bicarbonate in 800 mL of water. Add 200 mL of methanol, filter, and degas.

**Solution B**—Dissolve 0.8 g of ammonium bicarbonate in 150 mL of water. Add 850 mL of methanol, filter, and degas.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution in methanol containing 2.0 mg of 2-hydroxybenzyl alcohol per mL.

**Standard preparation**—Accurately weigh about 50 mg of USP Dimenhydrinate RS, add about 5 mL of *Ammonium bicarbonate solution* and 20.0 mL of *Internal standard solution*, and mix. To 1 mL of this solution add about 9 mL of *Diluent*, and mix.

**Assay preparation**—Transfer 5 Tablets into a 250-mL volumetric flask, add 25 mL of *Ammonium bicarbonate solution*, and shake gently to disperse, sonicating if necessary. Add 100.0 mL of *Internal standard solution*, shake vigorously for 30 minutes, and centrifuge. To 1 mL of the clear supernatant add about 9 mL of *Diluent*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–7.0	100	0	isocratic
7.0–7.1	100→0	0→100	linear gradient
7.1–15	0	100	isocratic
15–15.1	0→100	100→0	linear gradient
15.1–22.0	100	0	isocratic

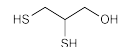
Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.3 for 8-chlorotheophylline, 0.5 for the internal standard, and 1.0 for diphenhydramine; the resolution,  $R$ , between 8-chlorotheophylline and the internal standard is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dimenhydrinate ( $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ ) per Tablet taken by the formula:

$$W(R_U / R_S)$$

in which *W* is the weight, in mg, of USP Dimenhydrinate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak area ratios of diphenhydramine to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dimercaprol



$C_3H_8OS_2$  124.23

1-Propanol, 2,3-dimercapto.

2,3-Dimercapto-1-propanol [59-52-9].

» Dimercaprol contains not less than 97.0 percent and not more than 100.5 percent of  $C_3H_8OS_2$ , and not more than 1.5 percent of 1,2,3-trimercaptopropane ( $C_3H_8S_3$ ).

**Packaging and storage**—Preserve in tight containers, in a cold place.

**Specific gravity** (841): between 1.242 and 1.244.

**Distilling range, Method I** (721): between 66° and 68°, under a pressure of 0.2 mm of mercury.

**Refractive index** (831): between 1.567 and 1.573.

**Limit of 1,2,3-trimercaptopropane and related impurities—**

**Adsorbent**—Use a suitable chromatographic grade of 100-mesh silicic acid.

**Standard buffer solution**—Prepare 100 mL of pH 6.0 Phosphate Buffer (see pH (791)), and dissolve in it 100 mg of sodium bisulfite.

**Acid-washed solvent hexane**—To 100 mL of solvent hexane contained in a separator add 10 mL of sulfuric acid, shake for not less than 12 hours, and allow the layers to separate. Transfer the acid-washed solvent to a distilling flask, and distill slowly, retaining only that portion distilling between 35° and 50°. Use only freshly distilled material.

**Diisopropyl ether**—Place 100 mL of diisopropyl ether in a distilling flask, and distill, retaining only that portion distilling between 68° and 69°. Use only freshly distilled material.

[*Caution—Do not evaporate to the point of near-dryness, since diisopropyl ether tends to form explosive peroxides*]

**Solvent hexane-diisopropyl ether mixture (mobile solvent)**—Mix 50 mL of Diisopropyl ether with 50 mL of Acid-washed solvent hexane.

**Chromatographic tube**—Insert a small plug of glass wool at the juncture of the tube and the stem of a 600- × 13-mm chromatographic tube.

**Chromatographic column**—Mix 20 g of Adsorbent with 20 mL of Standard buffer solution. Make into a slurry by mixing with 100 mL of chloroform. Transfer successive portions of the slurry into the Chromatographic tube, packing firmly and evenly with a close-fitting, ground-glass tamper after each addition. Keep a layer of liquid above the packed column to prevent the formation of air spaces. Wash the column free from chloroform with Solvent hexane-diisopropyl ether mixture, and allow the solvent to fall to the level of the Adsorbent.

**Procedure**—Place about 250 mg of Dimercaprol, accurately weighed and demonstrated to be free from hydrogen sulfide as directed in the Assay, in a 5-mL volumetric flask, add Solvent hexane-diisopropyl ether mixture to volume, and mix. Transfer 2.0 mL of the resulting solution to the prepared Chromatographic column. When the liquid has passed into the column, wash the wall of the tube with a 2-mL portion of Solvent hexane-diisopropyl ether mixture, and allow the washing to fall to the level of the Adsorbent. Fill the Chromatographic tube with solvent, and collect two successive fractions: (A) a 20-mL fraction containing all of the 1,2,3-trimercaptopropane, and (B) a 3-mL fraction that serves as a check on the separation. To each fraction add an equal volume of alcohol, and titrate with 0.1 N iodine VS until a permanent yellow color is produced. Perform a blank titration on 20 mL of the solvent mixture that has been passed through the column prior to introduction of the test specimen, and make any necessary correction. Fraction (B) does not decolorize 1 drop of 0.1 N iodine VS. Each mL of 0.1 N iodine added is equivalent to 4.676 mg of C<sub>3</sub>H<sub>8</sub>S<sub>3</sub>. Not more than 1.5% of 1,2,3-trimercaptopropane (C<sub>3</sub>H<sub>8</sub>S<sub>3</sub>) is found.

**Assay**—Test the Dimercaprol for the presence of hydrogen sulfide by examining the vapor above the assay specimen with moistened lead acetate test paper. If the paper darkens, bubble dry, oxygen-free nitrogen or carbon dioxide through the assay specimen until a fresh strip of test paper gives a negative test. Transfer about 2 mL of hydrogen sulfide-free Dimercaprol to a tared, glass-stoppered, 100-mL volumetric flask, weigh accurately, add methanol to volume, and mix. Pipet 10 mL of the solution into a 50-mL conical flask, and titrate with 0.1 N iodine VS until a permanent

yellow color is produced. Perform a blank titration, and make any necessary correction. Calculate the percentage of C<sub>3</sub>H<sub>8</sub>OS<sub>2</sub> taken by the formula:

$$0.6211V/W - 1.328T$$

in which *V* is the volume, in mL, of 0.1 N iodine used, *W* is the weight, in g, of specimen in the aliquot taken, and *T* is the percentage of C<sub>3</sub>H<sub>8</sub>S<sub>3</sub> found in the determination of the Limit of 1,2,3-trimercaptopropane and related impurities.

## Dimercaprol Injection

» Dimercaprol Injection is a sterile solution of Dimercaprol in a mixture of Benzyl Benzoate and vegetable oil. It contains, in each 100 g, not less than 9.0 g and not more than 11.0 g of dimercaprol (C<sub>3</sub>H<sub>8</sub>OS<sub>2</sub>).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.

**USP Reference standards** (11)—

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 1 USP Endotoxin Unit per mg of dimercaprol.

**Limit of 1,2,3-trimercaptopropane and related impurities—**

**Adsorbent, Standard buffer solution, Acid-washed solvent hexane, Diisopropyl ether, Solvent hexane-diisopropyl ether mixture, Chromatographic tube, and Chromatographic column**—Prepare as directed in the test for Limit of 1,2,3-trimercaptopropane and related impurities under Dimercaprol.

**Procedure**—Place about 1 g of Injection, accurately weighed and demonstrated to be free from hydrogen sulfide as directed in the Assay under Dimercaprol, in a 5-mL beaker, add 2 mL of Solvent hexane-diisopropyl ether mixture, and mix. Transfer the resulting solution to the prepared Chromatographic column. When the liquid has passed into the column, wash the beaker with two 2-mL portions of Solvent hexane-diisopropyl ether mixture, and allow the washings to fall to the level of the Adsorbent. Proceed as directed for Procedure in the test for Limit of 1,2,3-trimercaptopropane and related impurities under Dimercaprol. The limit of 1,2,3-trimercaptopropane (C<sub>3</sub>H<sub>8</sub>S<sub>3</sub>) is not more than 4.5%, by weight, of the content of dimercaprol.

**Other requirements**—It meets the requirements under Injections (1), except that at times it may be turbid or contain small amounts of flocculent material.

**Assay**—Transfer about 2 mL of Injection to a tared conical flask, and weigh accurately. Add 100 mL of a mixture of 1 volume of chloroform and 3 volumes of methanol, agitate to dissolve the Injection, and titrate with 0.1 N iodine VS to the production of a permanent yellow color. Perform a blank determination, and make any necessary correction. Calculate the percentage of dimercaprol (C<sub>3</sub>H<sub>8</sub>OS<sub>2</sub>) in the portion of Injection taken by the formula:

$$0.6211(V/W - v/w)$$

in which *V* is the volume, in mL, of 0.1 N iodine used; *W* is the weight, in g, of Injection taken; and *v* and *w* are the volume, in mL, of 0.1 N iodine and the weight, in g, of Injection, respectively, used in the test for Limit of 1,2,3-trimercaptopropane and related impurities.

## Dimethyl Sulfoxide



$\text{C}_2\text{H}_6\text{OS}$  78.13  
Methane, sulfinylbis-  
Methyl sulfoxide [67-68-5].

» Dimethyl Sulfoxide contains not less than 99.9 percent of  $\text{C}_2\text{H}_6\text{OS}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Dimethyl Sulfoxide RS

### Identification—

**A:** *Infrared Absorption* (197F), potassium bromide plates being used.

**B:** Add 1.5 mL cautiously and dropwise to 2.5 mL of hydriodic acid in a test tube cooled in ice. Filter the mixture rapidly, and collect the precipitate. Dry the precipitate in vacuum: a deep violet, crystalline solid is obtained, and it is soluble in chloroform, yielding a red solution.

**Specific gravity** (841): between 1.095 and 1.101.

**Refractive index** (831): between 1.4755 and 1.4775.

**Acidity**—Dissolve 50.0 g in 100 mL of water, and add phenolphthalein TS. If the solution remains colorless, titrate with 0.01 N sodium hydroxide until a pink color appears: not more than 5.0 mL of 0.01 N sodium hydroxide is consumed.

**Water, Method I** (921): not more than 0.1%. [NOTE—Weigh and transfer the test specimen in an environment of low humidity to minimize absorption of atmospheric water.]

**Ultraviolet absorbance**—Maintain Dimethyl Sulfoxide in a water bath at a temperature of less than 20° [NOTE—Do not freeze.], and purge with dry nitrogen for 30 minutes. Record the UV absorption spectrum between 270 and 350 nm in a 1-cm cell, using water as the blank: the spectrum is smooth with no absorption maxima; the absorbance at 275 nm is not more than 0.20, and the absorbance ratios,  $A_{285}/A_{275}$  and  $A_{295}/A_{275}$ , at the wavelengths indicated by the subscripts, are not more than 0.65 and 0.45, respectively.

**Limit of nonvolatile residue**—Evaporate about 100.0 g to dryness in a tared, preconditioned dish on a hot plate under a suitable fume hood. Evaporate gently so boiling does not occur. The weight of the residue does not exceed 0.01%.

### Related compounds—

**System suitability solution**—Prepare a solution containing about 0.15 mg of dimethyl sulfone per mL and 0.1 mg of dibenzyl per mL in Dimethyl Sulfoxide.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and contains a 0.32-mm  $\times$  15-m column bonded with a 3- $\mu$ m film of phase G2. Initially the column temperature is maintained at 100° for 15 minutes, then is increased at a rate of 10° per minute to a temperature of 170°, and maintained at 170° for 20 minutes. The injection port is maintained at a temperature of about 210°, and the detector block is maintained at a temperature of about 220°. Helium is used as the carrier gas, flowing at a rate of 1.7 mL per minute, and the split ratio is 33:1. [NOTE—The split ratio can be modified in order to optimize performance.] Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between dimethyl sulfone and dibenzyl is not less than 5.0;

and the column efficiency, determined from the dimethyl sulfoxide peak, is not less than 1000 theoretical plates.

**Procedure**—Inject about 1  $\mu$ L of Dimethyl Sulfoxide into the gas chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of total impurities in the portion of Dimethyl Sulfoxide taken by the formula:

$$100(r_U / r_S)$$

in which  $r_U$  is the sum of the areas of all the impurity peaks; and  $r_S$  is the sum of the areas of all the peaks: not more than 0.1% of total impurities is found.

**Assay**—Using the results from the test for *Limit of nonvolatile residue* and the test for *Related compounds*, calculate the percentage of  $\text{C}_2\text{H}_6\text{OS}$  in the portion of Dimethyl Sulfoxide taken by subtracting the percentage of nonvolatile residue and the percentage of total impurities found from 100.0%. [NOTE—A correction for percent water is not applied to the result.]

## Dimethyl Sulfoxide Gel

» Dimethyl Sulfoxide Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of  $\text{C}_2\text{H}_6\text{OS}$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—  
USP Dimethyl Sulfoxide RS

**Identification**—The retention time of the dimethyl sulfoxide peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 4.7 and 6.7, determined on a mixture of 5 g of Gel and 5 mL of water.

### Assay—

**Internal standard solution**—Transfer 1.0 mL of dimethylformamide to a 100-mL volumetric flask. Add about 75 mL of methanol, and shake for about 1 minute. Dilute with methanol to volume, and mix.

**Standard preparation**—Transfer about 2 g of USP Dimethyl Sulfoxide RS, accurately weighed, to a 100-mL volumetric flask, add 1.0 mL of dimethylformamide and 75 mL of methanol, and shake for about 1 minute. Dilute with methanol to volume, and mix. This solution contains about 20 mg of USP Dimethyl Sulfoxide RS per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Gel, equivalent to 2 g of dimethyl sulfoxide, to a 100-mL volumetric flask. Add 1.0 mL of dimethylformamide and about 75 mL of methanol. Dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.8-m column packed with 15% liquid phase G39 on support S1A. The column is maintained at about 160° and the injection port and detector block at about 200°. Helium is used as the carrier gas, flowing at a rate of about 30 mL per minute. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure*: examine the chromatogram to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms.

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for dimethylformamide and 1.0 for dimethyl sulfoxide; the tailing factor is not more than 2.0; the resolution,  $R_s$ , between the dimethylformamide peak and the dimethyl sulfoxide peak is not less than 4; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 40 minutes, and measure the peak heights for the dimethylformamide and dimethyl sulfoxide peaks. Calculate the percentage (w/w) of  $C_2H_6OS$  in the portion of Gel taken by the formula:

$$10(C / W)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation*;  $W$  is the weight, in g, of Gel taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the dimethyl sulfoxide peak response to the dimethylformamide peak response in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dimethyl Sulfoxide Irrigation

» Dimethyl Sulfoxide Irrigation is a sterile solution of Dimethyl Sulfoxide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_2H_6OS$ .

**Packaging and storage**—Preserve in single-dose containers, and store at controlled room temperature, protected from strong light.

**Labeling**—Label it to indicate prominently that it is not intended for injection.

### USP Reference standards (11)—

USP Dimethyl Sulfoxide RS

USP Endotoxin RS

**Identification**—A portion of the *Assay preparation*, chromatographed as directed in the *Assay*, exhibits a major peak for dimethyl sulfoxide, the retention time of which is identical with that exhibited by the *Standard preparation*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 Endotoxin Unit per mL.

**Sterility** (71): meets the requirements.

**pH** (791): between 5.0 and 7.0, when diluted with water to obtain a solution containing 50 mg of dimethyl sulfoxide per mL.

### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dimethyl Sulfoxide RS in acetone, and dilute quantitatively with acetone to obtain a solution having a known concentration of about 80 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Dimethyl Sulfoxide Irrigation, equivalent to about 2 g of dimethyl sulfoxide, to a 25-mL volumetric flask, dilute with acetone to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—Proceed as directed in the test for *Dimethyl sulfone* under *Dimethyl Sulfoxide*. The relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Introduce equal volumes (about 1.0  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the gas

chromatograph. Measure the peak responses, at corresponding retention times, so obtained, and calculate the quantity, in mg, of  $C_2H_6OS$  in each mL of Dimethyl Sulfoxide Irrigation taken by the formula:

$$25(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation*;  $V$  is the volume, in mL, of Dimethyl Sulfoxide Irrigation taken; and  $r_U$  and  $r_S$  are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

## Dimethyl Sulfoxide Topical Solution

» Dimethyl Sulfoxide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of  $C_2H_6OS$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Dimethyl Sulfoxide RS

### Identification—

**A:** The chromatogram of the *Assay preparation*, obtained as directed in the *Assay*, exhibits a major peak for dimethyl sulfoxide, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**B:** To 2 mL of Topical Solution in a test tube add 5 mL of cold hydriodic acid. Filter the mixture rapidly, and collect the precipitate. Dry the precipitate in vacuum: a deep violet, crystalline solid is obtained, and it is soluble in chloroform, yielding a red solution.

**C:** To 0.1 g of solid sodium hydroxide in a test tube add 10 mL of Topical Solution, and heat on a steam bath until the sodium hydroxide has dissolved. Add 10 mL of chloroform, and shake. Filter the chloroform layer through a filter funnel containing about 10 g of anhydrous sodium sulfate. Heat the filtrate on a steam bath to remove the chloroform. Determine the IR spectrum of the liquid residue as directed under *Spectrophotometric Identification Tests* (197F). The spectrum so obtained corresponds to that of a spectrum of USP Dimethyl Sulfoxide RS, similarly prepared.

**Assay**—[NOTE—Use glass equipment to prepare solutions.]

**Internal standard solution**—Transfer 3.0 mL of dimethylformamide to a 250-mL volumetric flask. Add about 200 mL of acetone, and shake for about 1 minute. Dilute with acetone to volume, and mix.

**Standard preparation**—Transfer about 2 g of USP Dimethyl Sulfoxide RS, accurately weighed, to a 250-mL volumetric flask, add 3.0 mL of dimethylformamide and 200 mL of acetone, and sonicate for about 1 minute. Dilute with acetone to volume, and mix to obtain a solution having a known concentration of about 8 mg of USP Dimethyl Sulfoxide RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Topical Solution, equivalent to about 2 g of dimethyl sulfoxide, to a 250-mL volumetric flask, add 3.0 mL of dimethylformamide and about 200 mL of acetone, and sonicate for about 1 minute. Dilute with acetone to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.8-m column packed with 10% liquid

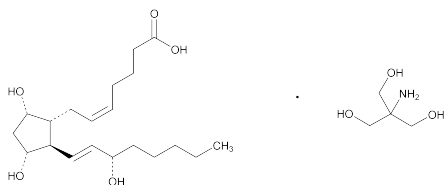
phase G16 on support S1A. The column is maintained at about 170°, the injection port at about 200°, and the detector block at about 300°. The carrier gas is helium, flowing at a rate of about 20 mL per minute. Chromatograph two portions of the acetone used to prepare the *Standard preparation*, the *Assay preparation*, and the *Internal standard solution*, and record the peak responses as directed under *Procedure*. Discard the first chromatogram, and examine the second to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure*: examine the chromatogram to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.2 for acetone, 0.5 for dimethylformamide, and 1.0 for dimethyl sulfoxide; the resolution,  $R$ , between the dimethylformamide peak and the dimethyl sulfoxide peak is not less than 2; the tailing factors for the dimethylformamide peak and the dimethyl sulfoxide peak are not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 20 minutes, and measure the responses for the dimethylformamide and dimethyl sulfoxide peaks. Calculate the percentage (v/v) of  $C_2H_6OS$  in the portion of Topical Solution taken by the formula:

$$(2.5 / 1.096)10(C / V)(R_U / R_S)$$

in which 1.096 is the specific gravity of dimethyl sulfoxide,  $C$  is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation*;  $V$  is the volume, in mL, of Topical Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the dimethyl sulfoxide peak response to the dimethylformamide peak response in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dinoprost Tromethamine



$C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$  475.62  
Prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-, (5Z,9 $\alpha$ ,11 $\alpha$ ,13E,15S)-, compd. with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).  
(E,Z)-(1R,2R,3R,5S)-7-[3,5-Dihydroxy-2-[(3S)-(3-hydroxy-1-octenyl)]cyclopentyl]-5-heptenoic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).  
Prostaglandin  $F_{2\alpha}$  tromethamine [38562-01-5].

» Dinoprost Tromethamine contains not less than 95.0 percent and not more than 105.0 percent of  $C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$ , calculated on the dried basis. [Caution—Great care should be taken to pre-

vent inhaling particles of Dinoprost Tromethamine and exposing the skin to it.]

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dinoprost Tromethamine RS

**Identification**, *Infrared Absorption* (197M).

**Specific rotation** (781S): between +19° and +26°.

*Test solution*: 20 mg per mL, in alcohol.

**Loss on drying** (731)—Dry it in vacuum at room temperature and at a pressure not exceeding 5 mm of mercury for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Chromatographic purity**—[NOTE—Prepare solutions immediately prior to use.]

*Mobile phase*—Proceed as directed in the *Assay*.

*Standard stock solution*—Prepare as directed for *Standard preparation* in the *Assay*.

*Standard solution*—Transfer 1.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Test solution*—Prepare as directed for the *Assay preparation*.

*Chromatographic system*—Proceed as directed in the *Assay*. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between dinoprost tromethamine and any other adjacent peak is not less than 1.0.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dinoprost Tromethamine taken by the formula:

$$2.5(C/W)F(r_i / r_s)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Dinoprost Tromethamine RS in the *Standard solution*;  $W$  is the weight, in mg, of Dinoprost Tromethamine taken to prepare the *Test solution*;  $F$  is the relative response factor and is equal to 0.25 for any peak at a relative retention time of about 0.30, 1.7 for any peak at a relative retention time of about 1.15, and 1.0 for any other peak;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_s$  is the peak response of dinoprost tromethamine obtained from the *Standard solution*: not more than 2.0% of any impurity having a relative retention time of about 0.94 is found; not more than 1.5% of any impurity having a relative retention time of about 0.84 is found; not more than 0.5% of any other impurity is found; and not more than 2.0% of all other impurities is found.

**Assay**—[NOTE—Prepare solutions immediately prior to use.]

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and phosphoric acid (750:250:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dinoprost Tromethamine RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.

*Assay preparation*—Transfer about 25.0 mg of Dinoprost Tromethamine, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the

*Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 6000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$  in the portion of Dinoprost Tromethamine taken by the formula:

$$25C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dinoprost Tromethamine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dinoprost Tromethamine Injection

» Dinoprost Tromethamine Injection is a sterile solution of Dinoprost Tromethamine in Water for Injection. It may contain a suitable preservative, such as benzyl alcohol. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dinoprost ( $C_{20}H_{34}O_5$ ).

*Caution*—Extreme care should be exercised when handling dinoprost tromethamine as it is readily absorbed through the skin; accidental spillage on the skin should be washed off immediately with soap and water.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Dinoprost Tromethamine RS

USP Endotoxin RS

**Identification**—The retention time of the derivatized dinoprost peak in the chromatogram of the *Assay preparation* corresponds to that of the derivatized dinoprost peak in the *Standard preparation*, both relative to the internal standard, obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 8 USP Endotoxin Units per 1.0 mg of dinoprost.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.0 and 9.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

*Mobile phase*—Prepare a solution consisting of methylene chloride, 1,3-butanediol, and water (496:3.5:0.25).

*Internal standard solution*—Prepare a solution in *Mobile phase* containing about 0.75 mg of guaifenesin per mL.

*Reagent preparations*—

A—Prepare a solution containing about 10 mg of  $\alpha$ -bromo-2'-acetophenone per mL of acetonitrile. Use a freshly prepared solution.

B—Prepare a solution containing 5  $\mu$ L of diisopropylethylamine per mL of acetonitrile. Use a freshly prepared solution.

C—Prepare a citrate buffer solution by dissolving 10.5 g of citric acid monohydrate in about 75 mL of water and adding 5 N sodium hydroxide until a pH of 4.0 is obtained. Dilute with water to 100 mL, and mix.

*Diluent*—Use *Sterile Water for Injection* containing 0.945% of benzyl alcohol.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dinoprost Tromethamine RS in *Diluent* to obtain a solution having a known concentration of about 0.67 mg of dinoprost tromethamine per mL (*Standard stock preparation*). Transfer 1.0 mL of this solution to a suitable container, and add 1.0 mL of *Reagent C* and 20.0 mL of methylene chloride. Shake and centrifuge. Transfer 5.0 mL of the lower layer into a suitable container, and evaporate with the aid of nitrogen to dryness. Wash the inside of the container with 200  $\mu$ L of *Reagent A*. Swirl to dissolve, add 100  $\mu$ L of *Reagent B*, and mix. Allow the solution to stand for about 1 hour at room temperature, evaporate to dryness, add 4.0 mL of *Internal standard solution*, and mix to obtain a *Standard preparation* having a known concentration of about 0.0419 mg of USP Dinoprost Tromethamine RS per mL.

*Assay preparation*—Dilute and mix an accurately measured volume of Injection with *Diluent* to obtain a solution having a known concentration of about 0.5 mg of dinoprost per mL (*Assay stock preparation*). Proceed as directed for *Standard preparation*, beginning with "Transfer 1.0 mL of this solution."

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the dinoprost tromethamine and internal standard peaks is not less than 10, and the relative standard deviation for replicate injections is not more than 2.0%.

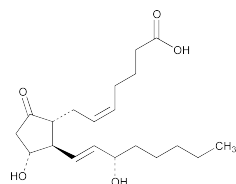
*Procedure*—Inject equal volumes (about 20  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses at equivalent retention times. The relative retention times are about 0.4, 0.5, 1.0, and 1.2 for the internal standard, the 15-*R* epimer, dinoprost tromethamine, and the 5,6-*trans* isomer, respectively. Calculate the quantity, in mg, of  $C_{20}H_{34}O_5$  in each mL of the Injection taken by the formula:

$$(354.48/475.62)(DC)(R_U / R_S)$$

in which 354.48 and 475.62 are the molecular weights of dinoprost and dinoprost tromethamine, respectively;  $D$  is the dilution factor used in preparing the *Assay stock preparation*;  $C$  is the concentration, in mg per mL, of USP Dinoprost Tromethamine RS in the *Standard stock preparation*; and  $R_U$  and  $R_S$  are the ratios of the responses for the dinoprost tromethamine and internal standard peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Dinoprostone



$C_{20}H_{32}O_5$  352.47  
 Prosta-5,13-dien-1-oic acid, 11,15-dihydroxy-9-oxo-, (5Z, 11 $\alpha$ ,13E,15S)-;  
 (E,Z)-(1R,2R,3R)-7-[3-Hydroxy-2-[(3S)-(3-hydroxy-1-octenyl)]-5-oxocyclopentyl]-5-heptenoic acid;  
 Prostaglandin  $E_2$  [363-24-6].

### DEFINITION

Dinoprostone contains NLT 97.0% and NMT 103.0% of  $C_{20}H_{32}O_5$ .

[NOTE—Prepare all solutions in all tests immediately before use.]

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol and 0.2% acetic acid (29:21)  
**Standard solution:** 2.5 mg/mL of USP Dinoprostone RS in *Mobile phase*

**Sample solution:** 2.5 mg/mL of Dinoprostone in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.0 between dinoprostone and any other adjacent peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{20}H_{32}O_5$  in the portion of Dinoprostone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Dinoprostone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0%

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.5%

#### Organic Impurities

##### PROCEDURE

**Mobile phase:** Proceed as directed in the *Assay*.

**Standard stock solution:** Prepare as directed for the *Standard solution* in the *Assay*.

**Standard solution:** Transfer 0.5 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** Prepare as directed in the *Assay*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard stock solution* and *Sample solution*

#### Suitability requirements

**Column efficiency:** NLT 6000 theoretical plates, *Standard stock solution*

**Relative standard deviation:** NMT 2.0%, *Standard stock solution*

**Resolution:** NLT 1.0 between dinoprostone and any other adjacent peak, *Sample solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Dinoprostone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of dinoprostone from the *Standard solution*

$C_S$  = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Dinoprostone in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
15-Oxo-dinoprostone	0.79	5	—*
15-Epi-dinoprostone	0.85	1.1	—*
8-Isodinoprostone	0.90	1.0	—*
5,6-trans-Di-noprostone	1.15	1.0	2.0
(5Z,13E,15S)-15-Hydroxy-9-oxoprost-5, 10, 13-triene-1-oic acid	1.80	5	1.0
(5Z,13E,15S)-15-Hydroxy-9-oxoprost-5, 8(12), 13-trien-1-oic acid	1.90	1.43	1.0
Any individual unspecified impurity	—	1.0	0.10

\* The sum of these three impurities is NMT 1.0%.

### SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation** (781S):  $-82.0^\circ$  to  $-90.0^\circ$ , at 20°

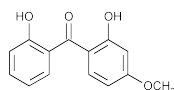
**Sample solution:** 5 mg/mL, in alcohol

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Dinoprostone RS

## Dioxybenzone



$C_{14}H_{12}O_4$  244.24

Methanone, (2-hydroxy-4-methoxyphenyl)(2-hydroxyphenyl)-

2,2'-Dihydroxy-4-methoxybenzophenone [131-53-3].

» Dioxybenzone contains not less than 97.0 percent and not more than 103.0 percent of  $C_{14}H_{12}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards (11)**—

USP Dioxybenzone RS

#### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 286 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Congeeing temperature (651):** not lower than 68.0°.

**Loss on drying (731)**—Dry it in vacuum at 40° for 2 hours: it loses not more than 2.0% of its weight.

**Assay**—Dissolve about 100 mg of Dioxybenzone, accurately weighed, in toluene in a 100-mL volumetric flask, dilute with toluene to volume, and mix. Pipet 1 mL of this solution into a second 100-mL volumetric flask, add methanol to volume, and mix. Similarly, prepare a Standard solution of USP Dioxybenzone RS, accurately weighed, having a known concentration of about 10  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 325 nm, with a suitable spectrophotometer, using a 1 in 100 solution of toluene in methanol as the blank. Calculate the quantity, in mg, of  $C_{14}H_{12}O_4$  in the Dioxybenzone taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Dioxybenzone RS in the Standard solution, and  $A_U$  and  $A_S$  are the absorbances of the solution of Dioxybenzone and the Standard solution, respectively.

## Dioxybenzone and Oxybenzone Cream

» Dioxybenzone and Oxybenzone Cream is a mixture of approximately equal parts of Dioxybenzone and Oxybenzone in a suitable cream base. It contains, in each 100 g, not less than

2.7 g and not more than 3.3 g each of dioxybenzone ( $C_{14}H_{12}O_4$ ) and oxybenzone ( $C_{14}H_{12}O_3$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Dioxybenzone RS

USP Oxybenzone RS

**Identification, Ultraviolet Absorption (197U)**—

*Solutions:* The solutions from the Cream employed for measurement of absorbance in the Assay.

**Minimum fill (755):** meets the requirements.

#### Assay—

**Standard preparations**—Transfer about 30 mg of USP Dioxybenzone RS, accurately weighed, to a 50-mL volumetric flask, dissolve in methanol, dilute with the same solvent to volume, and mix. Pipet 2 mL of this solution into a 100-mL volumetric flask, add methanol to volume, and mix. Similarly, prepare a *Standard preparation* of USP Oxybenzone RS, accurately weighed, having a known concentration of about 12  $\mu$ g per mL.

**Assay preparation**—Dissolve an accurately weighed portion of Cream, equivalent to about 25 mg each of dioxybenzone and oxybenzone, in methanol in a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pipet 1 mL of this solution into a 15-mL conical test tube, evaporate on a water bath just to dryness, using a gentle stream of air, and dissolve the residue in about 200  $\mu$ L of methanol.

**Procedure**—Prepare sheets of chromatographic paper (Whatman No. 1 or equivalent), each measuring about 23 × 28.5 cm, as follows. Immerse the sheets in a 1 in 20 solution of light mineral oil in solvent hexane, withdraw them immediately, and allow to dry in air. On one sheet mark a starting line about 2.5 cm from the long edge, and apply the entire *Assay preparation* as a uniform streak along the starting line, using a stream of air or an air blower, if necessary, to maintain the width of the streak between 5 mm and 10 mm. Rinse the conical test tube, which contained the *Assay preparation*, with about 100  $\mu$ L of methanol, and apply the rinse to the starting line. Similarly, repeat the rinsing and streaking with two additional portions of methanol, and then allow the paper to dry in air for 5 minutes.

Staple together the short edges of the paper to form a cylinder, and place it in a 12- × 25-cm cylindrical chromatographic chamber containing about 40 mL of a mobile solvent consisting of a mixture of equal volumes of acetone and water. Seal the chamber, and allow the chromatogram to develop for 2 hours.

Remove the paper from the chamber, air-dry, then remove the staples, and view the chromatogram under short-wavelength (254 nm) UV radiation. Mark the two bands representing the separated dioxybenzone and oxybenzone, respectively. [NOTE—Determine the relative position of each benzene on the chromatogram by applying suitable aliquots of each *Standard preparation* to another prepared chromatographic sheet, and developing the chromatogram in a manner similar to that described for the *Assay preparation*.] Cut the marked bands from the sheet, and then, keeping the band segments separate, cut each into several pieces to facilitate extraction. Place the pieces from each band in separate glass-stoppered, 50-mL conical flasks, add 20.0 mL of methanol to each flask, and shake gently for 30 minutes.

To provide the chromatographic blank, treat one of the prepared chromatographic sheets in the same manner as described above, but omit the application of the *Assay preparation*. Cut from the chromatographed paper the areas corresponding to the bands produced by the benzenes from the *Assay preparation*, and in the same manner extract the blank bands for 30 minutes with 20.0 mL of methanol.

Concomitantly determine the absorbance of each of the 4 solutions thus prepared, and of each of the *Standard preparations*, in a 1-cm cell at the wavelength of maximum absorbance at about 325 nm, with a suitable spectrophotometer.

ter, using methanol as the blank. Calculate the quantity, in mg, of dioxybenzone ( $C_{14}H_{12}O_4$ ) in the portion of Cream taken by the formula:

$$2C(A_U - A_B) / A_S$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Dioxybenzone RS in the dioxybenzone *Standard preparation*, and  $A_U$ ,  $A_B$ , and  $A_S$  are the absorbances of the dioxybenzone solution from the *Assay preparation*, the dioxybenzone chromatographic blank solution, and the dioxybenzone *Standard preparation*, respectively. In a similar manner, calculate the quantity, in mg, of oxybenzone ( $C_{14}H_{12}O_3$ ) in the portion of Cream taken, using as  $C$ ,  $A_U$ ,  $A_B$ , and  $A_S$  the respective values pertaining to the oxybenzone determination.

## Diphenhydramine Citrate

$C_{17}H_{21}NO \cdot C_6H_8O_7$  447.48

Ethanamine, 2-(diphenylmethoxy)-*N,N*-dimethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1).  
2-(Diphenylmethoxy)-*N,N*-dimethylethylamine citrate (1:1) [88637-37-0].

» Diphenhydramine Citrate contains not less than 98.0 percent and not more than 100.5 percent of  $C_{17}H_{21}NO \cdot C_6H_8O_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Diphenhydramine Citrate RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 700  $\mu\text{g}$  per mL.

*Medium:* water.

**C:** It responds to the test for *Citrate* (191).

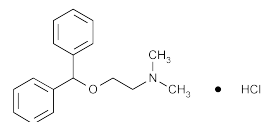
**Melting range** (741): between  $146^\circ$  and  $150^\circ$ , but the range between beginning and end of melting does not exceed  $2^\circ$ .

**Loss on drying** (731)—Dry it at  $105^\circ$  for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281)—To about 8 g, accurately weighed, add 5 mL of sulfuric acid, and char. After the substance is thoroughly charred, add 4 mL of nitric acid and a few drops of sulfuric acid, heat gently until fumes are no longer evolved, and ignite at  $800 \pm 25^\circ$  until the carbon is consumed. Place in a muffle furnace at  $550 \pm 50^\circ$  for about 1 hour. Continue the ignition until constant weight is attained: not more than 0.1% remains.

**Assay**—Dissolve about 1.6 g of Diphenhydramine Citrate, accurately weighed, in a mixture of 100 mL of glacial acetic acid and 20 mL of xylene. Add 20 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 44.75 mg of  $C_{17}H_{21}NO \cdot C_6H_8O_7$ .

## Diphenhydramine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$  291.82  
Ethanamine, 2-(diphenylmethoxy)-*N,N*-dimethyl-, hydrochloride;  
2-(Diphenylmethoxy)-*N,N*-dimethylethylamine hydrochloride [147-24-0].

### DEFINITION

Diphenhydramine Hydrochloride contains NLT 98.0% and NMT 102.0% of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, triethylamine, and water (100:1:100). Adjust with glacial acetic acid to a pH of 6.5.

**System suitability solution:** Dissolve 5 mg of benzophenone in 5 mL of acetonitrile, and dilute with water to 100 mL. Transfer 1.0 mL of this solution and 5 mg of diphenhydramine hydrochloride to a 10-mL volumetric flask, and dilute with water to volume.

**Standard solution:** 0.5 mg/mL of USP Diphenhydramine Hydrochloride RS in water

**Sample solution:** 0.5 mg/mL of Diphenhydramine Hydrochloride in water. Filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L10

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between the benzophenone and diphenhydramine peaks, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Sample solution* and *Standard solution*  
Calculate the percentage of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%, on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### SPECIFIC TESTS

- **ACIDITY OR ALKALINITY**

**Sample solution:** 50 mg/mL of Diphenhydramine Hydrochloride in carbon dioxide-free water

**Analysis:** To 10 mL of the *Sample solution*, add 0.15 mL of methyl red TS 2 and 0.25 mL of 0.01 N hydrochloric acid. The solution is pink. Titrate with 0.01 N sodium hydroxide.

**Acceptance criteria:** NMT 0.5 mL of 0.01 N sodium hydroxide is required to change the color of the solution to yellow.

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Diphenhydramine Hydrochloride RS

### Diphenhydramine Hydrochloride Capsules

» Diphenhydramine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{21}NO \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Diphenhydramine Hydrochloride RS

#### Identification—

**A:** The contents of the Capsules meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution, Procedure for a Pooled Sample (711)—

**Medium:** water; 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay*.

**Procedure**—Inject a measured volume (about 50  $\mu$ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the quantity of  $C_{17}H_{21}NO \cdot HCl$  dissolved in comparison with a *Standard solution* having a known concentration of USP Diphenhydramine Hydrochloride RS in the same medium and similarly chromatographed.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{21}NO \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase, Standard preparation, System suitability solution, and Chromatographic system**—Prepare as directed in the *Assay* under *Diphenhydramine Hydrochloride*.

**Assay preparation**—Weigh and combine the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the combined Capsule contents, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, mix, and filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diphenhydramine Hydrochloride*. Calculate the quantity, in mg, of  $C_{17}H_{21}NO \cdot HCl$  in the portion of Capsule contents taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Diphenhydramine Hydrochloride Injection

» Diphenhydramine Hydrochloride Injection is a sterile solution of Diphenhydramine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{21}NO \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

#### USP Reference standards (11)—

USP Diphenhydramine Hydrochloride RS

USP Endotoxin RS

#### Identification—

**A:** Dilute a volume of Injection, equivalent to about 50 mg of diphenhydramine hydrochloride, with 0.03 N sulfuric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator": the Injection meets the requirements of the test.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 3.4 USP Endotoxin Units per mg of diphenhydramine hydrochloride.

**pH** (791): between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase, Standard preparation, System suitability solution, and Chromatographic system**—Prepare as directed in the *Assay* under *Diphenhydramine Hydrochloride*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Diphenhydramine Hydrochloride*. Calculate the quantity, in mg, of  $C_{17}H_{21}NO \cdot HCl$  in each mL of the Injection taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and  $r_U$  and  $r_S$

are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diphenhydramine Hydrochloride Oral Solution

» Diphenhydramine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Diphenhydramine Hydrochloride RS

**Identification**—

**A:** Place a portion of Oral Solution, equivalent to 50 mg of diphenhydramine hydrochloride, in a separator, add 0.5 mL of 2 N sulfuric acid, and extract with three 15-mL portions of ether, discarding the extracts. Add 5 mL of water. In a second separator dissolve 50 mg of USP Diphenhydramine Hydrochloride RS in 25 mL of water. Treat each solution as follows. Add 2 mL of 1 N sodium hydroxide, and extract with 75 mL of *n*-heptane. Wash the *n*-heptane extract with 10 mL of water, evaporate the extract to dryness, and dissolve the residue in 4 mL of carbon disulfide. Pass through a dry filter to clarify the solution, if necessary, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Determine the absorption spectra of the filtered solutions": the Oral Solution meets the requirements of the test.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Alcohol content** (611): between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Assay**—

*Mobile phase, Standard preparation, System suitability solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Diphenhydramine Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Diphenhydramine Hydrochloride*. Calculate the quantity, in mg, of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diphenhydramine and Pseudoephedrine Capsules

» Diphenhydramine and Pseudoephedrine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts

of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Capsules to state both the contents of the active moieties and the contents of the salts used in formulating the article.

**USP Reference standards** (11)—

USP Diphenhydramine Hydrochloride RS

USP Pseudoephedrine Hydrochloride RS

**Identification**—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** *Developing solvent*—Prepare a mixture of acetonitrile, methylene chloride, and *n*-propylamine (56:40:4).

*Standard solution*—Dissolve about 12.5 mg of USP Diphenhydramine Hydrochloride RS and 30 mg of USP Pseudoephedrine Hydrochloride RS in 5.0 mL of water in a 50-mL centrifuge tube.

*Test solution*—Transfer an amount of Capsule contents, equivalent to about 12.5 mg of diphenhydramine hydrochloride and about 30.0 mg of pseudoephedrine hydrochloride, to a 50-mL centrifuge tube. Add 5.0 mL of water, and shake.

*Procedure*—To each centrifuge tube add 5.0 mL of a saturated solution of sodium carbonate and 10 mL of methylene chloride. Insert the stoppers into the tubes and shake for about 1 minute. Centrifuge at about 2000 rpm for about 10 minutes until the layers are well separated. Draw off and discard the top layer. Add 5 g of anhydrous sodium sulfate to the remaining solution, insert the stopper tightly, and shake. Separately apply 30  $\mu$ L ( $3 \times 10 \mu$ L) each of the *Test solution* and the *Standard solution* to a suitable unactivated thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* (621)) and allow the spots to dry. Develop the chromatograms in a paper lined chromatographic chamber equilibrated with the *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, place the plate in an iodine chamber, and allow to develop over several hours: the *R<sub>f</sub>* values of the principal spots obtained from the *Test solution* correspond to those obtained from the *Standard solution*.

**Dissolution, Procedure for a Pooled Sample** (711)—

*Medium:* water, 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 30 minutes.

*Procedure*—Inject a measured volume (about 50  $\mu$ L) of a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Determine the quantities of  $C_{17}H_{21}NO \cdot HCl$  and of  $C_{10}H_{15}NO \cdot HCl$  dissolved by employing the procedures set forth in the *Assay*, making any necessary modifications.

**Tolerances**—Not less than 75% (*Q*) of the labeled amounts of  $C_{17}H_{21}NO \cdot HCl$  and of  $C_{10}H_{15}NO \cdot HCl$  are dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—

*Mobile phase*—Prepare as directed in the *Assay*.

*Diluting solution*—Prepare a mixture of water, acetonitrile and methanol (64:26:10).

*Standard solution*—Transfer accurately weighed quantities of about 5 mg each of benzhydrol and benzophenone to a 500-mL volumetric flask, dissolve in *Diluting solution*, using heat and sonication if necessary, dilute with *Diluting solution*

to volume, and mix. Dilute 5.0 mL of this solution quantitatively with *Diluting solution* to 25.0 mL, mix, and filter.

**Test solution**—Transfer 10 Capsules to a 500-mL volumetric flask, add about 350 mL of *Diluting solution*, sonicate in warm water at about 40° to effect dissolution, and cool. Dilute with *Diluting solution* to volume, and mix. Dilute 5.0 mL of this solution with *Diluting solution* to 25.0 mL, mix, and filter.

**Resolution solution**—Prepare a solution in *Diluting solution* containing about 2 µg each of benzhydrol and benzophenone, and 100 µg of USP Diphenhydramine Hydrochloride RS, per mL.

**Chromatographic system**—Proceed as directed for *Chromatographic system* under *Assay*. Chromatograph 50 µL of the *Resolution solution*: the resolution,  $R$ , between benzhydrol and diphenhydramine is not less than 1.3 and the resolution,  $R$ , between diphenhydramine and benzophenone is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution*, and record the areas of the benzhydrol and benzophenone peaks. Calculate the amounts of benzhydrol and benzophenone in the Capsules taken by the formula:

$$C(r_U / r_S)$$

in which  $C$  is the concentration of either benzhydrol or benzophenone, in µg per mL, in the *Standard solution*, and  $r_U$  and  $r_S$  are the areas of the corresponding analyte peaks obtained from the *Test solution* and the *Standard solution*, respectively: the sum of the amounts of benzhydrol and benzophenone does not exceed 2% (w/w) of the diphenhydramine hydrochloride.

#### Assay—

**Aqueous solution**—Dissolve 1.7 g of sodium 1-heptanesulfonate and 0.8 mL of triethylamine in about 800 mL of water, adjust with glacial acetic acid to a pH of  $3.3 \pm 0.05$ , dilute with water to 1 L, mix, and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Aqueous solution*, acetonitrile, and methanol (64:26:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 25 mg of USP Diphenhydramine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask. Add 25/ mg of USP Pseudoephedrine Hydrochloride RS, accurately weighed,  $f$  being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of diphenhydramine hydrochloride per capsule, dissolve in 0.5% glacial acetic acid, dilute with 0.5% glacial acetic acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.5% glacial acetic acid to volume, and mix.

**Assay preparation**—Remove as completely as possible, the contents of not less than 10 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 25 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in 0.5% glacial acetic acid, dilute with 0.5% glacial acetic acid to volume, mix, and filter. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.5% glacial acetic acid to volume, and mix.

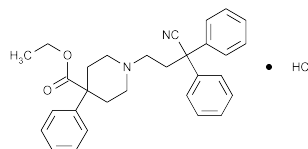
**Chromatographic system**—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the resolution,  $R$ , between the pseudoephedrine and diphenhydramine peaks is not less than 3.0. For each analyte peak, the tailing factor is not greater than 2.0, and the relative standard deviation for replicate injections is not greater than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the two analytes. The relative retention times are about 1.0 for pseudoephedrine and 3.0 for diphenhydramine. Calculate the quantity, in mg, of diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ) in the portion of Capsules taken by formula:

$$C(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Capsules taken by the same formula changing the terms to refer to pseudoephedrine hydrochloride.

## Diphenoxylate Hydrochloride



$C_{30}H_{32}N_2O_2 \cdot HCl$  489.05

4-Piperidinecarboxylic acid, 1-(3-cyano-3,3-diphenylpropyl)-4-phenyl-, ethyl ester, monohydrochloride.

Ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisopropylate monohydrochloride [3810-80-8].

» Diphenoxylate Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{30}H_{32}N_2O_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Diphenoxylate Hydrochloride RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 500 µg per mL.

*Medium:* hydrochloric acid in methanol (1 in 1000).

**C:** A saturated solution responds to the tests for *Chloride* (191).

**Melting range** (741): between 220° and 226°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

#### Ordinary impurities (466)—

*Test solution:* chloroform.

*Standard solution:* chloroform.

*Eluant:* a mixture of chloroform, cyclohexane, dehydrated alcohol, and formic acid (50:40:10:1).

*Visualization:* 17; then examine the plate immediately under short-wavelength UV light.

**Limits**—The sum of the intensities of all secondary spots obtained from the *Test solution* corresponds to not more than 1.0%.

**Assay**—Dissolve about 300 mg of Diphenoxylate Hydrochloride, accurately weighed, in 75 mL of glacial acetic acid, add 4 mL of mercuric acetate TS, and titrate with 0.1 N

perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 48.91 mg of  $C_{30}H_{32}N_2O_2 \cdot HCl$ .

## Diphenoxylate Hydrochloride and Atropine Sulfate Oral Solution

» Diphenoxylate Hydrochloride and Atropine Sulfate Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of diphenoxylate hydrochloride ( $C_{30}H_{32}N_2O_2 \cdot HCl$ ), and not less than 80.0 percent and not more than 120.0 percent of the labeled amount of atropine sulfate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ ].

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Atropine Sulfate RS

USP Diphenoxylate Hydrochloride RS

**Identification**—The retention times of two major peaks in the chromatogram of the *Assay preparation* correspond to the atropine and diphenoxylate peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements with respect to diphenoxylate hydrochloride.

### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.0 and 4.3, determined in a dilution of the Oral Solution with an equal volume of water.

**Alcohol content** (611): between 13.5% and 16.5% of  $C_2H_5OH$ .

### Assay—

**Solution A**—Transfer 192 mg of sodium 1-pentanesulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of water and 1.0 mL of phosphoric acid, and mix.

**Solution B**—Transfer 192 mg of sodium 1-pentanesulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of acetonitrile, 1.0 mL of phosphoric acid, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Solution B* and *Solution A* (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Atropine stock preparation**—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.04 mg per mL.

**Standard preparation**—Transfer about 20 mg of USP Diphenoxylate Hydrochloride RS to a 200-mL volumetric flask, add about 100 mL of dehydrated alcohol, and sonicate to dissolve. Accurately add 5.0 mL of *Atropine stock preparation* and 34 mL of water, and mix. Allow the solution to reach room temperature, and then dilute with dehydrated alcohol to volume. This solution contains about 0.1 mg of diphenoxylate hydrochloride and about 0.001 mg of atropine sulfate per mL.

**Assay preparation**—Transfer an accurately measured volume of the Oral Solution, equivalent to about 2.5 mg of diphenoxylate hydrochloride, based on the label claim, to a 25-mL volumetric flask, wash inside of the pipet with small

portions of dehydrated alcohol, add the washings to the flask, dilute with dehydrated alcohol to volume, and mix. Pass a portion of the solution obtained through a 0.45- $\mu$ m PTFE filter, discarding the first few mL, and use the clear filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L10. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.35 for atropine and 1.0 for diphenoxylate; the resolution,  $R$ , between atropine and diphenoxylate is not less than 5.0; the tailing factor is not more than 1.5 for atropine; and the relative standard deviation for replicate injections is not more than 2.0% for diphenoxylate and not more than 5.0% for atropine. [NOTE—If a significant tailing of the diphenoxylate peak is observed (greater than 2.5), it is recommended to maintain the column temperature at 25°, to stabilize the system.]

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diphenoxylate hydrochloride ( $C_{30}H_{32}N_2O_2 \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$25C_D (r_U / r_S)$$

in which 25 is the volume, in mL, of the *Assay preparation*;  $C_D$  is the concentration, in mg per mL, of USP Diphenoxylate Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the diphenoxylate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the quantity, in mg, of atropine sulfate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ ] in the portion of Oral Solution taken by the formula:

$$(694.83/676.83)(25)C_A (r_U / r_S)$$

in which 694.83 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; 25 is the volume, in mL, of the *Assay preparation*;  $C_A$  is the concentration, in mg per mL, of USP Atropine Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the atropine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diphenoxylate Hydrochloride and Atropine Sulfate Tablets

» Diphenoxylate Hydrochloride and Atropine Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diphenoxylate hydrochloride ( $C_{30}H_{32}N_2O_2 \cdot HCl$ ), and not less than 80.0 percent and not more than 120.0 percent of the labeled amount of atropine sulfate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ ].

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Atropine Sulfate RS

USP Diphenoxylate Hydrochloride RS

**Identification**—The retention times of two major peaks in the chromatogram of the *Assay preparation* correspond to the atropine and diphenoxylate peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—

*Medium:* 0.2 M acetic acid; 500 mL.

*Apparatus 1:* 150 rpm.

*Time:* 45 minutes.

Determine the amount of  $C_{30}H_{32}N_2O_2 \cdot HCl$  dissolved by employing the following method.

*Mobile phase*—Prepare a suitable degassed mixture of acetonitrile and 0.05 M monobasic potassium phosphate (65:35).

*Standard solution*—Dissolve an accurately weighed quantity of USP Diphenoxylate Hydrochloride RS in methanol to obtain a solution having a known concentration of about 250 µg per mL. Pipet 10 mL of this solution into a 500-mL volumetric flask, dilute with *Dissolution Medium* to volume, mix, and filter.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard solution* and of filtered portions of the solution under test into the chromatograph, record the chromatograms, measure the response for the major peak, and determine the amount of  $C_{30}H_{32}N_2O_2 \cdot HCl$  dissolved.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{30}H_{32}N_2O_2 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content uniformity* with respect to diphenoxylate hydrochloride.

**Assay**—

*Diluent*—Use a mixture of acetonitrile and water (1:1).

*Solution A*—Transfer 192 mg of sodium 1-pentane-sulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of water and 1.0 mL of phosphoric acid, and mix.

*Solution B*—Transfer 192 mg of sodium 1-pentane-sulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of acetonitrile and 1.0 mL of phosphoric acid, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of *Solution B* and *Solution A* (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Atropine stock preparation*—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

*Standard preparation*—Transfer about 20 mg of USP Diphenoxylate Hydrochloride RS to a 200-mL volumetric flask, add about 100 mL of *Diluent*, and sonicate to dissolve. Accurately add 5.0 mL of *Atropine stock preparation*, and mix. Allow the solution to reach room temperature, and then dilute with *Diluent* to volume. This solution contains about 0.1 mg of diphenoxylate hydrochloride and about 0.001 mg of atropine sulfate per mL.

*Assay preparation*—Transfer an accurately counted number of Tablets, equivalent to about 25 mg of diphenoxylate hydrochloride, based on the label claim, to a 250-mL volumetric flask, add approximately 100 mL of *Diluent*, and shake by mechanical means for at least 15 minutes or until the Tablets are completely disintegrated. Sonicate for an additional 15 minutes, allow the solution to reach room temperature, dilute with *Diluent* to volume, and mix. Pass a portion of the solution obtained through a 0.45-µm PTFE filter, discarding the first few mL, and use the clear filtrate.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector

and a 4.6-mm × 25-cm column that contains 5-µm packing L10. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.35 for atropine and 1.0 for diphenoxylate; the resolution, *R*, between atropine and diphenoxylate is not less than 5.0; the tailing factor is not more than 1.5 for atropine; and the relative standard deviation for replicate injections is not more than 2.0% for diphenoxylate and not more than 5.0% for atropine. [NOTE—If a significant tailing of the diphenoxylate peak is observed (greater than 2.5), it is recommended to maintain the column temperature at 25°, to stabilize the system.]

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diphenoxylate hydrochloride ( $C_{30}H_{32}N_2O_2 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$250C_D(r_U / r_S)$$

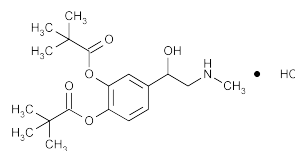
in which 250 is the volume, in mL, of the *Assay preparation*;  $C_D$  is the concentration, in mg per mL, of USP Diphenoxylate Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the diphenoxylate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the quantity, in mg, of atropine sulfate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ ] in the portion of the Tablets taken by the formula:

$$(694.83/676.83)(250)C_A(r_U / r_S)$$

in which 694.83 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; 250 is the volume, in mL, of the *Assay preparation*;  $C_A$  is the concentration, in mg per mL, of USP Atropine Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the atropine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dipivefrin Hydrochloride



$C_{19}H_{29}NO_5 \cdot HCl$  387.90

Propanoic acid, 2,2-dimethyl-, 4-[1-hydroxy-2-(methylamino)ethyl]-1,2-phenylene ester, hydrochloride, (±)-.

(±)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol 3,4-dipivalate hydrochloride [64019-93-8].

» Dipivefrin Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of  $C_{19}H_{29}NO_5 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Dipivefrin Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.



**C:** A solution (1 in 100) meets the requirements of the tests for *Chloride* (191).

**Melting range** (741): between 155° and 165°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it in a suitable vacuum drying tube over phosphorus pentoxide at 60° for 6 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.3%.

**Heavy metals, Method I** (231): not more than 0.0015%.

**Iron** (241): not more than 5 ppm.

**Standard iron solution**—Use the *Standard Iron Solution* prepared as directed under *Iron* (241).

**Hydroxylamine solution**—Dissolve 5 g of hydroxylamine hydrochloride in 50 mL of water.

**Triazine solution**—Dissolve 125 mg of 2,4,6-tri-(2-pyridyl)-S-triazine in 100 mL of methanol.

**Standard solution**—Into a 50-mL color-comparison tube pipet 1 mL of *Standard iron solution*, add 42.0 mL of water, and mix.

**Test solution**—Into a 50-mL color-comparison tube add 2.0 g of Dipivefrin Hydrochloride, 43.0 mL of water, and mix.

**Procedure**—To each of the tubes containing the *Standard solution* and the *Test solution*, add 5.0 mL of *Hydroxylamine solution*, 2.0 mL of *Triazine solution*, and mix: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution*.

#### Assay—

**Mobile phase**—Prepare a mixture of acetonitrile, 0.014 M sodium dodecyl sulfate, and glacial acetic acid (24:15:1).

**Standard preparation**—Dissolve a suitable quantity of USP Dipivefrin Hydrochloride RS, accurately weighed, in 0.0015 N hydrochloric acid to obtain a solution having a known concentration of about 5 mg per mL.

**Assay preparation**—Dissolve a suitable quantity of Dipivefrin Hydrochloride, accurately weighed, in 0.0015 N hydrochloric acid to obtain a solution having a known concentration of about 5 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 500 theoretical plates; the tailing factor for the major peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of C<sub>19</sub>H<sub>29</sub>NO<sub>5</sub> · HCl in the portion of Dipivefrin Hydrochloride taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of dipivefrin hydrochloride in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of dipivefrin hydrochloride in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dipivefrin Hydrochloride Ophthalmic Solution

» Dipivefrin Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Dipivefrin Hydrochloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of C<sub>19</sub>H<sub>29</sub>NO<sub>5</sub> · HCl. It contains a suitable antimicrobial agent and may contain stabilizers, suitable buffers, and chelating agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Dipivefrin Hydrochloride RS

**Identification**—It meets the requirements for *Identification test B* under *Dipivefrin Hydrochloride*.

**Sterility tests** (71): meets the requirements.

**pH** (791): between 2.5 and 3.5.

#### Assay—

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay* under *Dipivefrin Hydrochloride*.

**Standard preparation**—Dissolve a suitable quantity of USP Dipivefrin Hydrochloride RS, accurately weighed, in 0.0015 N hydrochloric acid to obtain a solution having a known concentration of about 1 mg per mL.

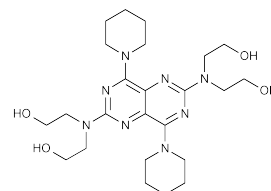
**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 25 mg of dipivefrin hydrochloride, to a 25-mL volumetric flask, dilute with 0.0015 N hydrochloric acid to volume, if necessary, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Dipivefrin Hydrochloride*. Calculate the quantity, in mg, of C<sub>19</sub>H<sub>29</sub>NO<sub>5</sub> · HCl in each mL of the Ophthalmic Solution taken by the formula:

$$(25C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dipivefrin Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dipyridamole



C<sub>24</sub>H<sub>40</sub>N<sub>8</sub>O<sub>4</sub> 504.63

Ethanol, 2,2',2'',2'''-[4,8-di-1-piperidinyl]pyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]tetraethanol.

2,2',2'',2'''-[4,8-Dipiperidinopyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]tetraethanol [58-32-2].

» Dipyridamole contains not less than 98.0 percent and not more than 102.0 percent of C<sub>24</sub>H<sub>40</sub>N<sub>8</sub>O<sub>4</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**USP Reference standards** (11)—

USP Dipyridamole RS

**Identification, Infrared Absorption** (197K).

**Melting range** (741): between 162° and 168°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it at 105° for 3 hours; it loses not more than 0.2% of its weight.

**Chloride**—Dissolve 500 mg in 5 mL of alcohol and 2 mL of 2 N nitric acid, and add 1 mL of silver nitrate TS: no turbidity or precipitate is produced.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

**Chromatographic purity**—

*Mobile phase and Chromatographic system*—Prepare as directed in the Assay under Dipyridamole Tablets.

*Test preparation A*—Prepare a solution of Dipyridamole in methanol having a known concentration of 1 mg per mL.

*Test preparation B*—Dilute 1.0 mL of Test preparation A with methanol to 100 mL, and mix.

*Procedure*—Inject 10 µL of Test preparation B into the chromatograph by means of a sampling valve, adjusting the operating parameters so that the response of the main peak (retention time about 6.5 minutes) obtained is about 5% full scale. Inject 10 µL of Test preparation A, and run the chromatograph for 10 minutes: the sum of responses of all secondary peaks obtained from Test preparation A is not greater than the response of the main peak obtained from Test preparation B (1.0%).

**Assay**—Transfer about 450 mg of Dipyridamole, accurately weighed, to a 250-mL beaker, and dissolve in 50 mL of glacial acetic acid. Stir for 30 minutes. Add 75 mL of acetone, and stir for an additional 15 minutes. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a silver-silver chloride reference electrode system. Perform a blank titration, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 50.46 mg of C<sub>24</sub>H<sub>40</sub>N<sub>8</sub>O<sub>4</sub>.

## Dipyridamole Injection

» Dipyridamole Injection is a sterile solution of Dipyridamole in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dipyridamole (C<sub>24</sub>H<sub>40</sub>N<sub>8</sub>O<sub>4</sub>).

**Packaging and storage**—Preserve in Containers for Injections as described under Injections (1). Protect from light, and avoid freezing.

**USP Reference standards** (11)—

USP Dipyridamole RS

USP Endotoxin RS

**Identification**—

**A: Thin-Layer Chromatographic Identification Test** (201)—

*Test solution*—Use the Injection.

*Standard solution*: 5 mg per mL in a mixture of methanol, water, and 0.1 N hydrochloric acid (5:4:1).

*Developing solvent system*: a mixture of butyl alcohol, water, and glacial acetic acid (34:10:5).

*Procedure*—Proceed as directed in the chapter. Locate the yellow spots on the plate: the R<sub>F</sub> value of the principal spot

obtained from the Test solution corresponds to that of the principal spot obtained from the Standard solution. Spray the plate lightly with a spray reagent prepared as follows. Transfer 1 g of iodine and 3 g of potassium iodide to a 100-mL volumetric flask. Add 10 mL of alcohol to dissolve (heat gently). Add 20 mL of 2 N sulfuric acid, dilute with water to volume, and mix. Store in a dark place. Observe the plate, and locate the brown spots: the R<sub>F</sub> value of the principal spot obtained from the Test solution corresponds to that of the principal spot obtained from the Standard solution.

**B:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Bacterial endotoxins** (85)—It contains not more than 8.8 USP Endotoxin Units per mg of dipyridamole.

**pH** (791): between 2.2 and 3.2.

**Chromatographic purity**—[NOTE—Protect dipyridamole solutions from exposure to light.]

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay.

*Test solution*—Use the Assay preparation prepared as directed in the Assay.

*Procedure*—Inject a volume (about 10 µL) of the Test solution into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 2.0% of any individual impurity is found; and not more than 4.5% of total impurities is found.

**Other requirements**—It meets the requirements under Injections (1).

**Assay**—[NOTE—Protect dipyridamole solutions from exposure to light.]

*Acetate buffer*—Dissolve a quantity of sodium acetate in water to obtain a concentration of about 6.8 mg per mL. Adjust with acetic acid to a pH of 5.1 ± 0.1.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and Acetate buffer (65:35). Make adjustments if necessary (see System Suitability under Chromatography (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dipyridamole RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 1.0 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of dipyridamole, to a 25-mL volumetric flask, dilute with Mobile phase to volume, and mix.

*Chromatographic system* (see Chromatography (621))—The liquid chromatograph is equipped with a 276-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency is not less than 2000 theoretical plates; the tailing factor is not greater than 1.7; and the relative standard deviation for replicate injections is not greater than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of dipyridamole ( $C_{24}H_{40}N_8O_4$ ) in the portion of Injection taken by the formula:

$$25C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dipyridamole RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dipyridamole Oral Suspension

### DEFINITION

Dipyridamole Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dipyridamole ( $C_{24}H_{40}N_8O_4$ ).

Prepare Dipyridamole Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Dipyridamole	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Place the required number of tablets into a suitable mortar and comminute to a fine powder, or add *Dipyridamole* powder to the mortar. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* in small portions, and mix well after each addition. Transfer, stepwise and quantitatively, to a graduated or calibrated bottle. Add *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### PROCEDURE

**Solution A:** 1 mg/mL of dibasic sodium phosphate. Adjust with dilute phosphoric acid (1 in 3) to a pH of 4.6.

**Mobile phase:** Methanol and *Solution A* (75:25). Pass through a membrane filter of a 0.5- $\mu$ m pore size, and degas.

**Standard solution:** 100  $\mu$ g/mL of USP Dipyridamole RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 288 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for dipyridamole is about 7.3 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 2.3% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of dipyridamole ( $C_{24}H_{40}N_8O_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of dipyridamole in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of dipyridamole in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

• **pH (791):** 3.8–4.8

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.

• **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded

• **USP REFERENCE STANDARDS (11)**  
USP Dipyridamole RS

## Dipyridamole Tablets

» Dipyridamole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{40}N_8O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards (11)**—

USP Dipyridamole RS

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of dipyridamole, with 10 mL of 0.1 N hydrochloric acid, and filter, collecting the filtrate in a beaker. Add 0.1 N sodium hydroxide until the solution is basic and a precipitate forms. Heat the mixture on a steam bath for 1 minute, cool, and filter. Dry the residue at  $105^\circ$  for 1 hour: the residue so obtained responds to the *Identification* test under *Dipyridamole*.

**Dissolution (711)**—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure**—Determine the amount of  $C_{24}H_{40}N_8O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 282 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Dipyridamole RS in the same *Medium*.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{24}H_{40}N_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Procedure for content uniformity**—Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, heat in a steam bath for 5 minutes, and shake by mechanical means for 30 minutes. Cool to room temperature, dilute with 1 N hydrochloric acid to volume, and mix. Filter, discarding the first 25 mL of the filtrate. Dilute an accurately

measured portion of the subsequent filtrate with 1 N hydrochloric acid to provide a solution containing about 10 µg of dipyrnidamole per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Dipyrnidamole RS in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 282 nm using 1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{24}H_{40}N_8O_4$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of dipyrnidamole in the Tablet; *C* is the concentration, in µg per mL, of USP Dipyrnidamole RS in the *Standard solution*; *D* is the concentration, in µg per mL, of dipyrnidamole in the solution from the Tablet based upon the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the *Standard solution*, respectively.

#### Assay—

**Mobile phase**—Dissolve 250 mg of dibasic sodium phosphate in 250 mL of water, and adjust with dilute phosphoric acid (1 in 3) to a pH of 4.6. Add 750 mL of methanol, mix, filter through a 0.5-µm membrane filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Using an accurately weighed quantity of USP Dipyrnidamole RS, prepare a solution in *Mobile phase* having a known concentration of about 15 µg per mL.

**Assay preparation**—Transfer not less than 20 Tablets to a 1000-mL volumetric flask, add 100 mL of water, and sonicate for 15 minutes. Add about 750 mL of methanol, and shake by mechanical means for 30 minutes. Dilute with methanol to volume, mix, and centrifuge. Dilute an accurately measured volume (*V<sub>S</sub>* mL) of the clear supernatant quantitatively with *Mobile phase* to obtain a solution (*V<sub>A</sub>* mL) containing about 15 µg of dipyrnidamole per mL.

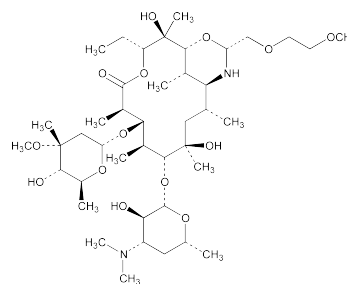
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 288-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1000 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{40}N_8O_4$  in the Tablets taken by the formula:

$$C(V_A / V_S)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Dipyrnidamole RS in the *Standard preparation*; *V<sub>A</sub>* is the volume, in mL, of the *Assay preparation*; *V<sub>S</sub>* is the volume, in mL, of supernatant taken for the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dirithromycin



$C_{42}H_{78}N_2O_{14}$  835.07

Erythromycin, 9-deoxy-11-deoxy-9,11-[imino[2-(2-methoxyethoxy)ethylidene]oxy]-, 9*S*(*R*)-, (9*S*)-9-Deoxy-11-deoxy-9,11-[imino[(1*R*)-2-(2-methoxyethoxy)ethylidene]oxy]erythromycin [62013-04-1].

» Dirithromycin contains not less than 96.0 percent and not more than 102.0 percent of  $C_{42}H_{78}N_2O_{14}$ , consisting of the 16*R*- and 16*S*-epimers, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Dirithromycin RS

**Identification**—

**A: Infrared Absorption** <197K>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method I* <921>: not more than 1.0%.

**Heavy metals**, *Method II* <231>: 0.002%.

**Limit of dirithromycin 16*S*-epimer**—Using the chromatogram obtained in the test for *Chromatographic purity*, calculate the percentage of dirithromycin 16*S*-epimer in the portion of Dirithromycin taken by the formula:

$$1000(C / W)(r_E / r_S)$$

in which *r<sub>E</sub>* is the response for dirithromycin 16*S*-epimer found in the chromatogram of the *Test solution*; and the other terms are as defined therein: not more than 1.5% of dirithromycin 16*S*-epimer is found.

**Chromatographic purity**—

*Potassium phosphate buffer*, *Mobile phase*, *System suitability solution*, *Solvent*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Test solution**—Transfer about 100 mg of Dirithromycin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms for a period of time that is not less than three times the retention time of dirithromycin (16*R*-epimer). Calculate the percentage of

each impurity found in the portion of Dirithromycin taken by the formula:

$$1000(C / W)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard solution*; *W* is the quantity, in mg, of Dirithromycin taken to prepare the *Test solution*; *r<sub>i</sub>* is the response for each impurity found in the chromatogram of the *Test solution*; and *r<sub>s</sub>* is the response of the dirithromycin (16*R*-epimer) in the chromatogram of the *Standard solution*: not more than 1.5% of 9-(*S*)-erythromycylamine is found; not more than 1.0% of any other individual impurity is found; and not more than 4.0% of total impurities is found. [NOTE—Do not regard dirithromycin 16*S*-epimer as an impurity.]

#### Assay—

**Potassium phosphate buffer**—Dissolve 1.41 g of monobasic potassium phosphate and 6.91 g of dibasic potassium phosphate in 1 L of water, and pass through a filter having a porosity of 0.5 μm or finer.

**Mobile phase**—Prepare a degassed mixture of acetonitrile, *Potassium phosphate buffer*, and methanol (44:37:19).

**System suitability solution**—Dissolve an accurately weighed quantity of USP Dirithromycin RS in *Mobile phase* to obtain a solution having a concentration of about 2.5 mg per mL. Store this solution at room temperature for about 24 hours. [NOTE—The solution then contains an equilibrated mixture of dirithromycin (16*R*-epimer), dirithromycin 16*S*-epimer, and 9-(*S*)-erythromycylamine. The solution may be used for 1 month when stored at room temperature.]

**Solvent**—Prepare a mixture of acetonitrile and methanol (70:30).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 2 mg per mL.

**Assay preparation**—Transfer about 20 mg of Dirithromycin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for 9-(*S*)-erythromycylamine, 1.0 for dirithromycin (16*R*-epimer), and 1.12 for dirithromycin 16*S*-epimer. The resolution, *R<sub>s</sub>*, between dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer is not less than 2.0, and between dirithromycin (16*R*-epimer) and 9-(*S*)-erythromycylamine is not less than 5.0, the tailing factor for the dirithromycin (16*R*-epimer) peak is not more than 2.0, and the relative standard deviation of the dirithromycin (16*R*-epimer) peak for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the percentage of C<sub>42</sub>H<sub>78</sub>N<sub>2</sub>O<sub>14</sub> in the portion of Dirithromycin taken by the formula:

$$1000(C / W)(r_U / r_s) + P_E$$

in which *C* is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard preparation*; *W* is the quantity, in mg, of Dirithromycin taken to prepare the *Assay preparation*; *r<sub>U</sub>* and *r<sub>s</sub>* are the area responses for dirithromycin (16*R*-epimer) obtained from the *Assay preparation* and the *Standard preparation*, respectively; and *P<sub>E</sub>* is the percentage of 16*S*-epimer as determined in the test for *Limit of dirithromycin 16S-epimer*.

## Dirithromycin Delayed-Release Tablets

» Dirithromycin Delayed-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dirithromycin (C<sub>42</sub>H<sub>78</sub>N<sub>2</sub>O<sub>14</sub>), consisting of the 16*R*- and 16*S*-epimers.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Dirithromycin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—Proceed as directed for *Procedure* for *Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

#### ACID STAGE—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 1:** 10-mesh basket; 100 rpm.

**XanthydroL TS**—[NOTE—Prepare this solution daily.] To about 150 mg of xanthydroL in a 100-mL volumetric flask, add 10 mL of glacial acetic acid, and swirl to dissolve. Dilute with hydrochloric acid to volume, and mix.

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.28 mg per mL.

**Procedure**—After 2 hours of operation, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessel, and subject each Tablet to the test in the *Buffer stage*. Separately add 0.50 mL of acetic anhydride to 0.50 mL of the filtered solution under test and to 0.50 mL of the *Standard solution*, and mix. Add 5.0 mL of glacial acetic acid, allow to stand for 5 minutes, then add 0.50 mL of *XanthydroL TS*, and allow 30 minutes for color development. Determine the amount of C<sub>42</sub>H<sub>78</sub>N<sub>2</sub>O<sub>14</sub>, including the 16*R*- and 16*S*-epimers, dissolved by employing UV absorption at the wavelength of maximum absorbance at about 540 nm.

#### BUFFER STAGE—

**Medium:** pH 6.8 phosphate buffer; 900 mL.

**Procedure**—Proceed as directed for *Acid stage* beginning with "Separately add 0.50 mL of acetic anhydride".

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of C<sub>42</sub>H<sub>78</sub>N<sub>2</sub>O<sub>14</sub>, including the 16*R*- and 16*S*-epimers, is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Water, Method I** <921>: not more than 5.0%.

#### Chromatographic purity—

**Potassium phosphate buffer, Mobile phase and System suitability solution**—Proceed as directed in the *Assay* under *Dirithromycin*.

**Phosphate buffer**—Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water, adjust with phosphoric acid to a pH of 8.0, and pass through a filter having a 0.5 μm or finer porosity.

**Solvent 1**—Prepare a mixture of acetonitrile and *Phosphate buffer* (60:40).

**Solvent 2**—Prepare a mixture of acetonitrile and *Phosphate buffer* (98:2). [NOTE—The mixture is cloudy.]

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent 1* to obtain a solution having a known concentration of about

0.2 mg per mL. [NOTE—Inject the *Standard solution* into the chromatograph immediately after preparation.]

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 745 mg of dirithromycin, to a 50-mL volumetric flask, dissolve in and dilute with *Solvent 2* to volume, and mix. Centrifuge a portion of this solution, transfer 6.0 mL of the clear supernatant to a 10-mL volumetric flask, dilute with *Phosphate buffer* to volume, and mix. [NOTE—Inject the *Test solution* into the chromatograph immediately after preparation.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for 9-(S)-erythromyclamine, 1.0 for dirithromycin (16R-epimer), and 1.12 for dirithromycin 16S-epimer; the resolution, *R*, between the dirithromycin (16R-epimer) and dirithromycin 16S-epimer is not less than 2.0, and between dirithromycin (16R-epimer) and 9-(S)-erythromyclamine is not less than 5.0; and the tailing factor for the dirithromycin (16R-epimer) peak is not more than 2.0.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is not less than three times the retention time of dirithromycin (16R-epimer), and measure the peak areas. Calculate the percentage of each impurity found in the portion of Tablets taken by the formula:

$$50,000(C / 6W)(L / T)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard solution*; *W* is the quantity, in mg, of Tablet powder taken to prepare the *Test solution*; *L* is the labeled amount, in mg, of dirithromycin in each Tablet; *T* is the average weight, in mg, of each Tablet; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response of dirithromycin (16R-epimer) obtained from the *Standard solution*: not more than 1.5% of 9-(S)-erythromyclamine is found; and not more than 5.0% of total impurities is found. [NOTE—Do not regard dirithromycin 16S-epimer as an impurity.]

#### Assay—

*Potassium phosphate buffer*, *Mobile phase*, *System suitability solution*, and *Solvent*—Proceed as directed in the Assay under *Dirithromycin*.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 2.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of dirithromycin, to a 100-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix. Centrifuge a portion of this solution, and use the clear supernatant as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for 9-(S)-erythromyclamine, 1.0 for dirithromycin (16R-epimer), and 1.12 for dirithromycin 16S-epimer; the resolution, *R*, between the dirithromycin (16R-epimer) and dirithromycin 16S-epimer is not less than 2.0, and between dirithromycin (16R-epimer) and 9-(S)-erythro-

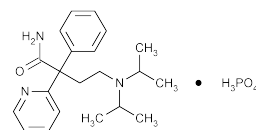
myclamine is not less than 5.0; the tailing factor for the dirithromycin (16R-epimer) peak is not more than 2.0; and the relative standard deviation determined from the dirithromycin (16R-epimer) peak for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dirithromycin (C<sub>42</sub>H<sub>78</sub>N<sub>2</sub>O<sub>14</sub>), which includes the 16R- and 16S-epimers, in the portion of Tablets taken by the formula:

$$40C(r_U + r_E) / r_S$$

in which *C* is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard preparation*; *r<sub>U</sub>* and *r<sub>E</sub>* are the peak responses for dirithromycin (16R-epimer) and dirithromycin 16S-epimer, respectively, obtained from the *Assay preparation*; and *r<sub>S</sub>* is the peak response of dirithromycin (16R-epimer) obtained from the *Standard preparation*.

## Disopyramide Phosphate



C<sub>21</sub>H<sub>29</sub>N<sub>3</sub>O · H<sub>3</sub>PO<sub>4</sub> 437.47

2-Pyridineacetamide, α-[2-[bis(1-methylethyl)amino]ethyl]-α-phenyl-, (±)-, phosphate (1:1).

(±)-α-[2-(Diisopropylamino)ethyl]-α-phenyl-2-pyridineacetamide phosphate (1:1) [22059-60-5].

» Disopyramide Phosphate contains not less than 98.0 percent and not more than 102.0 percent of C<sub>21</sub>H<sub>29</sub>N<sub>3</sub>O · H<sub>3</sub>PO<sub>4</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Disopyramide Phosphate RS

#### Identification—

**A:** *Infrared Absorption* <197M>.

**B:** A solution (1 in 200) meets the requirements of the tests for *Phosphate* <191>.

**pH** <791>: between 4.0 and 5.0 in a solution (1 in 20).

**Loss on drying** <731>—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Heavy metals**, *Method II* <231>: 0.002%.

#### Chromatographic purity—

**Standard solutions**—Prepare solutions *A* and *B* of USP Disopyramide Phosphate RS in methanol having concentrations of about 50 and 100 μg per mL, respectively.

**Test solution**—Prepare a solution of Disopyramide Phosphate in methanol having a concentration of about 10 mg per mL.

**Procedure**—Separately apply 10-μL portions of *Standard solutions A* and *B* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, dehydrated alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber,

allow to air-dry, and spray with potassium bismuth iodide TS: the  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from *Standard solution B*. Estimate the levels of any additional spots observed in the chromatogram of the *Test solution* by comparison with the principal spots in the chromatograms of *Standard solutions A* and *B*: the sum of the intensities of any additional spots observed is not greater than that obtained from *Standard solution B* (equivalent to 1%).

**Assay**—Dissolve about 160 mg of Disopyramide Phosphate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of  $C_{21}H_{29}N_3O \cdot H_3PO_4$ .

## Disopyramide Phosphate Capsules

» Disopyramide Phosphate Capsules contain an amount of Disopyramide Phosphate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of disopyramide ( $C_{21}H_{29}N_3O$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Disopyramide Phosphate RS

**Identification**—Transfer a portion of Capsule contents, equivalent to about 125 mg of disopyramide phosphate, to a 25-mL volumetric flask, add 20 mL of methanol, and shake by mechanical means for 20 minutes. Dilute with methanol to volume, mix, and filter through paper (Whatman No. 2 or equivalent), discarding the first 10 mL of the filtrate. Apply 10  $\mu$ L each of the subsequent filtrate and of a solution of USP Disopyramide Phosphate RS in methanol containing 6.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Dissolution** (711)—

Medium: water; 1000 mL.

Apparatus 2: 50 rpm.

Time: 20 minutes.

**Procedure**—Filter 15 mL of the solution under test, and transfer 10.0 mL of the filtrate to a 25-mL volumetric flask. Dilute with 2 N sulfuric acid to volume, and mix. Determine the amount of disopyramide ( $C_{21}H_{29}N_3O$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 268 nm of this solution, using water as the blank, in comparison with a Standard solution having a known concentration of USP Disopyramide Phosphate RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{21}H_{29}N_3O$  is dissolved in 20 minutes.

**Uniformity of dosage units** (905): meet the requirements.

## Assay—

**Methanolic sulfuric acid**—Cautiously add 5.4 mL of sulfuric acid to about 1800 mL of methanol with stirring, dilute with methanol to 2000 mL, and mix.

**Procedure**—Weigh the contents of not fewer than 20 Phosphate Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 125 mg of disopyramide phosphate, to a glass-stoppered, 125-mL flask. Add 50 mL of *Methanolic sulfuric acid*, and stir for 30 minutes. Filter through a medium-porosity, sintered-glass filter, and rinse thoroughly with *Methanolic sulfuric acid*. Transfer the combined filtrate and rinsings to a 100-mL volumetric flask, dilute with *Methanolic sulfuric acid* to volume, and mix. Dilute an accurately measured portion of this solution quantitatively and stepwise with the same solvent to obtain a solution having a concentration of about 40  $\mu$ g per mL. Dissolve an accurately weighed portion of USP Disopyramide Phosphate RS in *Methanolic sulfuric acid*, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 40  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using *Methanolic sulfuric acid* as the blank. Calculate the quantity, in mg, of  $C_{21}H_{29}N_3O$  in the portion of Capsules taken by the formula:

$$3.125(339.48 / 437.47)C(A_U / A_S)$$

in which 339.48 and 437.47 are the molecular weights of disopyramide and disopyramide phosphate, respectively; C is the concentration, in  $\mu$ g per mL, of USP Disopyramide Phosphate RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Capsules and the Standard solution, respectively.

## Disopyramide Phosphate Extended-Release Capsules

» Disopyramide Phosphate Extended-Release Capsules contain an amount of Disopyramide Phosphate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of disopyramide ( $C_{21}H_{29}N_3O$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The labeling indicates the *Dissolution Test* with which the product complies.

**USP Reference standards** (11)—  
USP Disopyramide Phosphate RS

**Identification**—Transfer a portion of Capsule contents, equivalent to about 195 mg of disopyramide phosphate, to a 25-mL volumetric flask, add 20 mL of methanol, and shake by mechanical means for 20 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 10 mL of the filtrate. Apply 20  $\mu$ L each of the subsequent filtrate and of a solution of USP Disopyramide Phosphate RS in methanol containing 7.7 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, absolute alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under

short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

#### Dissolution <711>—

TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

*pH 2.5, 0.1 M Phosphate buffer*—Dissolve 272 g of monobasic potassium phosphate in 20 L of water, and adjust with hydrochloric acid to a pH of  $2.50 \pm 0.04$ . [NOTE—Do not adjust back to pH 2.50 with base if too much acid is added. It is imperative that the ionic strength of the buffer be controlled.]

*Medium:* pH 2.5, 0.1 M Phosphate buffer; 1000 mL.

*Apparatus 1:* 100 rpm.

*Times:* 1 hour; 2 hours; 5 hours; 12 hours.

*Procedure*—Filter 10 mL of the solution under test at the required test points. Determine the amount of disopyramide ( $C_{21}H_{29}N_3O$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 261 nm of this solution, suitably diluted with *Medium*, if necessary, using *Medium* as the blank, in comparison with a Standard solution having a known concentration of USP Disopyramide Phosphate RS dissolved in *Medium*.

*Tolerances*—The percentage of the labeled amount of disopyramide ( $C_{21}H_{29}N_3O$ ) dissolved is within the range stated at each of the following times.

Time (hours)	Amount dissolved
1	between 5% and 25%
2	between 17% and 43%
5	between 50% and 80%
12	not less than 85%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*pH 2.5, 0.1 M Phosphate buffer*, and *Procedure*—Proceed as directed for *Test 1*.

*Medium*—Prepare as directed for *Test 1*; 900 mL.

*Apparatus 2:* 100 rpm.

*Times and Tolerances:*

Time (hours)	Amount dissolved
1	between 5% and 30%
4	between 40% and 65%
8	between 60% and 90%
12	not less than 75%

**Uniformity of dosage units <905>:** meet the requirements.

#### Assay—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Disopyramide Phosphate RS in 0.1 N sulfuric acid, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 40 µg per mL.

*Assay preparation*—Grind the contents of not fewer than 20 Capsules to a powder fine enough to pass through a 40-mesh screen. Transfer an accurately weighed portion of the powder, equivalent to about 650 mg of disopyramide phosphate, to a 500-mL volumetric flask. Add about 400 mL

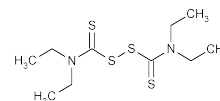
of 0.1 N sulfuric acid, and shake for 30 minutes. Dilute with 0.1 N sulfuric acid to volume, mix, and filter. Dilute an accurately measured portion of the filtrate quantitatively and stepwise with 0.1 N sulfuric acid to obtain a solution having a concentration of about 40 µg per mL.

*Procedure*—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 261 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of  $C_{21}H_{29}N_3O$  in the portion of Capsules taken by the formula:

$$16.25(339.48 / 437.47)C(A_U / A_S)$$

in which 339.48 and 437.47 are the molecular weights of disopyramide and disopyramide phosphate, respectively; C is the concentration, in µg per mL, of USP Disopyramide Phosphate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Disulfiram



$C_{10}H_{20}N_2S_4$  296.54  
Thioperoxydicarbonyl diamide  $[(H_2N)C(S)]_2S_2$ , tetraethyl-;  
Bis(diethylthiocarbamoyl) disulfide [97-77-8].

#### DEFINITION

Disulfiram contains NLT 98.0% and NMT 102.0% of  $C_{10}H_{20}N_2S_4$ .

#### IDENTIFICATION

- A. INFRARED ABSORPTION <197K>**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide solution to a pH of 7.0.

**Mobile phase:** Methanol and *Buffer* (7:3)

**Standard stock solution:** 1 mg/mL of USP Disulfiram RS in alcohol. [NOTE—Discard this solution after 5 days.]

**Standard solution:** 0.02 mg/mL of USP Disulfiram RS from the *Standard stock solution* diluted with *Mobile phase*. [NOTE—Prepare the *Standard solution* fresh daily.]

**Sample stock solution:** Transfer 50 mg of Disulfiram to a 50-mL volumetric flask, add 40 mL of alcohol, sonicate for 5 min to completely dissolve, cool, and dilute with alcohol to volume. [NOTE—Discard this solution after 5 days.]

**Sample solution:** 0.02 mg/mL of Disulfiram from the *Sample stock solution* diluted with *Mobile phase*. [NOTE—Prepare the *Sample solution* fresh daily.]



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 250 nm**Column:** 4-mm × 15-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 1800 theoretical plates**Tailing factor:** NMT 2**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>S<sub>4</sub> in the portion of Disulfiram taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Disulfiram RS in the *Standard solution* (μg/mL) $C_U$  = concentration of Disulfiram in the *Sample solution* (μg/mL)**Acceptance criteria:** 98.0%–102.0%**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

- **SELENIUM** <291>: 30 ppm

[NOTE— Perform this test only if selenium is a known inorganic process impurity.]

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE**, *Class I* <741>: 69°–72°

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** <11>—  
USP Disulfiram RS

**Disulfiram Tablets**

» Disulfiram Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>S<sub>4</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers.**USP Reference standards** <11>—

USP Disulfiram RS

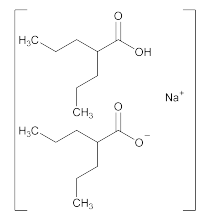
**Identification**—**A:** *Infrared Absorption* <197K> of a portion of powdered Tablets.**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.**Disintegration** <701>: 15 minutes, the use of disks being omitted.**Uniformity of dosage units** <905>: meet the requirements.**Assay**—*Buffer solution A*, *Buffer solution B*, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Disulfiram*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of disulfiram, to a 100-mL volumetric flask, add about 70 mL of alcohol and swirl, sonicate for 5 minutes, and shake by mechanical means for 30 minutes or until dissolved. Dilute with alcohol to volume, mix, and filter. [NOTE—Discard this solution after 5 days.] Quantitatively dilute this solution with *Mobile phase* to obtain the *Assay preparation* having a concentration of about 0.02 mg per mL. [NOTE—Prepare the *Assay preparation* fresh daily.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Disulfiram*. Calculate the quantity, in mg, of C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>S<sub>4</sub> in the portion of Tablets taken by the formula:

$$5C(r_U / r_S)$$

in which  $C$  is the concentration, in μg per mL, of USP Disulfiram RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Divalproex Sodium**

(C<sub>16</sub>H<sub>31</sub>NaO<sub>4</sub>)<sub>n</sub>  
 Pentanoic acid, 2-propyl-, sodium salt (2:1);  
 Sodium hydrogen bis(2-propylvalerate) oligomer.

310.41

**DEFINITION**

Divalproex Sodium contains NLT 98.0% and NMT 102.0% of available valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>
- **B. IDENTIFICATION TESTS—GENERAL**, *Sodium* <191>  
*Sample:* 100 mg  
*Analysis:* Ignite the *Sample*.  
*Acceptance criteria:* Meets the requirements

**ASSAY**• **PROCEDURE****Solution A:** 3.5 g of monobasic sodium phosphate monohydrate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.5. Dilute with water to 1 L.**Mobile phase:** Acetonitrile and *Solution A* (1:1)**Impurity stock solution:** 0.5 mg/mL of USP Valproic Acid Related Compound A RS in acetonitrile**Standard stock solution:** 5.0 mg/mL of USP Valproic Acid RS in *Mobile phase***System suitability solution:** 0.5 mg/mL of valproic acid from the *Standard stock solution*, and 5 μg/mL of valproic acid related compound A from the *Impurity stock solution*, in *Mobile phase***Standard solution:** 0.5 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in *Mobile phase***Sample solution:** 0.5 mg/mL of Divalproex Sodium in *Mobile phase*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L7**Flow rate:** 1 mL/min**Injection size:** 20 μL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for valproic acid related compound A and valproic acid are 0.69 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 5.0 between valproic acid related compound A and valproic acid**Tailing factor:** NMT 1.5 for the valproic acid peak**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of available valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>) in the portion of Divalproex Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight for divalproex sodium

repeating unit, 310.41

 $M_{r2}$  = molecular weight for valproic acid, 144.21 $F$  = number of moles of valproic acid per mole of divalproex sodium repeating unit, 2**Acceptance criteria:** 98.0%–102.0%**IMPURITIES****Inorganic Impurities**

- **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method I* <921>: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** <11>
  - USP Divalproex Sodium RS
  - Sodium hydrogen bis(2-propylvalerate), oligomer; pentanoic acid, 2-propyl-, sodium salt (2:1) (C<sub>16</sub>H<sub>31</sub>NaO<sub>4</sub>)<sub>n</sub> 310.41
  - USP Valproic Acid RS
  - USP Valproic Acid Related Compound A RS

## Divalproex Sodium Delayed-Release Capsules

**DEFINITION**

Divalproex Sodium Delayed-Release Capsules contain an amount of divalproex sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>
  - Diluent:** Acetonitrile and water (1:1)
  - Standard:** Prepare as directed in <197F> using USP Valproic Acid RS.
  - Sample:** Dissolve the contents of 20 Capsules in 30 mL of *Diluent* in a 50-mL volumetric flask. Sonicate for 30 min to dissolve. Dilute with *Diluent* to volume. Centrifuge the solution at 3000 rpm for about 20 min. Pipet

20 mL of the supernatant into a separatory funnel. Extract with 50 mL of *n*-hexane. Collect the *n*-hexane layer, and evaporate the solvent. Cast 1 mg of the liquid obtained after evaporation to NaCl windows.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE****Buffer:** 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.**Mobile phase:** Acetonitrile and *Buffer* (2:3)**Diluent:** Acetonitrile and water (1:1)**Standard solution:** Transfer a suitable amount of USP Valproic Acid RS to a suitable volumetric flask to obtain a solution having a final concentration of 2.5 mg/mL of valproic acid. Add 40% of the flask volume of *Diluent*. Sonicate for 5 min, and add 20% of the flask volume of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume.**Sample solution:** Transfer an amount of contents (from NLT 20 Capsules) to a suitable volumetric flask to obtain a nominal concentration of 2.5 mg/mL of valproic acid. Dissolve in 20% of the flask volume of 0.1 N hydrochloric acid, and sonicate for 5 min. Add 60% of the flask volume of *Diluent*, and sonicate for an additional 25 min. Dilute with *Diluent* to volume. Centrifuge at 4000 rpm for 10 min, and use the clear supernatant.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 1.8 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for valproic acid**Relative standard deviation:** NMT 2.0% for valproic acid**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of valproic acid in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0% of valproic acid**PERFORMANCE TESTS****Change to read:**• **DISSOLUTION** <711>**Test 1****Medium:** Phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water, adjusted with 0.08 N hydrochloric acid to a pH of 7.5); 500 mL, degassed**Apparatus 2:** 50 rpm, with sinkers**Time:** 2, 4, and 6 h**Buffer and Mobile phase:** Prepare as directed in the *Assay*.**Standard stock solution:** 1.6 mg/mL of USP Valproic Acid RS in *Mobile phase***Standard solution:** 0.26 mg/mL of valproic acid from the *Standard stock solution* and *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Replace the volume withdrawn with an equal volume of *Medium* previously heated at  $37.0 \pm 0.5^\circ$ .

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.8 mL/min

**Injection volume:** 40  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for valproic acid

**Relative standard deviation:** NMT 2.0% for valproic acid

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of valproic acid ( $C_8H_{16}O_2$ ) dissolved ( $D_i$ ) at each time interval:

$$D_i = (r_U/r_S) \times (C_S/L) \times V \times 100$$

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of the *Standard solution* (mg/mL)
- $L$  = label claim (mg/Capsule)
- $V$  = volume of *Medium*, 500 mL

Percentage of valproic acid dissolved at 2 h =  $D_1$

Percentage of valproic acid dissolved at 4 h =  $D_2 + [(D_1/V) \times V_S]$

Percentage of valproic acid dissolved at 6 h =  $D_3 + [(D_1/V) \times V_S] + [(D_2/V) \times V_S]$

- $V$  = volume of *Medium*, 500 mL
- $V_S$  = volume withdrawn at each sampling time (mL)

**Tolerances:** NLT 20% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 2 h; NLT 70% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 4 h; and NLT 85% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 6 h.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

#### Procedure A

**Medium:** 0.05 M phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water, adjusted with 2 N sodium hydroxide to a pH of 7.5); 500 mL

**Apparatus 2:** 50 rpm, contents of the Capsule

**Time:** 15 min

**Standard solution A:** 0.036 mg/mL of USP Valproic Acid RS in *Medium*. A volume of acetonitrile not exceeding 10% of the total volume may be used to dissolve valproic acid.

**Sample solution A:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Procedure B

**Medium:** 0.05 M phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water, adjusted with 2 N sodium hydroxide to a pH of 7.5); 900 mL

**Apparatus 2:** 50 rpm, with wire helix sinkers

**Time:** 4 h

**Buffer A:** 0.5 g/L of monohydrate citric acid and 0.4 g/L of dibasic sodium phosphate in water

**Buffer B:** 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water. Adjust with phosphoric acid to a pH of 7.4.

**Mobile phase:** Acetonitrile, *Buffer A*, and *Buffer B* (30:35:35). Adjust with phosphoric acid to a pH of 3.0.

**Standard solution B:** 0.13 mg/mL of USP Valproic Acid RS in *Medium*. A volume of acetonitrile not exceeding 10% of the total volume may be used to dissolve valproic acid.

**Sample solution B:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L11

**Flow rate:** 1.2 mL/min

**Injection volume:** 200  $\mu$ L for *Standard solution A* and *Sample solution A*, 50  $\mu$ L for *Standard solution B* and *Sample solution B*

#### System suitability

**Sample:** *Standard solution B*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for valproic acid

**Relative standard deviation:** NMT 2.0% for valproic acid

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at each time point:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

- $r_U$  = peak response from *Sample solution A* or *Sample solution B*
- $r_S$  = peak response from *Standard solution A* or *Standard solution B*
- $C_S$  = concentration of *Standard solution A* or *Standard solution B* (mg/mL)
- $L$  = label claim (mg/Capsule)
- $V$  = volume of *Medium*, 500 mL for *Sample solution A* and 900 mL for *Sample solution B*

**Tolerances:** NMT 20% of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 15 min (*Sample solution A*); and NLT 80% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 4 h (*Sample solution B*). The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at 4 h conforms to *Acceptance Table 1* in *Dissolution* <711>.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

#### Medium

**Acid stage medium:** 0.08 N hydrochloric acid; 900 mL

**Buffer stage medium:** pH 7.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate and 1.6 g/L of sodium hydroxide in water, prepared as follows. Transfer suitable quantities of monobasic potassium phosphate and sodium hydroxide to a suitable volumetric flask. Dissolve in 83% of the flask volume of water, and adjust with 0.1 N hydrochloric acid, if necessary, to a pH of 7.5. Dilute the resulting solution with water to volume); 900 mL

#### Time

**Acid stage time:** 2 h

**Buffer stage time:** 4 h

#### Apparatus 2: 50 rpm, with sinkers

**Buffer:** • 0.25 g/L of citric acid monohydrate, 0.2 g/L of anhydrous dibasic sodium phosphate, 3.4 g/L of monobasic potassium phosphate, and 0.85 g/L of sodium hydroxide in water. (ERR 1-May-2012)

**Mobile phase:** Acetonitrile and Buffer (45:55), mixed, degassed, and adjusted with phosphoric acid to a pH of 2.5

**Standard solution:** 0.14 mg/mL of USP Valproic Acid RS prepared as follows. Transfer a portion of USP Valproic Acid RS to a suitable volumetric flask. Dissolve in methanol using 5.0% of the final volume. Dilute to final volume with Buffer stage medium, and mix.

**Sample solution**

**Acid stage sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 3 mL of filtrate.

**Buffer stage sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 3 mL of filtrate.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L11

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution, Acid stage sample solution, and Buffer stage sample solution

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at each timepoint (Q):

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from Acid stage sample solution or Buffer stage sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of the Standard solution (mg/mL)

$L$  = label claim (mg/Capsule)

$V$  = volume of Acid stage medium or Buffer stage medium (900 mL)

**Tolerances:** The requirements for the Acid stage and the Buffer stage must be met.

**Acid stage:** NMT 30% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 2 h (Acid stage sample solution). The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at 2 h conforms to Table 1.

**Table 1**

Level	Number Tested	Criteria
A <sub>1</sub>	6	No individual value exceeds Q dissolved.
A <sub>2</sub>	6	Average of the 12 units (A <sub>1</sub> + A <sub>2</sub> ) is not more than Q dissolved; and no individual unit is greater than Q + 15% dissolved.
A <sub>3</sub>	12	Average of the 24 units (A <sub>1</sub> + A <sub>2</sub> + A <sub>3</sub> ) is not more than Q dissolved; and no individual unit is greater than Q + 15% dissolved.

**Buffer stage:** NLT 80% (Q) of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved in 4 h (Buffer stage sample solution). The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at 4 h conforms to Acceptance Table 2 in Dissolution <711>.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.

- **LABELING:** Divalproex Delayed-Release Capsules may be swallowed whole or may be administered by carefully opening the Capsule and sprinkling the entire contents on a small amount of soft food. This drug/food mixture should be swallowed immediately and not chewed. It should not be stored for future use. When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.

- **USP REFERENCE STANDARDS <11>**  
USP Valproic Acid RS

## Divalproex Sodium Delayed-Release Tablets

» Divalproex Sodium Delayed-Release Tablets contain an amount of divalproex sodium equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards <11>**—

USP Valproic Acid RS

**Identification**—The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Dissolution <711>**—

ACID STAGE—

**Medium:** 0.08 N hydrochloric acid (prepared by adding 40 mL of hydrochloric acid to 5000 mL of water, adjusting with 2 N hydrochloric acid to a pH of 1.2, and diluting with water to 6000 mL); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 1 hour.

**Procedure**—At the end of 1 hour, carefully transfer the Tablet to a dissolution vessel containing the Medium of the Buffer stage. [NOTE—Do not perform an analysis of the Medium in the Acid stage.]

BUFFER STAGE—

**Medium:** pH 7.5 phosphate buffer (prepared by dissolving 40.83 g of monobasic potassium phosphate and 9.84 g of sodium hydroxide in 5000 mL of water, adjusting with 0.08 N hydrochloric acid to a pH of 7.5, and diluting with water to 6000 mL); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 1 hour.

Determine the amount of  $C_8H_{16}O_2$  dissolved in the Buffer stage by employing the following method.

**Citrate buffer**—Dissolve 0.5 g of citric acid monohydrate and 0.4 g of dibasic sodium phosphate in 1.0 L of water.

**Potassium phosphate buffer**—Dissolve 6.8 g of monobasic potassium phosphate and 1.7 g of sodium hydroxide in 1.0 L of water. Adjust with phosphoric acid to a pH of 7.4  $\pm$  0.1.

**Mobile phase**—Prepare a mixture of Citrate buffer, Potassium phosphate buffer, and acetonitrile (35:35:30). Adjust with phosphoric acid to a pH of 3.0  $\pm$  0.1, and mix. Filter and degas. Make adjustments if necessary (see System Suitability under Chromatography <621>).

**Standard solution**—Prepare a solution of USP Valproic Acid RS in the Medium used in the Buffer stage, having a known concentration of about 0.12 mg per mL. [NOTE—A volume of acetonitrile not exceeding 10.0% of the total volume may be used to dissolve the USP Valproic Acid RS.]

**Test solution**—If necessary, dilute a portion of each filtered solution under test with the *Medium* used in the *Buffer stage* to obtain a solution having a concentration of about 0.12 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 15-cm column that contains 4-μm packing L11. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>) dissolved by the formula:

$$900CD(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Valproic Acid RS in the *Standard solution*; *D* is the dilution factor used to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas of valproic acid obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of C<sub>8</sub>H<sub>16</sub>O<sub>2</sub> is dissolved in 1 hour in the *Buffer stage*.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Citrate buffer**—Dissolve 2.0 g of citric acid monohydrate and 1.6 g of dibasic sodium phosphate in 4.0 L of water.

**Mobile phase**—Prepare a mixture of *Citrate buffer* and acetonitrile (7:3). Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and mix. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Prepare a solution of USP Valproic Acid RS in *Mobile phase* having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer a number of whole Tablets containing the equivalent of about 2500 mg of valproic acid into a 250-mL volumetric flask. Add 150 mL of *Mobile phase*, and sonicate with frequent swirling for 30 minutes or until the Tablets completely disintegrate. Allow the solution to cool down to room temperature, and then dilute with *Mobile phase* to volume. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 15-cm column that contains 4-μm packing L11. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>) in the portion of Tablets taken by the formula:

$$5000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Valproic Acid RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas of valproic acid obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Divalproex Sodium Extended-Release Tablets

### DEFINITION

Divalproex Sodium Extended-Release Tablets contain an amount of divalproex sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 0.5 g of citric acid monohydrate and 0.4 g of dibasic sodium phosphate in 1 L of water

**Mobile phase:** Methanol and *Buffer* (11:9). Adjust with phosphoric acid to a pH of 5.0.

**Diluent:** *Buffer*, adjusted with phosphoric acid to a pH of 2.0

**Standard stock solution:** 2.5 mg/mL of USP Valproic Acid RS in methanol

**Standard solution:** 1.0 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in *Diluent*

**Sample stock solution:** Transfer an amount of powder (from NLT 20 Tablets) to a suitable volumetric flask to obtain a nominal concentration of 2.5 mg/mL of valproic acid. Dissolve in 50% of the flask volume of methanol by shaking for 1 h. Dilute with methanol to volume, and pass through a suitable filter.

**Sample solution:** 1.0 mg/mL of valproic acid from the filtrate of the *Sample stock solution* in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm × 15-cm; 4-μm packing L11

**Flow rate:** 0.7 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for valproic acid

**Relative standard deviation:** NMT 2.0% for valproic acid

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of valproic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of valproic acid

### PERFORMANCE TESTS

#### Change to read:

#### DISSOLUTION <711>

##### Test 1

**Acid stage medium:** 0.1 N hydrochloric acid; 500 mL

**Buffer stage medium:** 21.6 g of sodium dodecyl sulfate, 6.9 g of sodium dihydrogen phosphate monohydrate, and 0.12 g of sodium hydroxide in 1 L of water.

Adjust with diluted sodium hydroxide or phosphoric acid to a pH of 5.5; 900 mL.

**Apparatus 2:** 100 rpm, with three-prong sinkers only for 250-mg Tablets, if necessary

**Times:** 45 min in the *Acid stage medium*; 3, 9, 12, and 24 h in the *Buffer stage medium* (RB 1-May-2012)

**Analysis:** After 45 min in the *Acid stage medium*, withdraw a sample from the solution, and immediately filter. Replace the *Acid stage medium* with the *Buffer stage medium*, and run the test for the times specified.

**Buffer:** 1.42 g of dibasic sodium phosphate and 0.5 mL of glacial acetic acid in 1 L of water. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Methanol and *Buffer* (13:7)

**Standard stock solution:** 2.5 mg/mL of USP Valproic Acid RS in methanol

**Standard solution:** 0.15 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in the *Buffer stage medium*. [NOTE—Add 40% of the flask volume of methanol before diluting with *Buffer stage medium* to volume.]

**Sample solutions:** Pass a portion of the solution under test through a suitable filter of 20- $\mu$ m pore size. Use the *Sample solution* from the *Acid stage medium* as is. Dilute the *Sample solution* from the *Buffer stage medium* with methanol by a factor of 2.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm  $\times$  15-cm; 10- $\mu$ m packing L11

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 80  $\mu$ L

**Run time:** 6 min

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution*, *Sample solution* from the *Acid stage medium*, and *Sample solutions* from the *Buffer stage medium*

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V_A \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V_A$  = volume of the *Acid stage medium*, 500 mL

Calculate the concentration of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Buffer stage medium* at the time interval,  $t$ , in mg/mL:

$$C_t = (r_U/r_S) \times (C_S \times D_U)$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

$D_U$  = dilution factor of the *Sample solution* in the *Buffer stage medium*, 2

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) in the *Buffer stage medium* at the first time interval:

$$\text{Result} = C_1 \times V_B \times (100/L)$$

$C_1$  = concentration of valproic acid in the *Buffer stage medium* at the first time interval (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$L$  = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Buffer stage medium* at the second time interval:

$$\text{Result} = [C_2 \times (V_B - V_S)] + (C_1 \times V_S) \times (100/L)$$

$C_2$  = concentration of valproic acid in the *Buffer stage medium* at the second time interval (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$V_S$  = volume of the sample taken at each time interval (mL)

$C_1$  = concentration of valproic acid in the *Buffer stage medium* at the first time interval (mg/mL)

$L$  = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Buffer stage medium* at the  $n^{\text{th}}$  time interval:

$$\text{Result} = C_n \times [V_B - (n - 1) \times V_S] + [(C_1 + C_2 + \dots + C_{n-1}) \times V_S] \times (100/L)$$

$C_n$  = concentration of valproic acid in the *Buffer stage medium* at the  $n^{\text{th}}$  time interval (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$V_S$  = volume of the sample taken (mL)

$C_1$  = concentration of valproic acid dissolved in the first time interval in the *Buffer stage medium* (mg/mL)

$C_2$  = concentration of valproic acid dissolved in the second time interval in the *Buffer stage medium* (mg/mL)

$C_{n-1}$  = concentration of valproic acid dissolved in the  $(n - 1)^{\text{th}}$  time interval in the *Buffer stage medium* (mg/mL)

$L$  = label claim (mg/Tablet)

#### Tolerances

**Acid stage:** NMT 10% of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) is dissolved.

**Buffer stage:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved (Tablets labeled to contain 500 mg of valproic acid)	Amount Dissolved (Tablets labeled to contain 250 mg of valproic acid)
3	10%–30%	10%–30%
9	35%–55%	35%–60%
12	45%–70%	45%–75%
24	NLT 75%	NLT 75%

The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Acid stage medium:** 0.1 N hydrochloric acid; 500 mL

**Buffer stage concentrate:** 15.53 g/L of monobasic sodium phosphate monohydrate, 5.45 g/L of sodium hydroxide, and 48.65 g of sodium lauryl sulfate per L in water (final pH approximately 11); 400 mL

**Buffer stage medium:** Mix 400 mL of *Buffer stage concentrate* with 500 mL of *Acid stage medium* to a pH of  $5.5 \pm 0.05$ . [NOTE—If necessary, adjust the pH of the *Buffer stage concentrate* with 1 N hydrochloric acid or 1 N sodium hydroxide to assure that the final pH of

the mixture of media is 5.5.] Retain this solution to dilute the solutions prepared later.

**Apparatus 2:** 100 rpm, with wire helix sinkers

**Times:** 45 min in the *Acid stage medium*; 3, 9, 12, and 21 h in the *Buffer stage medium*. • The times in the *Buffer stage medium* include the time in the *Acid stage medium*. • (RB 1-May-2012)

**Procedure:** After 45 min in *Acid stage medium*, stop and lift the paddles from the vessels. Do not perform an analysis of the *Acid stage medium*. Transfer 400 mL of *Buffer stage concentrate* to the vessels containing the *Acid stage medium*, and run the test for the times specified.

**Buffer:** 3.5 g/L of monobasic sodium phosphate monohydrate in water. Adjust with phosphoric acid to a pH of 3.5.

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard stock solution:** 28 mg/mL of USP Valproic Acid RS in a suitable volumetric flask. Dissolve with 20% of the flask volume of 1 N sodium hydroxide, and dilute with water to volume. Dilute this solution with *Buffer stage medium* to obtain a final concentration of about 2.8 mg/mL.

**Standard solutions:** Prepare a series of dilutions in *Buffer stage medium* from the *Standard stock solution* in the concentrations of 0.028, 0.11, 0.22, 0.50, and 0.70 mg/mL.

**Sample solution:** Withdraw 10 mL of the solution under test, and pass through a suitable filter of 35-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

#### System suitability

**Samples:** 0.028, 0.11, 0.22, 0.50, and 0.70 mg/mL of the *Standard solutions*

#### Suitability requirements

**Tailing factor:** NMT 2.0, using the 0.50-mg/mL *Standard solution*

**Relative standard deviation:** NMT 2.0%

**Correlation coefficient:** NLT 0.999, using the five concentrations of the *Standard solution*

#### Analysis

**Samples:** *Sample solutions*

From the standard curve, determine the amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at each time interval using the response of each *Sample solution*.

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Buffer stage medium* at the first time interval:

$$\text{Result} = (C_1 \times V_B) \times (100/L)$$

$C_1$  = concentration of valproic acid in the *Buffer stage medium* at the 3-h time interval (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$L$  = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Buffer stage medium* at the  $n^{\text{th}}$  time interval:

$$\text{Result} = C_n \times [V_B - (n - 1) \times V_S] + [(C_1 + C_2 + \dots + C_{n-1}) \times V_B] \times (100/L)$$

$C_n$  = concentration of valproic acid in the *Buffer stage medium* at the  $n^{\text{th}}$  time interval (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$V_S$  = volume of the sample taken (mL)

$C_1$  = concentration of valproic acid dissolved in the first time interval in the *Buffer stage medium* (mg/mL)

$C_2$  = concentration of valproic acid dissolved in the second time interval in the *Buffer stage medium* (mg/mL)

$C_{n-1}$  = concentration of valproic acid dissolved in the  $(n - 1)^{\text{th}}$  time interval in the *Buffer stage medium* (mg/mL)

$L$  = label claim (mg/Tablet)

**Tolerances:** The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at the times specified conform to the following acceptance table (*Table 2*).

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Acid stage medium:** 0.1 N hydrochloric acid; 250 mL (row 1)

**Buffer stage medium:** pH 6.8 buffer (6.8 g of monobasic potassium phosphate and 0.92 g of sodium hydroxide in 1 L of water. Adjust with phosphoric acid or sodium hydroxide to a pH of  $6.8 \pm 0.05$ ; 250 mL (rows 2–4)

**Apparatus 3:** 30 dips/min, 20-mesh polypropylene screen on top and bottom; 30-s drip time

**Times:** 1 h in acid stage (row 1); 2, 12, and 24 h in buffer stage (rows 2–4). • The times in the *Buffer stage medium* include the time in the *Acid stage medium*. • (RB 1-May-2012)

**Buffer:** 0.25 g of citric acid monohydrate, 0.2 g of anhydrous dibasic sodium phosphate, 3.4 g of monobasic potassium phosphate, and 0.85 g of sodium hydroxide in 1 L of water. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.05$ .

**Mobile phase:** Acetonitrile and *Buffer* (30:70)

**Acid stage standard stock solution:** 1 mg/mL of USP Valproic Acid RS in *Acid stage medium*. Dissolve a suitable amount of USP Valproic Acid RS in a suitable volumetric flask in 10% of the flask volume of methanol to solubilize the valproic acid. Dilute with *Acid stage medium* to volume.

**Buffer stage standard stock solution:** 1 mg/mL of USP Valproic Acid RS in *Buffer stage medium*. Dissolve a suitable amount of USP Valproic Acid RS in a suitable volumetric flask in 10% of the flask volume of methanol to solubilize the valproic acid. Dilute with *Buffer stage medium* to volume.

**Table 2**

		<b>3 h</b>	<b>9 h</b>	<b>12 h</b>	<b>21 h</b>
L1	Individual Tablets	10%–27%	35%–70%	44%–92%	NLT 87%
L2	Average	10%–27%	35%–70%	44%–92%	NLT 87%
L2	Individual Tablets	0%–37%	25%–80%	34%–102%	NLT 77%
L3	Average	10%–27%	35%–70%	44%–92%	NLT 87%
L3	Individual Tablets	NMT 2 Tablets are outside the range of 0%–37% and no individual Tablet is outside the range of 0%–47%	NMT 2 Tablets are outside the range of 25%–80% and no individual Tablet is outside the range of 15%–90%	NMT 2 Tablets are outside the range of 34%–102% and no individual Tablet is outside the range of 24%–112%	NMT 2 Tablets release less than 77% and no individual Tablet releases less than 67%

**Acid stage standard solution:** ( $L/2500$ ) mg/mL of valproic acid from *Acid stage stock solution* in *Acid stage medium*, where  $L$  is the Tablet label claim, in mg

**Buffer stage standard solution:** ( $L/700$ ) mg/mL of valproic acid from *Buffer stage stock solution* in *Buffer stage medium*, where  $L$  is the Tablet label claim, in mg

**Sample solutions:** Centrifuge a portion of the solution under test at about 3000 rpm for about 20 min. Use the supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm  $\times$  15-cm; 5- $\mu$ m packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 100  $\mu$ L for Tablets labeled to contain 250 mg; 50  $\mu$ L for Tablets labeled to contain 500 mg

#### System suitability

**Samples:** *Acid stage standard solution* and *Buffer stage standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solutions*, and *Buffer stage sample solutions*

Calculate the percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at each time point  $Q_i$ :

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_2 = [(r_U/r_S) \times (C_S/L) \times V \times 100] + Q_1$$

$$Q_{12} = [(r_U/r_S) \times (C_S/L) \times V \times 100] + Q_2$$

$$Q_{24} = [(r_U/r_S) \times (C_S/L) \times V \times 100] + Q_{12}$$

$r_U$  = peak response of the *Sample solution* from the *Acid stage* or *Buffer stage* time points

$r_S$  = peak response of the *Acid stage standard solution* or *Buffer stage standard solution*

$C_S$  = concentration of valproic acid in the *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of the *Acid stage medium* or *Buffer stage medium*, 250 mL

**Tolerances:** See *Table 3*.

**Table 3**

Time (h)	Amount Dissolved (Tablets labeled to contain 500 mg of valproic acid)	Amount Dissolved (Tablets labeled to contain 250 mg of valproic acid)
1	NMT 10%	NMT 10%
2	5%–25%	5%–25%
12	55%–75%	65%–85%
24	NLT 80%	NLT 80%

The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

• **Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Acid stage medium:** 0.1 N hydrochloric acid; 500 mL

**Buffer stage stock medium:** 19.0 g/L of trisodium phosphate dodecahydrate in water adjusted with hydrochloric acid to a pH of 5.5

**Buffer stage medium:** 21.6 g/L of sodium lauryl sulfate in *Buffer stage stock medium*; 900 mL

**Apparatus 2:** 100 rpm, with sinkers for 250 mg and 500 mg

**Times:** 45 min in *Acid stage medium*; 3, 9, 12, and 18 h in *Buffer stage medium*. The times in the *Buffer stage medium* include the time in the *Acid stage medium*.

**Buffer:** 1.36 g/L of monobasic potassium phosphate and triethylamine (99.5: 0.5). Adjust with phosphoric acid to a pH of 2.75.

**Solution A:** 1.0 g/L of sodium lauryl sulfate in *Buffer*

**Mobile phase:** Acetonitrile and *Solution A* (50:50), degassed

**Acid stage standard stock solution:** 1 mg/mL of USP Valproic Acid RS prepared as follows. Transfer a suitable amount of USP Valproic Acid RS to a volumetric flask, and dissolve in 20% of the flask volume of acetonitrile to solubilize valproic acid. Dilute with *Acid stage medium* to volume.

**Acid stage standard solution:** ( $L/5000$ ) mg/mL of valproic acid from *Acid stage stock solution* in *Acid stage medium*, where  $L$  is the Tablet label claim, in mg

**Buffer stage standard solution:** ( $L/900$ ) mg/mL of USP Valproic Acid RS, prepared as follows. Transfer a suitable amount of USP Valproic Acid RS to a volumetric flask, and dissolve in ( $L/50$ )% of the flask volume of acetonitrile. Dilute with *Buffer stage medium* to volume.  $L$  is the Tablet label claim, in mg.

**Acid stage sample solution:** Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter of 45- $\mu$ m pore size.

**Buffer stage sample solution:** Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter of 45- $\mu$ m pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *Acid stage standard solution* and *Buffer stage standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solutions*

Calculate the percentage of the labeled amount ( $Q_A$ ) of valproic acid ( $C_8H_{16}O_2$ ) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times (C_S) \times V_A \times (1/L) \times 100$$

$r_U$  = peak response of the *Acid stage sample solution*

$r_S$  = peak response of the *Acid stage standard solution*

$C_S$  = concentration of USP Valproic Acid RS in the *Acid stage standard solution* (mg/mL)

$V_A$  = volume of the *Acid stage medium*, 500 mL

$L$  = label claim (mg/Tablet)

Calculate the concentration ( $C_i$ ) of valproic acid ( $C_8H_{16}O_2$ ) in the sample withdrawn from the vessel at each *Buffer stage* time point ( $i$ ):

$$\text{Result}_i = (r_U/r_S) \times (C_S) \times 100$$

$r_U$  = peak response of the *Buffer stage sample solution*



$r_s$  = peak response of the *Buffer stage standard solution*

$C_s$  = concentration of USP Valproic Acid RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount ( $Q_i$ ) of valproic acid ( $C_8H_{16}O_2$ ) dissolved at each *Buffer stage* time point ( $i$ ):

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + Q_A$$

$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_s)] \times (1/L) \times 100\} + Q_A$$

$$\text{Result}_3 = \{[(C_3 \times V_B) + [(C_2 + C_1) \times V_s]] \times (1/L) \times 100\} + Q_A$$

$$\text{Result}_4 = \{[(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_s]] \times (1/L) \times 100\} + Q_A$$

$C_i$  = concentration of valproic acid in the *Buffer stage sample solution* withdrawn at time point  $i$  (mg/mL)

$V_B$  = volume of the *Buffer stage medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the *Buffer stage sample solution* withdrawn from the vessel (mL)

$Q_A$  = percentage of the labeled amount of valproic acid dissolved in the *Acid stage*

Tolerances: See *Table 4*.

**Table 4**

Time Point (i)	Time (h)	Amount Dissolved (Tablets labeled to contain 500 mg of valproic acid)	Amount Dissolved (Tablets labeled to contain 250 mg of valproic acid)
1	3	10%–30%	10%–30%
2	9	40%–70%	35%–60%
3	12	60%–90%	50%–80%
4	18	NLT 85%	NLT 85%

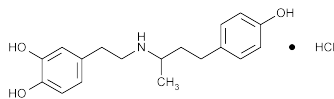
The percentage of the labeled amount of valproic acid ( $C_8H_{16}O_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>. (RB 1-May-2012)

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** <11>  
USP Valproic Acid RS

## Dobutamine Hydrochloride



$C_{18}H_{23}NO_3 \cdot HCl$  337.84

1,2-Benzenediol, 4-[2-[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]ethyl]-, hydrochloride, ( $\pm$ )-  
( $\pm$ )-4-[2-[[3-(*p*-Hydroxyphenyl)-1-methylpropyl]amino]ethyl]-pyrocatechol hydrochloride [49745-95-1].

» Dobutamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{23}NO_3 \cdot HCl$ , calculated on the anhydrous basis.

**Caution**—Great care should be taken to prevent inhaling particles of Dobutamine Hydrochloride and exposing the skin to it. Protect the eyes.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Dobutamine Hydrochloride RS

**Color of solution**—Transfer 500 mg of Dobutamine Hydrochloride to a 25-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, heating at 30° to 35° to dissolve the sample, if necessary. Immediately cool the solution to room temperature and read the absorbance: the absorbance, determined in a 1-cm cell at 480 nm in a suitable spectrophotometer, water being used as the blank, is not greater than 0.04.

#### Identification—

**A:** *Infrared Absorption* <197K>.

**B:** It responds to the test for dry chlorides in *Chloride* <191>.

**Water, Method I** <921>: not more than 1.0%.

**Residue on ignition** <281>: not more than 0.2%.

**Heavy metals, Method II** <231>: 0.003%.

#### Chromatographic purity—

**Solution A**—Dissolve 2.60 g of sodium 1-octanesulfonate in 1000 mL of water, pipet 3 mL of triethylamine into the solution, and mix. Adjust the solution with phosphoric acid to a pH of 2.5. Filter and degas before use.

**Solution B**—Prepare a filtered and degassed mixture of methanol and acetonitrile (82:18). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluting solution**—Prepare a mixture of *Solution A* and *Solution B* (1:1).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments to either *Solution* if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Dissolve an accurately weighed quantity of USP Dobutamine Hydrochloride RS in *Diluting solution* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Test solution**—Transfer about 50.0 mg of Dobutamine Hydrochloride, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	65	35	equilibration
0–5	65	35	isocratic
5–20	65→20	35→80	linear gradient
20–25	20	80	isocratic
25–26	20→65	80→35	linear gradient
26–30	65	35	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not

more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Dobutamine Hydrochloride taken by the formula:

$$100(C/D)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Dobutamine Hydrochloride RS in the *Standard solution*; *D* is the concentration, in mg per mL, of Dobutamine Hydrochloride in the *Test solution*; *r<sub>i</sub>* is the peak response for each impurity found in the *Test solution*; and *r<sub>s</sub>* is the dobutamine response obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found, and not more than 1.0% of total impurities is found.

#### Assay—

**Phosphate buffer**—Transfer about 23 g of monobasic ammonium phosphate to a 2-liter volumetric flask, add 1900 mL of water, and mix. Adjust with phosphoric acid to a pH of 2.2, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dobutamine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.5 mg per mL. [NOTE—Prepare fresh daily, and refrigerate until injected.]

**System suitability solution**—Dissolve suitable quantities of 5-(hydroxymethyl)furfural and USP Dobutamine Hydrochloride RS in water to obtain a solution containing about 0.01 and 0.5 mg per mL, respectively.

**Assay preparation**—Transfer about 50 mg of Dobutamine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. [NOTE—Refrigerate until injected, and use within 8 hours.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the relative retention times are 1.0 for dobutamine and not more than 0.62 for 5-(hydroxymethyl)furfural, and the retention time of dobutamine is not more than 5.3 minutes. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{23}NO_3 \cdot HCl$  in the portion of Dobutamine Hydrochloride taken by the formula:

$$100C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Dobutamine Hydrochloride RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dobutamine Injection

» Dobutamine Injection is a sterile solution of Dobutamine Hydrochloride in Water for Injection. It contains an amount of Dobutamine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dobutamine ( $C_{18}H_{23}NO_3$ ). It may contain one or more suitable antioxidants, chelating agents, or preservatives.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate that it is to be diluted to appropriate strength with a suitable parenteral vehicle prior to administration.

#### USP Reference standards <11>—

USP Dobutamine Hydrochloride RS

USP Endotoxin RS

**Identification**—A 10- $\mu$ L volume of it responds to the *Identification* test under *Dobutamine for Injection*.

**Bacterial endotoxins** <85>—It contains not more than 2.08 USP Endotoxin Units per mg of dobutamine.

**pH** <791>: between 2.5 and 5.5.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* <1>.

#### Assay—

**Ion-pair solution**—Dissolve 3.38 g of sodium 1-octanesulfonate in 1000 mL of water, pipet 3 mL of triethylamine into the solution, and mix. Adjust the solution with phosphoric acid to a pH of 2.5.

**Mobile phase**—Prepare a filtered and degassed mixture of the ion-pair solution, acetonitrile, and methanol (58:28:14). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>). [NOTE—The ratio of acetonitrile to methanol is critical to the elution order of the *System suitability solution* components.]

**System suitability solution**—Dissolve suitable quantities of 4-(4-hydroxyphenyl)-2-butanone and USP Dobutamine Hydrochloride RS in *Mobile phase* to obtain a solution containing about 0.3 and 0.56 mg per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dobutamine Hydrochloride RS in *Mobile phase* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.56 mg per mL (equivalent to about 0.5 mg of dobutamine per mL).

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of dobutamine, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m, base-deactivated packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.9 for 4-(4-hydroxyphenyl)-2-butanone and 1.0 for dobutamine, the resolution, *R*, between 4-(4-hydroxyphenyl)-2-butanone and dobutamine is not less than 1.5; the tailing factor for dobutamine is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{23}NO_3$  in the portion of Injection taken by the formula:

$$(301.39 / 337.84)(50C)(r_U / r_S)$$

in which 301.39 is the molecular weight of dobutamine; 337.84 is the molecular weight of dobutamine hydrochloride; C is the concentration, in mg per mL, of USP Dobutamine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dobutamine for Injection

» Dobutamine for Injection is a sterile mixture of Dobutamine Hydrochloride with suitable diluents. It contains an amount of dobutamine hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dobutamine ( $C_{18}H_{23}NO_3$ ).

**Caution**—Great care should be taken to prevent inhaling particles of Dobutamine for Injection and exposing the skin to it. Protect the eyes.

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under *Injections* <1>, at controlled room temperature.

**USP Reference standards** <11>—  
USP Dobutamine Hydrochloride RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>. Do not use the constituted solution if it is brown or contains a precipitate.

**Identification**—Prepare a solution in methanol, clarified by centrifugation, to contain 10 mg of dobutamine hydrochloride per mL. Apply 10  $\mu$ L of this solution and 10  $\mu$ L of a freshly prepared Standard solution of USP Dobutamine Hydrochloride RS in methanol containing 10 mg per mL to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, *n*-propyl alcohol, water, and glacial acetic acid (100:40:15:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate at room temperature. Observe the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Bacterial endotoxins** <85>—It contains not more than 5.56 USP Endotoxin Units per mg of dobutamine.

**Uniformity of dosage units** <905>: meets the requirements.

**pH** <791>: between 2.5 and 5.5, the contents of 1 vial being dissolved in 10 mL of water.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

*Ion-pair solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Dobutamine Injection*.

**Assay preparation**—Inject about 10 mL of *Mobile phase* into 1 vial of Dobutamine for Injection, taking care not to let pressure build up in the vial. Shake to dissolve the specimen completely. Transfer the solution to an appropriate volumetric flask, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of 0.5 mg of dobutamine per mL.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{23}NO_3$  in each container of Dobutamine for Injection taken by the formula:

$$(301.39 / 337.84)(10CD)(r_U / r_S)$$

in which 301.39 and 337.84 are the molecular weights of dobutamine and dobutamine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Dobutamine Hydrochloride RS in the *Standard preparation*; D is the dilution factor in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dobutamine in Dextrose Injection

» Dobutamine in Dextrose Injection is a sterile solution of Dobutamine Hydrochloride and Dextrose in Water for Injection. It contains an amount of Dobutamine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dobutamine ( $C_{18}H_{23}NO_3$ ) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ). It may contain one or more suitable antioxidants or chelating agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type II glass, or of a suitable plastic material, and store at room temperature, avoid excessive heat, and protect from freezing.

**Labeling**—The label states the total osmolar concentration in mOsmol per L.

**USP Reference standards** <11>—  
USP Dobutamine Hydrochloride RS  
USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**B:** It meets the requirements for the *Identification* test under *Dextrose*.

**Bacterial endotoxins** <85>—It contains not more than 5.56 USP Endotoxin Units per mg of dobutamine.

**pH** <791>: between 2.5 and 5.5.

**Particulate matter** <788>—It meets the requirements for large-volume injections.

**Chromatographic purity**—

*Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay under *Dobutamine Hydrochloride*.

**Test solution**—Transfer an accurately measured portion of Dobutamine in Dextrose Injection, equivalent to about

44.6 mg of dobutamine to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity, excluding 5-hydroxymethylfurfural from all calculations, in the portion of Dobutamine in Dextrose Injection taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 1.0% of any individual impurity is found, and not more than 2.0% of the total impurities is found.

#### Limit of 5-hydroxymethylfurfural—

**Ion-exchange column**—Fill an 8-mm chromatographic tube to a height of about 40 mm with a 100- to 200-mesh, strongly acidic, styrene-divinylbenzene cation-exchange resin. Wash the column with about 30 mL of water, discarding the eluate. [NOTE—Prepare a new column for each *Test solution* and *Blank solution*, and use each column only once.]

**Test solution**—Transfer 2 mL of Injection to the ion-exchange column, and collect the eluate in a 50-mL volumetric flask. Pass 25 mL of water through the column, and collect the eluate in the same volumetric flask. Dilute the eluate with water to volume, and mix. Remove the stopper from the flask, and allow the solution to stand for about 30 minutes in order to oxidize any bisulfite ions present. The solution so obtained is the *Test solution*.

**Blank solution**—Prepare a *Blank solution* in a similar manner to the *Test solution* by passing 27 mL of water through an ion-exchange column, and collecting the eluate in a 50-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—Determine the absorbance of the *Test solution* in a 1-cm cell at 284 nm, with a suitable spectrophotometer, after correcting for the *Blank solution*: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for dextrose**—Determine the angular rotation of Injection (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

#### Assay for dobutamine—

**Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system**—Proceed as directed in the Assay under *Dobutamine Hydrochloride*.

**Assay preparation**—Transfer an amount of Dobutamine in Dextrose Injection, equivalent to about 44.6 mg of dobutamine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Refrigerate until injected, and use within 8 hours.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into

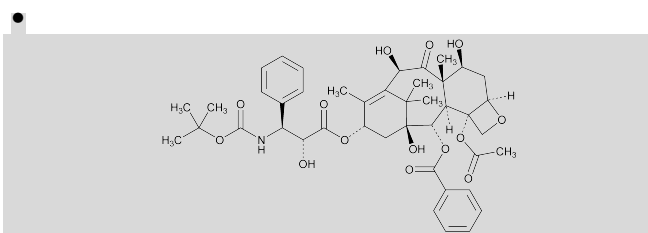
the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dobutamine ( $C_{18}H_{23}NO_3$ ) in the portion of Dobutamine in Dextrose Injection taken by the formula:

$$(301.39/337.84)100C(r_U / r_S)$$

in which 301.39 and 337.84 are the molecular weights of dobutamine and dobutamine hydrochloride, respectively;  $C$  is the concentration, in mg per mL, of USP Dobutamine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Docetaxel

### Change to read:



● (RB 1-May-2012)

$C_{43}H_{53}NO_{14} \cdot 3H_2O$

861.93

Anhydrous

807.88

Benzenepropanoic acid,  $\beta$ -[[[(1,1-dimethylethoxy)carbonyl]amino]- $\alpha$ -hydroxy-, 12b-(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester, ● (RB 1-May-2012) [2aR-[2a $\alpha$ ,4 $\beta$ ,4a $\beta$ ,6 $\beta$ ,9 $\alpha$ ( $\alpha$ R\*, $\beta$ S\*),11 $\alpha$ ,12 $\alpha$ ,12a $\alpha$ ,12b $\alpha$ ]]-; (2R,3S)-N-Carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5 $\beta$ ,20-epoxy-1,2 $\alpha$ ,4,7 $\beta$ ,10 $\beta$ ,13 $\alpha$ -hexahydroxytax-11-en-9-one 4-acetate 2-benzoate

● Trihydrate: [148408-66-6].

Anhydrous: [114977-28-5]. ● (RB 1-May-2012)

### DEFINITION

Docetaxel contains NLT 97.5% and NMT 102.0% of docetaxel ( $C_{43}H_{53}NO_{14}$ ), calculated on the anhydrous and solvent-free basis. [CAUTION—Docetaxel is cytotoxic. Great care should be taken to prevent inhaling particles of Docetaxel and exposing the skin to it.]

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197)

[NOTE—Methods described in *Infrared Absorption* (197K), (197M), or (197S) may be used. Use a solution containing 60 mg/mL of Docetaxel in methylene chloride for (197S).]

#### B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
9.0	72	28
39.0	28	72
39.1	72	28
50	72	28

**Diluent:** Acetonitrile, water, and acetic acid (100:100:0.1)

**System suitability solution:** 1 mg/mL of USP Docetaxel Identification RS in *Diluent*. [NOTE—USP Docetaxel Identification RS contains docetaxel and small amounts of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. See Table 2.]

**Standard solution:** 1.0 mg/mL made by transferring a quantity of USP Docetaxel RS to a suitable volumetric flask, dissolving in alcohol, equivalent to about 5% of the final volume, and diluting with *Diluent* to volume

**Sample solution:** 1.0 mg/mL made by transferring a quantity of Docetaxel to a suitable volumetric flask, dissolving in alcohol, equivalent to about 5% of the final volume, and diluting with *Diluent* to volume

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 232 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

**Temperatures**

Refrigerated autosampler: 10°

Column: 45°

Flow rate: 1.2 mL/min

Injection volume: 10 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 4 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of docetaxel (C<sub>43</sub>H<sub>53</sub>NO<sub>14</sub>) in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of docetaxel in the *Standard solution* (mg/mL)

$C_U$  = concentration of Docetaxel in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES**• **RESIDUE ON IGNITION** <281>: NMT 0.1%**Delete the following:**• **HEAVY METALS**, *Method I* <231>

**Sample solution:** Dissolve 1 g in 20 mL of a mixture of dimethylformamide and water (17:3). To 12 mL of this solution, add 2 mL of pH 3.5 Acetate Buffer, and mix.

Add 1.2 mL of thioacetamide–glycerin base TS, and mix.

**Acceptance criteria:** NMT 20 ppm • (IRA 1-May-2012)

**Add the following:**• **HEAVY METALS**

**Lead nitrate stock solution:** Prepare as directed in *Heavy Metals* <231>, *Special Reagents*.

**pH 3.5 acetate buffer:** Prepare as directed in *Heavy Metals* <231>, *Method I*.

**Diluent:** Dimethylformamide and water (17:3)

**Standard lead solution:** 1 μg/mL of lead in *Diluent* from the *Lead nitrate stock solution*, prepared on the day of use

**Test stock preparation:** Dissolve 1 g of Docetaxel in 20 mL of *Diluent*.

**Test preparation:** Place 12 mL of the *Test stock preparation* in a color-comparison tube, add 2 mL of pH 3.5 acetate buffer and 1.2 mL of thioacetamide–glycerin base TS, and mix.

**Monitor preparation:** Place 10 mL of the *Standard lead solution* in a color-comparison tube, add 2 mL of the *Test stock preparation*, and mix. Add 2 mL of pH 3.5 acetate buffer and 1.2 mL of thioacetamide–glycerin base TS, and mix.

**Blank preparation:** Place 10 mL of *Diluent* in a color-comparison tube, add 2 mL of the *Test stock preparation*, and mix. Add 2 mL of pH 3.5 acetate buffer and 1.2 mL of thioacetamide–glycerin base TS, and mix.

**Analysis:** Allow the *Test preparation*, *Monitor preparation*, and *Blank preparation* to stand for 2 min, and view downward over a white surface.

**Acceptance criteria:** Compared to the *Blank preparation*, the *Monitor preparation* shows a slight brown color. Any brown color in the *Test preparation* is not more intense than in the *Monitor preparation* (NMT 20 ppm) • (IRA 1-May-2012)

**Change to read:**• **ORGANIC IMPURITIES**, • **PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 1* is recommended if 10-deacetyl baccatin and 2-debenzoxyl 2-pentenoyl docetaxel are specified impurities. *Procedure 2* is recommended if O-BOC N-pyruvyl docetaxel is a specified impurity.] • (RB 1-May-2012)

**System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Sensitivity solution:** 0.5 μg/mL of USP Docetaxel RS in *Diluent* from the *Standard solution*

**System suitability**

**Samples:** *System suitability solution* and *Sensitivity solution*

**Suitability requirements**

**Resolution:** NLT 4 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*

**Signal-to-noise ratio:** NLT 10 for the docetaxel peak, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of the responses of all peaks from the *Sample solution*

$F$  = relative response factor for each individual impurity (see Table 2)

**Acceptance criteria:** See Table 2.

Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
• 10-Deacetyl baccatin (if present) <sup>a</sup>	0.25	1.5	0.15 • (RB 1-May-2012)
2-Debenzoxy-2-pentenyl docetaxel <sup>b</sup>	0.97	0.63	0.5
Docetaxel	1.00	—	—
6-Oxodocetaxel <sup>c</sup>	1.08	1.0	0.3
4-Epidocetaxel <sup>d</sup>	1.13	1.0	0.3
4-Epi-6-oxodocetaxel <sup>e</sup>	1.18	1.0	0.2
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate. • (RB 1-May-2012)

<sup>b</sup> (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-[(E)-2-methylbut-2-enoate], 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>c</sup> (2aR,4S,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>d</sup> (2aR,4R,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>e</sup> (2aR,4R,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

#### Add the following:

#### • ORGANIC IMPURITIES, PROCEDURE 2

**Solution A:** Water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
25	45	55
35	20	80
45	20	80
45.1	65	35
53	65	35

**Standard solution:** 5.0 µg/mL of USP Docetaxel RS in acetonitrile

**System suitability solution:** 1 mg/mL of USP Docetaxel System Suitability Mixture RS in acetonitrile. [NOTE—USP Docetaxel System Suitability Mixture RS contains docetaxel and a small amount of 6-oxodocetaxel, O-BOC N-pyruvyl docetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. See Table 4.]

**Sample solution:** 1.0 mg/mL of Docetaxel in acetonitrile

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

**Temperatures**

**Column:** 40°

**Sample:** 4°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** System suitability solution

#### Suitability requirements

**Resolution:** NLT 2.0 between O-BOC N-pyruvyl docetaxel and 4-epidocetaxel

**Tailing factor:** 0.8–1.5 for the docetaxel peak

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each individual impurity from the Sample solution

$r_S$  = peak area of docetaxel from the Standard solution

$C_S$  = concentration of USP Docetaxel RS in the Standard solution (mg/mL)

$C_U$  = concentration of Docetaxel in the Sample solution (mg/mL)

$F$  = relative response factor for each individual impurity (see Table 4)

**Acceptance criteria:** See Table 4.

Disregard any impurity peaks less than 0.05%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Docetaxel	1.0	—	—
6-Oxodocetaxel <sup>a</sup>	1.19	1.1	0.15
O-BOC N-pyruvyl docetaxel <sup>b</sup>	1.24	0.80	0.15
4-Epidocetaxel <sup>c</sup>	1.29	0.96	0.15
4-Epi-6-oxodocetaxel <sup>d</sup>	1.42	1.2	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.0

<sup>a</sup> (2aR,4S,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>b</sup> (2aR,4R,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-2-(tert-butoxycarbonyloxy)-3-(2-oxopropylamido)-3-phenylpropanoic acid.

<sup>c</sup> (2aR,4R,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>d</sup> (2aR,4R,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

• (RB 1-May-2012)

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial limit does not exceed  $10^2$  cfu/g. The total molds and yeasts count does not exceed 10 cfu/g.

**Change to read:**

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.4 • (RB 1-May-2012) USP Endotoxin Unit/mg.

**Change to read:**

- **WATER DETERMINATION, Method 1c** (921): 5.0%–7.0%. • If labeled as an anhydrous form: NMT 1.5%. • (RB 1-May-2012)

**Change to read:**

- **OPTICAL ROTATION, Specific Rotation** (781S)  
Sample solution: 10 mg/mL in methanol  
Acceptance criteria:  $-39^\circ$  to  $-41^\circ$  ( $t = 20^\circ$ ), calculated on the anhydrous and solvent-free basis. • If labeled as an anhydrous form:  $-35^\circ$  to  $-45^\circ$  ( $t = 20^\circ$ ), calculated on the as-is basis. • (RB 1-May-2012)

**ADDITIONAL REQUIREMENTS****Add the following:**

- **LABELING:** Where it is an anhydrous form, the label so indicates. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies. • (RB 1-May-2012)
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.

**Change to read:**

- **USP REFERENCE STANDARDS** (11)  
USP Docetaxel RS  
USP Docetaxel Identification RS  
It contains docetaxel and small amounts of the following:  
2-Debenzoxyl 2-pentenoyl docetaxel: (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-[(E)-2-methylbut-2-enoate], 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.  
 $C_{41}H_{55}NO_{14}$  785.87  
6-Oxodocetaxel: (2aR,4S,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.  
 $C_{43}H_{51}NO_{14}$  805.86  
4-Epidocetaxel: (2aR,4R,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.  
 $C_{43}H_{53}NO_{14}$  807.88  
4-Epi-6-oxodocetaxel: (2aR,4R,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.  
 $C_{43}H_{51}NO_{14}$  805.86

- USP Docetaxel System Suitability RS  
Contains docetaxel and a small amount of 6-oxodocetaxel, O-BOC N-pyruvyl docetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. • (RB 1-May-2012)  
USP Endotoxin RS

**Docetaxel Injection****DEFINITION**

Docetaxel Injection is a sterile solution of Docetaxel. It contains NLT 90.0% and NMT 110.0% of the labeled amount of docetaxel (anhydrous) ( $C_{43}H_{53}NO_{14}$ ). It contains polysorbate 80 and/or other suitable solubilizing agents in the infusion vehicle. It may also contain dehydrated alcohol.

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.4 mg/mL of USP Docetaxel RS in methylene chloride containing 1% (v/v) of polysorbate 80

**Sample solution:** 0.4 mg/mL of docetaxel (anhydrous) in methylene chloride from Injection

**Chromatographic system**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator

**Developing solvent system:** Methylene chloride and methanol (23:2)

**TLC tank:** Lined with filter paper

**Analysis:** After removing the plate from the tank, allow to dry in a fume hood, and view under UV light at 254 nm.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

**Solution A:** Water

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	72	28
9.0	72	28
39.0	28	72
39.1	0	100
49.0	0	100
49.1	72	28
60	72	28

**Diluent:** Acetonitrile, acetic acid, and water (100:0.1:100)

**System suitability solution:** 1 mg/mL of USP Docetaxel Identification RS in *Diluent*

**Standard solution:** 0.2 mg/mL of USP Docetaxel RS. Transfer USP Docetaxel RS into a suitable volumetric flask, and dissolve in alcohol equivalent to 5% of the final volume. Dilute with *Diluent* to volume.

**Sample solution** (for the Injection labeled as one-vial formulation): Dilute a portion of the Injection with *Diluent* to obtain a solution containing 0.2 mg/mL of docetaxel (anhydrous).

**Sample solution** (for the Injection labeled as two-vial formulation): Transfer the content of the vial containing the Injection concentrate to a suitable volumetric flask. Dissolve in an amount of alcohol equivalent to 5% of

the final volume, and dilute with *Diluent* to volume to obtain a solution having a concentration of 0.2 mg/mL of docetaxel (anhydrous).

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 232 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L1

**Temperatures**

**Refrigerated autosampler:** 10°

**Column:** 45°

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docetaxel (C<sub>43</sub>H<sub>53</sub>NO<sub>14</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Docetaxel RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of docetaxel (anhydrous) in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## IMPURITIES

### Change to read:

#### • ORGANIC IMPURITIES

**Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sensitivity solution:** 0.2 μg/mL of USP Docetaxel RS in *Diluent* from the *Standard solution*

#### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*

**Signal-to-noise ratio:** NLT 10 for the docetaxel peak, *Sensitivity solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak area of each individual impurity from the *Sample solution*

$r_T$  = sum of all of the peak areas from the *Sample solution*

$F$  = relative response factor for each individual impurity (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard any impurity peak less than 0.1% and any peak with a relative retention time less than 0.2 or greater than 1.3.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Deacetyl baccatin <sup>a</sup>	0.27	1.5	0.30
2-Debenzoxyl 2-pentenoyl docetaxel <sup>b</sup>	0.97	—	—
Docetaxel	1.00	—	—
Crotonaldehyde analog <sup>c</sup>	1.05	1.0	1.3
6-Oxodocetaxel <sup>d</sup>	1.08	1.0	1.5
4-Epidocetaxel <sup>e</sup>	1.13	1.0	1.0 • (RB 1-May-2012)
4-Epi-6-oxodocetaxel <sup>f</sup>	1.18	1.0	0.5
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.5

<sup>a</sup> (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate.

<sup>b</sup> (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-[(E)-2-methylbut-2-enolate], 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-epoxy-1,7β,10β-trihydroxy-9-oxotax-11-ene-2α,4,13α-triyl 4-acetate 13-[(2R,3S)-3-[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate] 2-[(2E)-2-methylbut-2-enolate]. It is a process impurity and is listed in *Table 2* for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the *Total Impurities*.

• (RB 1-May-2012)

<sup>c</sup> (1S,2S,3R,9S,E)-3-[(S,E)-2-Acetoxy-1-hydroxy-5-oxopent-3-en-2-yl]-1,5,9-trihydroxy-4,8,11,11-tetramethyl-6-oxobicyclo[5.3.1]undeca-4,7-dien-2-yl benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

<sup>d</sup> (2aR,4S,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

The alternative chemical name is 5β,20-epoxy-1,7β-dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2R,3S)-3-[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate]. • (RB 1-May-2012)

<sup>e</sup> (2aR,4R,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-epoxy-1,7α,10β-trihydroxy-9-oxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2R,3S)-3-[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate]. • (RB 1-May-2012)

<sup>f</sup> (2aR,4R,4aS,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine.

The alternative chemical name is 5β,20-epoxy-1,7α-dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2R,3S)-3-[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate]. • (RB 1-May-2012)

## SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 1.94 USP Endotoxin Units/mg of docetaxel (anhydrous).

• **STERILITY TESTS (71):** It meets the requirements when tested as directed in the *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections* (1)

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.

• **LABELING:** Label it to indicate whether it is a one-vial formulation or two-vial formulation (Injection concentrate)



and diluent), and also label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.

• **USP REFERENCE STANDARDS** <11>

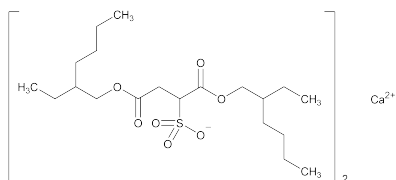
USP Docetaxel RS

USP Docetaxel Identification RS

[NOTE—USP Docetaxel Identification RS contains docetaxel and small amounts of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel.]

USP Endotoxin RS

## Docusate Calcium



$C_{40}H_{74}CaO_{14}S_2$  883.22

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, calcium salt;

1,4-Bis(2-ethylhexyl) sulfosuccinate, calcium salt [128-49-4].

### DEFINITION

Docusate Calcium contains NLT 91.0% and NMT 100.5% of docusate calcium ( $C_{40}H_{74}CaO_{14}S_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

• **A.**

**Sample:** Place a small piece of Docusate Calcium on a salt plate, add 1 drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.

**Acceptance criteria:** The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Calcium RS.

• **B.**

**Sample:** 25 mg

**Analysis:** Dissolve the *Sample* in 2 mL of acetone. Add 2 mL of water, and add 2 drops of sulfuric acid.

**Acceptance criteria:** A white precipitate is formed.

• **C. THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 10 mg/mL of USP Docusate Calcium RS in isopropyl alcohol

**Sample solution:** 10 mg/mL of Docusate Calcium in isopropyl alcohol

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10  $\mu$ L. [NOTE—Apply with the aid of a stream of nitrogen.]

**Developing solvent system:** Ethyl acetate, ammonium hydroxide, and alcohol (5:2:2)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Ex-

pose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

• **PROCEDURE**

**Solution A:** 2.500 g/L of tetra-*n*-butylammonium iodide in water

**Solution B:** A mixture of 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

**Sample:** 50 mg

**Analysis:** Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500  $\mu$ L of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in 2-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand for 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 2.989 mg of docusate calcium ( $C_{40}H_{74}CaO_{14}S_2$ ).

**Acceptance criteria:** 91.0%–100.5% on the anhydrous basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): 14.5%–16.5%, calculated on the anhydrous basis

• **HEAVY METALS**, *Method I* (231)

**Sample:** 2.0 g

**Analysis:** Transfer the *Sample* to a platinum crucible, and ignite until free from carbon. Cool, moisten the residue with 1 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Add 2 mL of 1 N acetic acid, digest on a steam bath for 5 min, filter into one of a pair of matched 50-mL, color-comparison tubes, and wash the residue with sufficient water to make 25 mL.

**Acceptance criteria:** NMT 10 ppm

• **LIMIT OF BIS(2-ETHYLHEXYL) MALEATE**

**Mobile phase:** Alcohol and water (78:22), filtered and degassed

**Standard solution:** 80  $\mu$ g/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

**Sample solution:** 20 mg/mL of Docusate Calcium in alcohol. [NOTE—If necessary, warm the mixture using the steam bath to achieve a complete dissolution.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  3-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 3  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Calcium taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

$r_s$  = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

$C_s$  = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Docusate Calcium in the  
Sample solution (mg/mL)

Acceptance criteria: NMT 0.4%

#### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 2.0%

- **CLARITY OF SOLUTION**

Sample solution: 25 g in 94 mL of alcohol

Acceptance criteria: The Sample solution does not develop a haze within 24 h when maintained at a temperature of  $25 \pm 1^\circ$ .

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Bis(2-ethylhexyl) Maleate RS
  - $C_{20}H_{36}O_4$  340.51
  - USP Docusate Calcium RS

### Docusate Calcium Capsules

#### DEFINITION

Docusate Calcium Capsules contain NLT 85.0% and NMT 115.0% of the labeled amount of docusate calcium ( $C_{40}H_{74}CaO_{14}S_2$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, Calcium (191)
  - Sample solution: Ash the contents of 1 Capsule, dissolve the residue in 10 mL of dilute hydrochloric acid (1 in 12), and filter.
  - Acceptance criteria: The filtrate meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHY**
  - Standard solution: 10 mg/mL of USP Docusate Calcium RS in isopropyl alcohol
  - Sample solution: Empty the contents of 1 Capsule into a conical flask, and add isopropyl alcohol to obtain a solution containing 10 mg/mL of docusate calcium.
  - Chromatographic system**  
(See Chromatography (621), Thin-Layer Chromatography.)
  - Mode: TLC
  - Adsorbent: 0.25-mm layer of chromatographic silica gel
  - Application volume: 10  $\mu$ L. [NOTE—Apply with the aid of a stream of nitrogen.]
  - Developing solvent system: Ethyl acetate, ammonium hydroxide, and alcohol (5:2:2)

#### Analysis

Samples: Standard solution and Sample solution

Allow the spots to dry, and develop the chromatogram in the Developing solvent system until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

Acceptance criteria: The  $R_f$  value of the principal spot of the Sample solution corresponds to that of the Standard solution.

#### ASSAY

- **PROCEDURE**

**Basic fuchsin solution:** Dissolve 100 mg of basic fuchsin, previously recrystallized from alcohol, in 3 mL of methanol in a 100-mL volumetric flask, and dilute with water to volume. Filter before use.

**Standard stock solution:** 5 mg/mL of USP Docusate Calcium RS in isopropyl alcohol

**Standard solution:** Transfer 5 mL of the Standard stock solution to a 500-mL volumetric flask containing 90 mL

of a mixture of water and isopropanol (1:1) with swirling. Dilute with water to volume. The concentration of USP Docusate Calcium RS in this solution is 50  $\mu$ g/mL.

**Sample stock solution:** To 10 Capsules in a 400-mL beaker add 300 mL of hot water. Heat on a steam bath, with occasional stirring, until the Capsules are completely disintegrated. Cool, and transfer with the aid of 100 mL of warm water to a 1000-mL volumetric flask. Rinse the beaker with 100 mL of isopropyl alcohol, add the rinsing to the volumetric flask, and shake to dissolve any previously undissolved particles. Dilute with a mixture of water and isopropyl alcohol (1:1) to volume.

**Sample solution:** Transfer 10.0 mL of the Sample stock solution to a 100-mL volumetric flask, and dilute with water to volume.

#### Instrumental conditions

(See Spectrophotometry and Light-Scattering (851).)

Mode: Vis

Analytical wavelength: 545 nm

Cell: 1 cm

Blank: Chloroform

**Analysis:** Transfer 4.0 mL of the Standard solution to a 125-mL separator. To a second 125-mL separator transfer a volume of the Sample solution, equivalent to 200–240  $\mu$ g of docusate calcium, and add a solution of isopropyl alcohol (1 in 100), if necessary, to bring the volume of the solution in the separator to 4.0 mL. To each separator transfer 20.0 mL of pH 1.2 Hydrochloric Acid Buffer (see Reagents, Indicators, and Solutions—Solutions) and 2.0 mL of Basic fuchsin solution. Add 20 mL of chloroform to each separator, and shake for 1 min. Allow the phases to separate, and transfer the chloroform extracts, through separate pledgets of absorbent cotton, into separate 100-mL volumetric flasks. Extract each of the solutions in the separators in the same manner with additional 20-mL portions of chloroform until no more color is visible in the extract, and pass each extract through the pledget of cotton used for the preceding extract into the flask containing the preceding extract. Dilute the contents of each flask to volume by passing chloroform through the cotton pledgets that had been used to filter the extracts, and mix.

Calculate the percentage of the labeled amount of docusate calcium ( $C_{40}H_{74}CaO_{14}S_2$ ) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the Sample solution

$A_S$  = absorbance of the Standard solution

$C_S$  = concentration of anhydrous USP Docusate Calcium RS in the Standard solution ( $\mu$ g/mL)

$C_U$  = nominal concentration of docusate calcium in the Sample solution ( $\mu$ g/mL)

Acceptance criteria: 85.0%–115.0%

#### PERFORMANCE TESTS

- **DISSOLUTION** (711)

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

**Analysis:** Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances:** The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

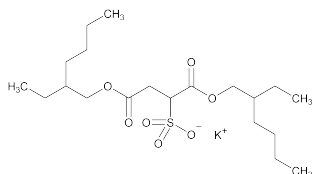
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for Content Uniformity for solid-filled capsules and

meet the requirements for *Weight Variation* for solution-filled capsules

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature in a dry place.
- **USP REFERENCE STANDARDS** <11>  
USP Docusate Calcium RS

## Docusate Potassium



$C_{20}H_{37}KO_7S$  460.67  
Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, potassium salt;  
Potassium 1,4-bis(2-ethylhexyl) sulfosuccinate [7491-09-0].

#### DEFINITION

Docusate Potassium contains NLT 95.0% and NMT 100.5% of docusate potassium ( $C_{20}H_{37}KO_7S$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A.**  
**Sample:** Place a small piece of Docusate Potassium on a salt plate, add 1 drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.  
**Acceptance criteria:** The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Potassium RS.
- **B. IDENTIFICATION TESTS—GENERAL, Potassium** <191>:  
Meets the requirements of the flame test

#### ASSAY

- **PROCEDURE**  
**Solution A:** 2.500 g/L of tetra-*n*-butylammonium iodide in water  
**Solution B:** A mixture of 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water  
**Sample:** 100 mg  
**Analysis:** Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500  $\mu$ L of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in 2-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand for 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 3.118 mg of docusate potassium ( $C_{20}H_{37}KO_7S$ ).  
**Acceptance criteria:** 95.0%–100.5% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** <281>: 18.0%–20.0%, calculated on the dried basis
- **HEAVY METALS, Method I** <231>  
**Sample:** 2.0 g  
**Analysis:** Transfer the *Sample* to a platinum crucible, and ignite until free from carbon. Cool, moisten the residue with 1 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Add 2 mL of 6 N acetic acid,

digest on a steam bath for 5 min, filter into one of a pair of matched 50-mL, color-comparison tubes, and wash the residue with sufficient water to make 25 mL.

**Acceptance criteria:** NMT 10 ppm

#### • LIMIT OF BIS(2-ETHYLHEXYL) MALEATE

**Mobile phase:** Alcohol and water (78:22), filtered and degassed

**Standard solution:** 80  $\mu$ g/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

**Sample solution:** 20 mg/mL of Docusate Potassium in alcohol. [NOTE—If necessary, warm the mixture using the steam bath to achieve a complete dissolution.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  3-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 3  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

$r_S$  = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

$C_S$  = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Docusate Potassium in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.4%

#### SPECIFIC TESTS

##### • LOSS ON DRYING <731>

**Analysis:** Dry the sample in a glass container at 105° for 4 h.

**Acceptance criteria:** NMT 3.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Bis(2-ethylhexyl) Maleate RS  
 $C_{20}H_{36}O_4$  340.51  
USP Docusate Potassium RS

## Docusate Potassium Capsules

#### DEFINITION

Docusate Potassium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate potassium ( $C_{20}H_{37}KO_7S$ ).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Diluent:** Transfer 180 mL of water to a 1000-mL volumetric flask, and dilute with methanol to volume.

**Tetrabutylammonium hydroxide solution:** 250 mg/mL of tetrabutylammonium hydroxide in methanol

**Mobile phase:** Mix 180 mL of water, 6.0 mL of glacial acetic acid, and 8.0 mL of *Tetrabutylammonium hydroxide solution* in a 1000-mL volumetric flask, and dilute with methanol to volume. The water concentration may be varied to meet *System suitability* requirements and to provide a suitable elution time (about 5 min) for docusate potassium.

**Standard solution:** 4 mg/mL of USP Docusate Sodium RS, calculated on the anhydrous basis, in *Diluent*

**Sample solution**

**Solid-filled capsules:** Open and empty into a suitable container the contents of a counted number of Capsules, nominally equivalent to 1000 mg of docusate potassium. Place the Capsule shells in the container, and add 250.0 mL of *Diluent*. Shake by mechanical means for 20 min, and clarify a portion of the mixture by centrifuging. Pass a portion of the clear supernatant through a membrane filter of 0.5- $\mu$ m or finer pore size.

**Solution-filled capsules:** Cut open a counted number of Capsules, nominally equivalent to 2.5 g of docusate potassium, and place the shells and contents in a suitable container. Add 25 mL of methanol, agitate for NLT 2 min, and decant the liquid into a 200-mL volumetric flask. Repeat the addition of methanol, agitation, and decanting NLT four times. Dilute with methanol to volume. Pipet 8 mL of this solution into a 25-mL volumetric flask, add 4.5 mL of water, and dilute with methanol to volume.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.8 mL/min

**Injection volume:** 100  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 3.0% for five replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate potassium ( $C_{20}H_{37}KO_7S$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of anhydrous USP Docusate Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of docusate potassium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of docusate potassium, 460.67

$M_{r2}$  = molecular weight of docusate sodium, 444.56

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISSOLUTION** <711>

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Analysis:** Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances:** The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Content Uniformity* for solid-filled capsules and meet the requirements for *Weight Variation* for solution-filled capsules

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Docusate Potassium RS

USP Docusate Sodium RS

## Docusate Sodium

$C_{20}H_{37}NaO_7S$  444.56

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt;

Sodium 1,4-bis(2-ethylhexyl) sulfosuccinate [577-11-7].

**DEFINITION**

Docusate Sodium contains NLT 99.0% and NMT 100.5% of  $C_{20}H_{37}NaO_7S$ , calculated on the anhydrous basis.

**IDENTIFICATION**

• **PROCEDURE**

**Sample:** Place a small piece of Docusate Sodium on a salt plate, add one drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.

**Acceptance criteria:** The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Sodium RS.

**ASSAY**

• **PROCEDURE**

**Solution A:** 2.5 g/L of tetra-*n*-butylammonium iodide in water

**Solution B:** A mixture of 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

**Sample:** 50 mg

**Analysis:** Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500  $\mu$ L of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in two-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 3.009 mg of  $C_{20}H_{37}NaO_7S$ .

**Acceptance criteria:** 99.0%–100.5% on the anhydrous basis

**IMPURITIES**

**Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: Between 15.5% and 16.5%, calculated on the anhydrous basis

**Procedure:** Transfer about 1 g, accurately weighed, to a tared crucible, ignite until thoroughly charred, and cool. Moisten the ash with 1 mL of sulfuric acid, and complete the ignition by heating at  $800 \pm 25^\circ$  for 15-min periods to constant weight.

- **HEAVY METALS** <231>

**Sample:** 2.0 g

**Analysis:** Transfer the *Sample* to a platinum crucible, and ignite until free from carbon. Cool, moisten the residue with 1 mL of hydrochloric acid, and evaporate on a steam bath to dryness. Add 2 mL of 6 N acetic acid, digest on a steam bath for 5 min, filter into one of a pair of matched 50-mL, color-comparison tubes,

and wash the residue with sufficient water to make 25 mL.

Acceptance criteria: NMT 10 ppm

#### Organic Impurities

##### • PROCEDURE: LIMIT OF BIS(2-ETHYLHEXYL) MALEATE

**Mobile phase:** Alcohol and water (78:22), filtered and degassed

**Standard solution:** 80 µg/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

**Sample solution:** 20 mg/mL of Docusate Sodium in alcohol. [NOTE—If necessary, warm the mixture using the steam bath to achieve a complete dissolution.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 3-cm; 3.5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 3 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

$r_S$  = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

$C_S$  = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Docusate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4%

#### SPECIFIC TESTS

##### • WATER DETERMINATION, Method I <921>: NMT 2.0%

##### • CLARITY OF SOLUTION

**Sample solution:** 25 g in 100 mL of alcohol

**Acceptance criteria:** The *Sample solution* does not develop a haze within 24 h.

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS <11>

USP Bis(2-ethylhexyl) Maleate RS

$C_{20}H_{36}O_4$  340.51

USP Docusate Sodium RS

## Docusate Sodium Capsules

#### DEFINITION

Docusate Sodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ).

#### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium dihydrogen phosphate (66:34)

**Diluent:** Acetonitrile and water (50:50)

**Standard solution:** 0.1 mg/mL of USP Docusate Sodium RS in *Diluent*. Filter the solution, discarding the first 6 mL of the filtrate.

**Sample stock solution:** Transfer a number of Capsules, equivalent to 250 mg of docusate sodium, to a 250-mL volumetric flask, and add 50 mL of water. Heat the mixture with occasional swirling until the Capsule shells have ruptured and dissolved. [NOTE—Take special care to ensure that all of the Capsules have ruptured.] Remove from heat, and add 50 mL of acetonitrile. Allow this solution to cool to room temperature, and dilute with *Diluent* to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and filter, discarding the first 6 mL of filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm × 15-cm; packing L1 that has been highly deactivated (carbon loading of 30%)

**Flow rate:** 1.5 mL/min

**Injection volume:** 25 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of anhydrous docusate sodium in the *Standard solution*, as determined from the concentration of USP Docusate Sodium RS corrected for moisture by a titrimetric water determination (mg/mL)

$C_U$  = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Analysis:** Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances:** The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* for solid-filled capsules and meet the requirements for *Weight Variation* for solution-filled capsules

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Docusate Sodium RS

**Docusate Sodium Solution****DEFINITION**

Docusate Sodium Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium dihydrogen phosphate (66:34)

**Diluent:** Acetonitrile and water (50:50)

**Standard solution:** 0.1 mg/mL of USP Docusate Sodium RS in *Diluent*. Filter the solution, discarding the first 6 mL of the filtrate.

**Sample stock solution:** Transfer a volume of Solution, equivalent to 100 mg of docusate sodium, to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and filter, discarding the first 6 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm × 15-cm; packing L1 that has been highly deactivated (carbon loading of 30%)

**Flow rate:** 1.5 mL/min

**Injection volume:** 25 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ) in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of anhydrous docusate sodium in the *Standard solution*, as determined from the concentration of USP Docusate Sodium RS corrected for moisture by a titrimetric water determination (mg/mL)

$C_U$  = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** <791>: 4.5–6.9

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
USP Docusate Sodium RS

**Docusate Sodium Syrup****DEFINITION**

Docusate Sodium Syrup contains NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ).

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 2 mg/mL of USP Docusate Sodium RS in isopropyl alcohol

**Sample solution:** Dilute a volume of Syrup, equivalent to about 10 mg of docusate sodium, with isopropyl alcohol to obtain a preparation containing about 2 mg/mL, and mix. Use the upper layer of this preparation.

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 50 µL. [NOTE—Apply with the aid of a stream of nitrogen.]

**Developing solvent system:** Ethyl acetate, alcohol, water, and ammonium hydroxide (25:10:20:1). [NOTE—This is a two-phase system.]

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system*, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

**Acceptance criteria:** The *Sample solution* produces a spot at the same  $R_f$  value and of approximately the same size as that obtained from the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Standard stock solution:** 1.0 mg/mL of USP Docusate Sodium RS, first dissolved in alcohol using about 2.5% of the final volume, and then diluted with water to volume. This solution contains 1 mg/mL of USP Docusate Sodium RS.

**Standard solution:** 10 µg/mL in water from the *Standard stock solution*

**Sample solution:** Transfer an accurately measured volume of Syrup, equivalent to about 100 mg of docusate sodium, to a 1000-mL volumetric flask, allowing the pipet to drain for 15 min. Dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Vis

**Analytical wavelength:** 650 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Transfer 20.0 mL each of the *Standard solution* and the *Sample solution* to two individual separators. Place

20 mL of water in a third separator (*Blank solution*). To each separator add 5 drops of hydrochloric acid, mix by swirling, add 1.0 mL of methylene blue solution (1 in 1000), and mix by swirling. To each separator add 20.0 mL of chloroform, and shake vigorously for 5 min. Wash each chloroform solution, in clean separators, with 20 mL of water, shaking vigorously for 60 s. Discard the washings, and filter each chloroform solution through a layer of 3 g of anhydrous granular sodium sulfate, supported on glass wool, into a 100-mL volumetric flask, washing each separator with two 10-mL portions of chloroform, and filtering the washings into each flask. Dilute each flask with chloroform to volume. Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*, using the *Blank solution* to set the instrument. Calculate the percentage of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ) in the portion of Syrup taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Docusate Sodium RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of docusate sodium in the *Sample solution* ( $\mu\text{g/mL}$ )  
**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **PH** (791): 5.5–6.5

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Docusate Sodium RS

### Docusate Sodium Tablets

#### DEFINITION

Docusate Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)  
**Sample:** Finely divide a suitable number of Tablets, extract with solvent hexane, filter, and evaporate the solvent hexane extract on a steam bath. Use the dry residue.  
**Acceptance criteria:** Meet the requirements

#### ASSAY

- **PROCEDURE**  
**Mobile phase:** Acetonitrile and 7 mM ammonium acetate (1:1)  
**Standard solution:** Dissolve the USP Docusate Sodium RS in alcohol, and dilute with water to obtain a solution containing 1.0 mg/mL of USP Docusate Sodium RS.  
**Methylparaben solution:** 0.15 mg/mL of methylparaben in water  
**System suitability solution:** Mix 0.1 mL of *Methylparaben solution* and 10 mL of *Standard solution*.  
**Sample solution:** Transfer 10 Tablets to a 1-L volumetric flask, add 200 mL of alcohol and 300 mL of water, and shake by mechanical means for NLT 90 min to completely disintegrate the Tablets. Dilute with water to volume, and filter, discarding the first 3 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC  
**Detector:** UV 210 nm  
**Column:** 4.6-mm  $\times$  10-cm; packing L1  
**Column temperature:** 40°  
**Flow rate:** 1 mL/min  
**Injection volume:** 40  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*  
 [NOTE—The relative retention times for methylparaben and docusate are about 0.74 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between methylparaben and docusate, *System suitability solution*  
**Tailing factor:** NMT 2.5, *Standard solution*  
**Relative standard deviation:** NMT 1.8%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Docusate Sodium RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0%

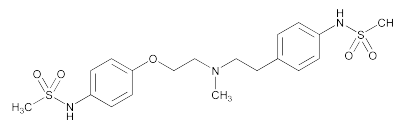
#### PERFORMANCE TESTS

- **DISINTEGRATION** (701)  
**Medium:** Proceed as directed in the chapter, except substitute simulated gastric fluid TS for water in the test for *Uncoated Tablets*.  
**Time:** 1 h  
**Acceptance criteria:** Meet the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Docusate Sodium RS

### Dofetilide



$C_{19}H_{27}N_3O_5S_2$  441.56  
 Methanesulfonamide, N-[4-[2-[methyl[2-[4-[(methylsulfonyl)amino]phenoxy]ethyl]amino]ethyl]phenyl]-;  
 $\beta$ -[(p-Methanesulfonamidophenethyl)methylamino]methanesulfono-p-phenetidine [115256-11-6].

#### DEFINITION

Dofetilide contains NLT 97.0% and NMT 103.0% of  $C_{19}H_{27}N_3O_5S_2$ , calculated on the anhydrous, solvent-free basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197K>
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Potassium hydroxide solution:** 0.56 g/mL of potassium hydroxide

**Buffer:** 1.36 g of monobasic potassium phosphate and 5 mg of ascorbic acid in 1 L of water. Adjust with *Potassium hydroxide solution* to a pH of 7.0.

**Mobile phase:** Acetonitrile and *Buffer* (1:3)

**System suitability solution:** 25 µg/mL of USP Dofetilide RS and 0.5 µg/mL of USP Dofetilide Related Compound A RS in *Mobile phase*

**Standard solution:** 25 µg/mL of USP Dofetilide RS in *Mobile phase*

**Sample solution:** 25 µg/mL of Dofetilide in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L7

**Temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 8.0 between dofetilide and dofetilide related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>19</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub> in the portion of Dofetilide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of dofetilide from the *Sample solution*

$r_S$  = peak response of dofetilide from the *Standard solution*

$C_S$  = concentration of dofetilide in the *Standard solution* (µg/mL)

$C_U$  = concentration of Dofetilide in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–103.0% on the anhydrous and solvent-free basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS, Method II** <231>: NMT 20 ppm

**Organic Impurities**• **PROCEDURE**

**Buffer:** 0.78 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1.

**Diluent:** Acetonitrile and *Buffer* (3:22)

**Solution A:** *Buffer*

**Solution B:** Methanol

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	88	12
5	88	12
25	70	30
30	70	30

**System suitability solution:** 1.25 µg/mL each of USP Dofetilide RS and USP Dofetilide Related Compound A RS in *Diluent*

**Standard solution:** 1.25 µg/mL of USP Dofetilide RS in *Diluent*

**Diluted standard solution:** 0.125 µg/mL of USP Dofetilide RS in *Diluent* from the *Standard solution*

**Sample solution:** 0.25 mg/mL of Dofetilide in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 3.9-mm × 15-cm; 5-µm packing L1

**Temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

**System suitability**

**Samples:** *System suitability solution* and *Diluted standard solution*

**System suitability requirements**

**Resolution:** NLT 5.0 between dofetilide and dofetilide related compound A, *System suitability solution*

**Column efficiency:** NLT 35,000 theoretical plates for the dofetilide peak, *System suitability solution*

**Tailing factor:** NMT 1.5 for the dofetilide peak, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Diluted standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Dofetilide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of dofetilide from the *Standard solution*

$C_S$  = concentration of USP Dofetilide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Dofetilide in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dofetilide related compound A <sup>a</sup>	0.9	1.04	0.5
Dofetilide	1.0	—	—
Any other individual unspecified impurity	—	1.00 <sup>b</sup>	0.1
Total impurities	—	—	0.5

<sup>a</sup> N-[4-(2-(2-[4-(Methanesulfonamido)phenoxy]ethylamino)ethyl)phenyl]methanesulfonamide.

<sup>b</sup> Unless otherwise determined.

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** <921>: NMT 1.0%

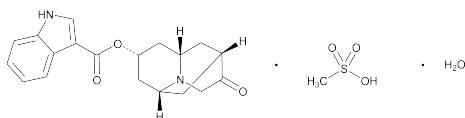
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.



- **USP REFERENCE STANDARDS** <11>
  - USP Dofetilide RS
  - USP Dofetilide Related Compound A RS
  - N*-[4-(2-[2-[4-(Methanesulfonamido)phenoxy]ethylamino]ethyl)phenyl]methanesulfonamide.
  - 427.54

## Dolasetron Mesylate



$C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$  438.49  
 1*H*-Indole-3-carboxylic acid, octahydro-3-oxo-2,6-methano-2*H*-quinolizin-8-yl ester, (2 $\alpha$ ,6 $\alpha$ ,8 $\alpha$ ,9 $\alpha\beta$ )-, monomethanesulfonate monohydrate;  
 Indole-3-carboxylic acid, ester with (8*r*)-hexahydro-8-hydroxy-2,6-methano-2*H*-quinolizin-3(4*H*)-one, monomethanesulfonate monohydrate [115956-13-3].

### DEFINITION

Dolasetron Mesylate contains NLT 98.0% and NMT 102.0% of  $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ , calculated on the as-is basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B. PROCEDURE**

**Sample solution:** 1 mg/mL

**Analysis:** Transfer 5–10 mg of 5,5'-methylenedisalicylic acid to a clean crucible, and heat in an oven at 150° for 5 min. Remove from the oven, and add 10 drops of the *Sample solution*. Return to the oven, and evaporate to dryness.

**Acceptance criteria:** A red or pink color (presence of methanesulfonic acid) develops in the white residue.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, water, and 1 M ammonium formate (450:440:110), adding 0.19 mL of triethylamine to the acetonitrile portion

**Standard solution:** 0.04 mg/mL and 0.004 mg/mL respectively of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*

**Sample solution:** 0.04 mg/mL of Dolasetron Mesylate in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 285 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 4 between indole-3-carboxylic acid and dolasetron mesylate

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 1.5% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$  in the Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of USP Dolasetron Mesylate RS in the *Standard solution* (mg/mL)
- $C_U$  = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the as-is basis

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**0.01 M Dibasic ammonium phosphate solution:** 1.32 g/L of dibasic ammonium phosphate. Adjust with 2.0 M phosphoric acid to a pH of 7.0.

**Diluent:** Acetonitrile and water (1:4)

**Solution A:** Acetonitrile and 0.01 M *Dibasic ammonium phosphate solution* (53:1000), filtered and degassed

**Solution B:** Acetonitrile and 0.01 M *Dibasic ammonium phosphate solution* (795:295), filtered and degassed

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
28	0	100
38	0	100
40	100	0
50	100	0

**System suitability solution:** 0.004 mg/mL and 0.03 mg/mL, respectively, of indole and USP Dolasetron Mesylate RS in *Diluent*

**Standard solution A:** 0.03 mg/mL of USP Dolasetron Mesylate RS in *Diluent*

**Standard solution B:** 6 mg/mL and 0.0072 mg/mL, respectively, of USP Dolasetron Mesylate RS and USP Dolasetron Mesylate Related Compound A RS in *Diluent*

**Sample solution:** 6 mg/mL of Dolasetron Mesylate in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 1.5 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

##### Suitability requirements

**Resolution:** NLT 1.5 between the first eluting peak, indole, and the second eluting peak, dolasetron mesylate from the *System suitability solution*. [NOTE— If the dolasetron mesylate peak is found to elute before the indole peak, condition the column as follows: fill up the column with *Solution A*, plug the column, and place the column in a convection oven at 105° for about 16 h. Retest the column.]

**Relative standard deviation:** NMT 5.0% for replicate injections of *Standard solution A*

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of dolasetron mesylate related compound A in the Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of dolasetron mesylate related compound A from the *Sample solution*

$r_S$  = peak response of dolasetron mesylate related compound A from the *Standard solution B*

$C_S$  = concentration of USP Dolasetron Mesylate Related Compound A RS in the *Standard solution B* (mg/mL)

$C_U$  = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of dolasetron mesylate related compound A base, 181.2

$M_{r2}$  = molecular weight of dolasetron mesylate related compound A hydrochloride, 217.8

Calculate the percentage of each impurity (other than dolasetron mesylate related compound A) in the portion of Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of dolasetron mesylate from the *Standard solution A*

$C_S$  = concentration of USP Dolasetron Mesylate RS in the *Standard solution A* (mg/mL)

$C_U$  = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** NMT 0.1%

**Total impurities:** NMT 0.3%

[NOTE—The reporting level for impurities is 0.05%.]

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method Ia (921):** Between 3.5% and 4.7%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **USP REFERENCE STANDARDS (11)**
  - USP Dolasetron Mesylate RS
  - USP Dolasetron Mesylate Related Compound A RS
  - Hexahydro-8-hydroxy-2,6-methano-2H-quinolizin-3 (4H)-one, hydrochloride.

### Dolasetron Mesylate Injection

» Dolasetron Mesylate Injection is a sterile solution, suitable for intravenous administration, containing Dolasetron Mesylate in a buffer solution. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ ).

**Packaging and storage**—Preserve in a single-dose container, protected from light. Store at controlled room temperature.

**Labeling**—Label it to indicate that it may be diluted with a suitable parenteral vehicle prior to intravenous infusion.

#### USP Reference standards (11)—

USP Dolasetron Mesylate RS

USP Endotoxin RS

#### Identification, Infrared Absorption (197K)—

**Test specimen**—Transfer a portion of Injection, equivalent to about 100 mg of dolasetron mesylate, to a 150-mL beaker. Add about 20 mL of water and 10 mL of a sodium hydroxide solution (1 in 10). Mix, and allow to stand at room temperature for 30 minutes. Pass through a filtering crucible with fritted disk having a medium porosity, using about 100 mL of water to aid in the transfer. Dry the precipitate in a vacuum oven at 105° for 4 hours. Prepare a 1.5% mixture of the dried powder with potassium bromide.

**Bacterial endotoxins (85)**—It contains not more than 2.7 USP Endotoxin Units per mg of dolasetron mesylate.

**pH (791):** between 3.2 and 3.8.

**Particulate matter (788):** meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Proceed as directed in the *Assay* under *Dolasetron Mesylate*.

**System suitability preparation**—Dissolve accurately weighed quantities of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1 mg per mL and 0.02 mg per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dolasetron Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Using a “to contain” pipet, transfer 2.5 mL of Injection to a 50-mL volumetric flask. Rinse the pipet with several portions of *Mobile phase*, and collect the rinses in the same flask. Dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the *Assay* under *Dolasetron Mesylate*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between indole-3-carboxylic acid and dolasetron mesylate is not less than 4; and the tailing factor for the dolasetron mesylate peak is not more than 1.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ ) in each mL of Injection taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dolasetron Mesylate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Dolasetron Mesylate Oral Solution

#### DEFINITION

Dolasetron Mesylate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$ ).

Prepare Dolasetron Mesylate Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Dolasetron Mesylate powder	1 g
Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , a sufficient quantity to make	100 mL

Add *Dolasetron Mesylate powder* and 15 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix.

**ASSAY****• PROCEDURE**

**Solution A:** 0.05 M ammonium acetate adjusted with diluted ammonium hydroxide to a pH of 7.5

**Mobile phase:** Acetonitrile and *Solution A* (24:76). Filter, and degas.

**Diluent:** Acetonitrile and water (24:76)

**Standard stock solution:** 500 µg/mL of USP Dolasetron Mesylate RS in *Diluent*

**Standard solution:** 10 µg/mL of USP Dolasetron Mesylate RS from *Standard stock solution* in *Mobile phase*

**Sample solution:** 10 µg/mL of dolasetron mesylate prepared from Oral Solution and *Diluent*. Shake each sample thoroughly by hand for 15 s, centrifuge at 1000 rpm for 2 min, and use the supernatant.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L10

**Column temperature:** 30°

**Flow rate:** 0.8 mL/min

**Injection volume:** 5 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for dolasetron mesylate is about 6.9 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.4% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of dolasetron mesylate in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of dolasetron mesylate in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH <791>:** 3.6–4.6

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.

**• LABELING:** Label it to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded

**• USP REFERENCE STANDARDS <11>**

USP Dolasetron Mesylate RS

**Dolasetron Mesylate Oral Suspension****DEFINITION**

Dolasetron Mesylate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$ ).

Prepare Dolasetron Mesylate Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Dolasetron Mesylate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

If using tablets, place the required number in a suitable mortar, and comminute to a fine powder, or add *Dolasetron Mesylate powder* to the mortar. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions, and mix well after each addition. Transfer, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Solution A:** 0.05 M ammonium acetate adjusted with diluted ammonium hydroxide to a pH of 7.5

**Mobile phase:** Acetonitrile and *Solution A* (24:76). Filter and degas.

**Diluent:** Acetonitrile and water (24:76)

**Standard stock solution:** 500 µg/mL of USP Dolasetron Mesylate RS in *Diluent*

**Standard solution:** 10 µg/mL of USP Dolasetron Mesylate RS prepared from the *Standard stock solution* in *Mobile phase*

**Sample solution:** 10 µg/mL of dolasetron mesylate prepared from Oral Suspension and *Diluent*. Shake each sample thoroughly by hand for 15 s, centrifuge at 1000 rpm for 2 min, and use the supernatant.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L10

**Column temperature:** 30°

**Flow rate:** 0.8 mL/min

**Injection volume:** 5 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for dolasetron mesylate is about 6.9 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.4% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of dolasetron mesylate in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of dolasetron mesylate in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH <791>:** 3.6–4.6

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.

- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded
- **USP REFERENCE STANDARDS** <11>  
USP Dolasetron Mesylate RS

## Dolasetron Mesylate Tablets

» Dolasetron Mesylate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ ).

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**USP Reference standards** <11>—  
USP Dolasetron Mesylate RS

**Identification, Infrared Absorption** <197K>—

*Test specimen*—Transfer a quantity of powdered Tablets, equivalent to about 200 mg of dolasetron mesylate, to a capped tube. Add 10 mL of acetonitrile, and shake for about 5 minutes. Allow to settle, filter, and collect the filtrate in a glass vial. Evaporate the solvent at 85°. Add an additional 5 mL of acetonitrile to the vial, and evaporate at 85°. Then evaporate to dryness in a vacuum oven at 80°. Mix about 2 mg of the crystals obtained with about 300 mg of potassium bromide.

**Dissolution** <711>—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Dolasetron Mesylate RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

*Mobile phase*—Proceed as directed in the Assay under *Dolasetron Mesylate*.

*System suitability preparation*—Dissolve accurately weighed quantities of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1 mg and 0.05 mg per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dolasetron Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer 10 Tablets to a 500-mL volumetric flask, add about 400 mL of *Mobile phase*, and stir vigorously with a magnetic stirrer for at least 40 minutes to dissolve. Dilute with *Mobile phase* to volume, mix, and allow the insoluble excipients to settle. Quantitatively dilute a portion of the supernatant with *Mobile phase* to obtain a solution having a concentration of about 0.1 mg of dolasetron mesylate per mL.

*Chromatographic system* (see *Chromatography* <621>)—Prepare as directed in the Assay under *Dolasetron Mesylate*. Chromatograph the *System suitability preparation*, and re-

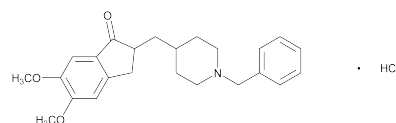
cord the peak responses as directed for *Procedure*: the resolution, *R*, between indole-3-carboxylic acid and dolasetron mesylate is not less than 4; and the tailing factor for the dolasetron mesylate peak is not more than 1.8. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dolasetron mesylate ( $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ ) in each Tablet taken by the formula:

$$(TC/D)(r_U / r_S)$$

in which *T* is the labeled quantity, in mg, of dolasetron mesylate in the Tablet; *C* is the concentration, in mg per mL, of USP Dolasetron Mesylate RS in the *Standard preparation*; *D* is the concentration, in mg per mL, of dolasetron mesylate in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Donepezil Hydrochloride



$C_{24}H_{29}NO_3 \cdot HCl$  415.95  
(±)-2-[(1-Benzyl-4-piperidyl)methyl]-5,6-dimethoxy-1-indanone hydrochloride [120011-70-3].

### DEFINITION

Donepezil Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{24}H_{29}NO_3 \cdot HCl$ , calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION <197K>

[NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the USP Donepezil Hydrochloride RS separately in dichloromethane, evaporate to dryness, and record new spectra using the residues.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### • C. IDENTIFICATION TESTS—GENERAL, Chloride <191>

**Sample solution:** 10 mg/mL

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Buffer:** 3.9 g/L of sodium 1-decane sulfonate in water  
**Mobile phase:** Acetonitrile and *Buffer* (35:65). Adjust with perchloric acid to a pH of 1.8.

**System suitability solution:** 0.4 mg/mL of USP Donepezil Hydrochloride RS and 0.016 mg/mL of USP Donepezil Related Compound A RS prepared as follows. Dissolve suitable quantities of USP Donepezil Hydrochloride RS and USP Donepezil Related Compound A RS using 40% of the flask volume of methanol, and dilute with water to volume.

**Standard solution:** 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.4 mg/mL of Donepezil Hydrochloride in *Mobile phase*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 271 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 35°**Flow rate:** 1.4 mL/min**Injection size:** 20 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*[NOTE—Refer to *Table 1* under *Organic Impurities, Procedure 1* for the relative retention times.]**Suitability requirements****Resolution:** NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of donepezil hydrochloride (C<sub>24</sub>H<sub>29</sub>NO<sub>3</sub> · HCl) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of donepezil hydrochloride from the *Sample solution* $r_S$  = peak response of donepezil hydrochloride from the *Standard solution* $C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES**

- **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if any of the impurities included in *Table 3* are potential related compounds.]**Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.**Standard solution:** 0.8 μg/mL of USP Donepezil Hydrochloride RS in *Mobile phase***System suitability****Samples:** *System suitability solution* and *Standard solution*[NOTE—Refer to *Table 1* for the relative retention times.]**Suitability requirements****Resolution:** NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Donepezil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of any individual impurity from the *Sample solution* $r_S$  = peak response of donepezil hydrochloride from the *Standard solution* $C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desbenzyl donepezil <sup>a</sup>	0.33	0.2
Hydroxydonepezil <sup>b</sup>	0.54	0.2
Donepezil related compound A <sup>c</sup>	0.92	0.1
Donepezil hydrochloride	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.<sup>b</sup> 2-[(1-Benzylpiperidin-4-yl)(hydroxy)methyl]-5,6-dimethoxyindan-1-one.<sup>c</sup> (E)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.**• ORGANIC IMPURITIES, PROCEDURE 2****Diluent:** Acetonitrile and water (25:75)**Solution A:** Add 1 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a filter of 0.45-μm or finer pore size.**Solution B:** Acetonitrile**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	40	60
40	40	60
41	75	25
50	75	25

**Standard solution:** 0.01 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Sonication may be used to aid the dissolution.**Sample solution:** 1.0 mg/mL of Donepezil Hydrochloride in *Diluent*. Sonication may be used to aid the dissolution.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 286 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 50°**Flow rate:** 1.5 mL/min**Injection size:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 40,000 theoretical plates**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%, for five replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Donepezil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of any individual impurity from the *Sample solution* $r_S$  = peak response of donepezil hydrochloride from the *Standard solution* $C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

- $C_U$  = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)  
 $F$  = relative response factor for the corresponding impurity peak from Table 3

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time*	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil <sup>a</sup>	0.23	1.5	0.15
Donepezil pyridine analog (DPMI) <sup>b</sup>	0.49	1.9	0.15
Donepezilbenzyl bromide <sup>c</sup>	0.68	0.73	0.15
Donepezil hydrochloride	1.0	1.0	—
Dehydrodeoxy donepezil <sup>d</sup>	1.72	2.0	0.15
Deoxydonepezil <sup>e</sup>	2.12	0.67	0.15
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

\* Relative retention times are based on 1-mL gradient delay volume.

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.

<sup>b</sup> 5,6-Dimethoxy-2-(pyridin-4-ylmethyl)indan-1-one.

<sup>c</sup> 1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidinium.

<sup>d</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine.

<sup>e</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine.

### SPECIFIC TESTS

- **WATER DETERMINATION, Method 1a (921):** NMT 0.4% for the anhydrous form; NMT 7.0% for the monohydrate form

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** Label to indicate whether it is the monohydrate form. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS (11)**  
 USP Donepezil Hydrochloride RS  
 USP Donepezil Related Compound A RS  
 (E)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.  
 $C_{24}H_{27}NO_3$  377.48

## Donepezil Hydrochloride Tablets

### DEFINITION

Donepezil Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of donepezil hydrochloride ( $C_{24}H_{29}NO_3 \cdot HCl$ ).

### IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**  
 Wavelength range: 220–360 nm  
 Sample solution: Crush a suitable number of Tablets, and transfer an amount of powder, equivalent to 10 mg of donepezil hydrochloride, to a 100-mL volumetric flask. Add 80 mL of 0.1 N hydrochloric acid, and sonicate for 5 min. Cool the solution to room temperature, and dilute with 0.1 N hydrochloric acid to volume. Transfer a portion of this solution to a centrifuge tube, and centrifuge for 15 min. Transfer 5 mL of the clear

supernatant to a 25-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

**Analysis:** Using a 1-cm cell, record the UV spectrum of the *Sample solution*.

**Acceptance criteria:** The solution exhibits absorption maxima at 230, 271, and 315 nm.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Diluent:** Methanol and 0.1 N hydrochloric acid (3:1)

**Mobile phase:** Dissolve 2.5 g of sodium decanesulfonate in 650 mL of water, and add 1.0 mL of perchloric acid and 350 mL of acetonitrile. If necessary, adjust with an additional 0.5 mL of perchloric acid to a pH of about 1.8.

**System suitability solution:** 0.2 mg/mL of USP

Donepezil Hydrochloride RS and 0.008 mg/mL of USP Donepezil Related Compound A RS. [NOTE—Dissolve in 40% of the flask volume of methanol, swirl, and dilute with water to volume.]

**Standard solution:** 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. [NOTE—Dissolve in 60% of the flask volume of *Diluent*, swirl, and dilute with *Diluent* to volume.]

**Sample solution:** Nominally, 0.4 mg/mL of donepezil hydrochloride prepared as follows. Dissolve a suitable number of Tablets in 75% of the flask volume of *Diluent*, and sonicate in an ultrasonic bath for 20 min. Swirl the mixture for 30 s, allow to cool to room temperature, and dilute with *Diluent* to volume. [NOTE—If necessary, add a magnetic stirring bar to the flask, and mix for 10 min on the magnetic stirrer, to aid in dissolution.] Allow a few min for the solids to settle. Pass through a suitable filter, discarding the first 2–3 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 271 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 1.4 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

**Tailing factor:** NMT 1.5 for the donepezil peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of donepezil hydrochloride ( $C_{24}H_{29}NO_3 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of donepezil hydrochloride from the *Sample solution*

$r_S$  = peak response of donepezil hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of donepezil hydrochloride ( $C_{24}H_{29}NO_3 \cdot HCl$ ) dissolved, by using one of the following methods.

### Chromatographic method

Diluent: Methanol and 0.1 N hydrochloric acid (3:1)

Mobile phase: Acetonitrile, water, and perchloric acid (350:650:1)

Standard stock solution: 1.1 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Dilute this solution with *Medium* to obtain a final concentration of 0.11 mg/mL.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of ( $L/1000$ ) mg/mL, where  $L$  is the label claim in mg/Tablet.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discarding the first few mL of the filtrate.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 271 nm

Column: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection size: 50  $\mu$ L

### System suitability

Sample: *Standard solution*

### Suitability requirements

Tailing factor: NMT 1.5

Column efficiency: NLT 5000 theoretical plates

Relative standard deviation: NMT 2.0%

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of donepezil hydrochloride ( $C_{24}H_{29}NO_3 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

### Spectrometric method

Standard stock solution: 0.11 mg/mL of USP Donepezil Hydrochloride RS in water

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of ( $L/900$ ) mg/mL, where  $L$  is the label claim in mg/Tablet.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

Mode: UV

Analytical wavelength: 230 nm

Blank: *Medium*

Calculate the percentage of the labeled amount of donepezil hydrochloride ( $C_{24}H_{29}NO_3 \cdot HCl$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_U$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of donepezil hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES, PROCEDURE 1

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if any of the impurities included in *Table 3* are potential degradation products.]

Diluent, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.8  $\mu$ g/mL of USP Donepezil Hydrochloride RS in *Diluent*

### System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

### Suitability requirements

Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

Relative standard deviation: NMT 8.0%, *Standard solution*

### Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Identify the impurities using the relative retention times given in *Table 1*.]

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of donepezil hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil <sup>a</sup>	0.33	1.0	0.5
Donepezil open ring <sup>b</sup>	0.70	0.6	0.5
Donepezil hydrochloride	1.0	—	—
Donepezil N-oxide <sup>c</sup>	1.2	1.0	0.5
Any individual unspecified degradation product	—	—	0.2

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.

<sup>b</sup> 2-(3-(1-Benzylpiperidin-4-yl)-2-oxopropyl)-4,5-dimethoxybenzoic acid.

<sup>c</sup> 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxyindan-1-one N-oxide.

### • ORGANIC IMPURITIES, PROCEDURE 2

Diluent: Acetonitrile and water (25:75)

Solution A: Add 1 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a filter of 0.45- $\mu$ m or finer pore size.

**Solution B:** Acetonitrile  
**Mobile phase:** See Table 2.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	40	60
40	40	60
41	75	25
50	75	25

**Standard solution:** 0.01 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Sonication may be used to aid the dissolution.

**Sample solution:** Nominally 1.0 mg/mL of donepezil hydrochloride in *Diluent*. Sonication may be used to aid the dissolution.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 50°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%, for five replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity or any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of donepezil hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for the corresponding impurity peak from Table 3

**Acceptance criteria:** See Table 3.

**Table 3**

Name	Relative Retention Time*	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil <sup>a</sup>	0.23	1.5	0.15
Donepezil pyridine analog (DPMI) <sup>b</sup>	0.49	1.9	0.15
Donepezil benzyl bromide <sup>c</sup>	0.68	0.73	0.15

\* Relative retention times are based on 1-mL gradient delay volume.

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one hydrochloride.

<sup>b</sup> 5,6-Dimethoxy-2-(pyridin-4-ylmethyl)indan-1-one.

<sup>c</sup> 1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidinium bromide.

<sup>d</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine hydrochloride.

<sup>e</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine hydrochloride.

**Table 3 (Continued)**

Name	Relative Retention Time*	Relative Response Factor	Acceptance Criteria, NMT (%)
Donepezil hydrochloride	1.0	1.0	—
Dehydrodeoxy donepezil <sup>d</sup>	1.72	2.0	0.15
Deoxydonepezil <sup>e</sup>	2.12	0.67	0.15
Any individual degradation product	—	1.0	0.1
Total impurities	—	—	0.75

\* Relative retention times are based on 1-mL gradient delay volume.

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one hydrochloride.

<sup>b</sup> 5,6-Dimethoxy-2-(pyridin-4-ylmethyl)indan-1-one.

<sup>c</sup> 1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidinium bromide.

<sup>d</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine hydrochloride.

<sup>e</sup> 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine hydrochloride.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS** <11>

USP Donepezil Hydrochloride RS

USP Donepezil Related Compound A RS

(*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.

C<sub>24</sub>H<sub>27</sub>NO<sub>3</sub> 377.48

## Donepezil Hydrochloride Orally Disintegrating Tablets

**DEFINITION**

Donepezil Hydrochloride Orally Disintegrating Tablets contains NLT 93.0% and NMT 107.0% of the labeled amount of donepezil hydrochloride (C<sub>24</sub>H<sub>29</sub>NO<sub>3</sub> · HCl).

**IDENTIFICATION**• **A. ULTRAVIOLET ABSORPTION** <197U>

**Sample solution:** Crush a suitable number of Tablets, and transfer an amount of powder, equivalent to 10 mg of donepezil hydrochloride, to a 100-mL volumetric flask. Add 80 mL of 0.1 N hydrochloric acid, and sonicate for 5 min. Cool to room temperature, and dilute with 0.1 N hydrochloric acid to volume. Transfer a portion to a centrifuge tube, and centrifuge for 15 min. Transfer 5 mL of the clear supernatant to a 25-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

**Analysis**

**Wavelength range:** 220–360 nm

**Acceptance criteria:** 230, 271, and 315 nm

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol and 0.1 N hydrochloric acid (3:1)

**Mobile phase:** Dissolve 2.5 g of sodium decanesulfonate in 650 mL of water, and add 1.0 mL of perchloric acid and 350 mL of acetonitrile. If necessary, adjust with an additional 0.5 mL of perchloric acid to a pH of about 1.8.



**System suitability solution:** 0.4 mg/mL of USP Donepezil Hydrochloride RS and 0.016 mg/mL of USP Donepezil Related Compound A RS, prepared by dissolving in 40% of the flask volume of methanol and diluting with water to volume.

**Standard solution:** 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*

**Sample solution:** 0.4 mg/mL of donepezil hydrochloride in *Diluent*, prepared by transferring a suitable number of Tablets to an appropriate volumetric flask containing 10 mL of 0.1 N hydrochloric acid. Shake to disintegrate the Tablets. Add 60% of the flask volume of *Diluent*, sonicate for 10 min, allow to cool to room temperature, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 271 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 35°

**Flow rate:** 1.4 mL/min

**Injection size:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

**Tailing factor:** NMT 1.5 for donepezil, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{24}H_{29}NO_3 \cdot HCl$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of donepezil hydrochloride from the *Sample solution*

$r_S$  = peak response of donepezil hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION <701>

**Time:** NMT 60 s

##### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Diluent:** Methanol and 0.1 N hydrochloric acid (3:1)

**Mobile phase:** Acetonitrile, perchloric acid, and water (350:1:650)

**Standard stock solution:** 1.1 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Dilute with *Medium* to obtain a concentration of 0.11 mg/mL.

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a concentration of L/1000 mg/mL, where L is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 271 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 50 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 5000 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{24}H_{29}NO_3 \cdot HCl$  dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{24}H_{29}NO_3 \cdot HCl$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE

**Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.8 μg/mL of USP Donepezil Hydrochloride RS in *Diluent*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

**Relative standard deviation:** NMT 8.0%, *Standard solution*

#### Analysis

**Samples:** *Sample solution* and *Standard solution*.

[NOTE—Identify the impurities, using the relative retention times given in *Impurity Table 1*.]

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of any individual impurity from the *Sample solution*

$r_S$  = peak response of donepezil hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

- $C_U$  = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)  
 $F$  = relative response factor of each related compound, as listed in *Impurity Table 1*

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil <sup>a</sup>	0.35	1.0	0.5
Donepezil open ring <sup>b</sup>	0.70	0.6	0.5
Donepezil hydrochloride	1.0	—	—
Donepezil <i>N</i> -oxide <sup>c</sup>	1.2	1.0	0.5
Individual unspecified degradation impurity	—	—	0.2
Total impurities	—	—	1.0

<sup>a</sup> 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.

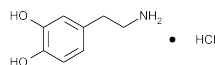
<sup>b</sup> 2-(3-(1-Benzylpiperidin-4-yl)-2-oxopropyl)-4,5-dimethoxybenzoic acid.

<sup>c</sup> 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxyindan-1-one *N*-oxide.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Donepezil Hydrochloride RS  
 USP Donepezil Related Compound A RS  
 (*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.  
 $C_{24}H_{27}NO_3$  377.48

## Dopamine Hydrochloride



$C_8H_{11}NO_2 \cdot HCl$  189.64

1,2-Benzenediol, 4-(2-aminoethyl)-, hydrochloride.

4-(2-Aminoethyl)pyrocatechol hydrochloride [62-31-7].

» Dopamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_8H_{11}NO_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at room temperature.

**USP Reference standards (11)**—

USP Dopamine Hydrochloride RS

**Clarity and color of solution**—A solution of 400 mg in 10 mL of sodium bisulfite solution (1 in 1000) is clear and colorless or practically colorless.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 40 µg per mL.

*Medium:* sodium bisulfite in water (1 in 1000).

**C:** It responds to the tests for *Chloride* (191).

**pH** (791): between 3.0 and 5.5, in a solution (1 in 25).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method I** (231)—Dissolve 1 g in 25 mL of water: the limit is 0.002%.

**Sulfate** (221)—Dissolve 500 mg in 40 mL of water: any turbidity produced is not more than that produced in a solution containing 0.10 mL of 0.020 N sulfuric acid.

**Readily carbonizable substances** (271)—Dissolve 100 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

**Chromatographic purity**—

**Standard solution and Standard dilutions**—Prepare a solution of USP Dopamine Hydrochloride RS in methanol to contain 30 mg per mL (*Standard solution*). Prepare a series of dilutions of the *Standard solution* in methanol to contain 0.6 mg, 0.3 mg, and 0.15 mg per mL (*Standard dilutions*), corresponding to 2.0%, 1.0%, and 0.5% of impurities, respectively.

**Test solution**—Transfer 150 mg of Dopamine Hydrochloride to a 5-mL volumetric flask, dilute with methanol to volume, and mix.

**Procedure**—In a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* (621)), place a solvent system consisting of 13 volumes of chloroform, 9 volumes of methanol, and 4 volumes of dilute glacial acetic acid (3 in 10). Line the chamber with filter paper, and allow to equilibrate. Apply 10-µL portions of the *Standard solution*, the *Standard dilutions*, and the *Test solution* to a 20- × 20-cm chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, allow to dry at room temperature for several minutes, and spray evenly with a freshly prepared mixture containing equal volumes of ferric chloride solution (1 in 10) and potassium ferricyanide solution (1 in 20). [Dopamine and its related impurities appear as blue spots under visible light.] The *Test solution* exhibits its principal spot at an  $R_f$  value corresponding to that of the *Standard solution* and not more than three secondary spots. Estimate the concentration of any secondary spots exhibited by the *Test solution* by comparison with the *Standard dilutions*: the sum of the impurities is not greater than 1.0%.

**Assay**—Dissolve about 75 mg of Dopamine Hydrochloride, accurately weighed, in 5 mL of formic acid, add 25 mL of acetic anhydride, and mix. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.96 mg of  $C_8H_{11}NO_2 \cdot HCl$ .

## Dopamine Hydrochloride Injection

» Dopamine Hydrochloride Injection is a sterile solution of Dopamine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_8H_{11}NO_2 \cdot HCl$ . It may contain a suitable antioxidant.

**NOTE**—Do not use the Injection if it is darker than slightly yellow or discolored in any other way.

**Packaging and storage**—Preserve in single-dose containers of Type I glass.

**Labeling**—Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

**USP Reference standards** (11)—

USP Dopamine Hydrochloride RS

USP Endotoxin RS

**Identification**—Transfer a volume of Injection to a suitable container, and dilute if necessary, with dilute methanol (1 in 5) to obtain a test solution having a known concentration of 1.6 mg per mL. Prepare a Standard solution of USP Dopamine Hydrochloride RS in dilute methanol (1 in 5) having the same concentration as the test solution. The test solution so obtained responds to the *Thin-layer Chromatographic Identification Test* (201), a mixture of *n*-butyl alcohol, glacial acetic acid, and water (4:1:1) being used as the developing solvent and 5  $\mu$ L each of the test solution and Standard solution being applied to the thin-layer chromatographic plate.

**Bacterial endotoxins** (85)—It contains not more than 16.67 USP Endotoxin Units per mg of dopamine hydrochloride.

**pH** (791): between 2.5 and 5.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.005 M sodium 1-octanesulfonate in 1% glacial acetic acid and acetonitrile (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve a suitable quantity of USP Dopamine Hydrochloride RS, accurately weighed, in *Mobile phase* to obtain a solution having a concentration of about 1.6 mg per mL. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.16 mg of dopamine hydrochloride per mL.

**System suitability solution**—Prepare a solution of benzoic acid in methanol containing about 20 mg per mL. Dilute 1 volume of this solution with 3 volumes of the *Mobile phase* to obtain a solution having a final concentration of about 5 mg per mL. Transfer 10.0 mL of this solution and 10.0 mL of a Standard solution containing 1.6 mg of USP Dopamine Hydrochloride RS per mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 16 mg of dopamine hydrochloride, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm  $\times$  30-cm column packed with packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the resolution, *R*, between benzoic acid and dopamine hydrochloride is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 40  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_8H_{11}NO_2 \cdot HCl$  in each mL of the Injection taken by the formula:

$$(100C / V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dopamine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of dopamine hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dopamine Hydrochloride and Dextrose Injection

» Dopamine Hydrochloride and Dextrose Injection is a sterile solution of Dopamine Hydrochloride and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dopamine hydrochloride ( $C_8H_{11}NO_2 \cdot HCl$ ) and of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ).

NOTE—Do not use the Injection if it is darker than slightly yellow or discolored in any other way.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Dopamine Hydrochloride RS

USP Endotoxin RS

**Identification**—

A: It responds to the *Identification* test under *Dextrose*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dopamine hydrochloride*.

**Bacterial endotoxins** (85)—It contains not more than 16.67 USP Endotoxin Units per mg of dopamine hydrochloride.

**pH** (791): between 2.5 and 4.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Limit of 5-hydroxymethylfurfural and related substances**—

**Diluting solution**—Prepare a solution containing approximately 0.022 N sodium hydroxide in water.

**Cation-exchange column**—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* (621)), using a chromatographic tube capable of providing a 0.8-cm  $\times$  4-cm bed volume (or about 2 mL) of 100- to 200-mesh, strongly acidic, styrene-divinylbenzene cation-exchange resin. Condition the column by washing with about 30 mL of water, discarding the eluate.

**Procedure**—Pass a volume of Injection containing about 100 mg of hydrous dextrose through the resin bed in the *Cation-exchange column*, allowing the specimen to flow down the wall of the column so as not to disturb the resin bed, and collect the eluate in a 50-mL volumetric flask. Wash the column with 25 mL of water, and collect the eluate in the same 50-mL volumetric flask. Dilute the eluate with *Diluting solution* to volume, and mix to obtain the test solution. In a similar manner, prepare a blank by passing 27 mL of water through a freshly conditioned *Cation-exchange column*, collecting the eluate in a 50-mL volumetric flask, diluting with *Diluting solution* to volume, and mixing. Determine the absorbance of the test solution against the blank in a 1-cm cell at 284 nm, with a suitable spectrophotometer: the absorbance is not more than 0.25.

**Other requirements**—It meets the requirements under *Injections* (1).

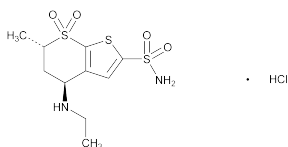
**Assay for dopamine hydrochloride**—Proceed with Injection as directed in the *Assay* under *Dopamine Hydrochloride Injection*.

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* <781>). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

## Dorzolamide Hydrochloride



$C_{10}H_{16}N_2O_4S_3 \cdot HCl$  360.90

4*H*-Thieno[2,3-*b*]thiopyran-2-sulfonamide, 4-(ethylamino)-5,6-dihydro-6-methyl-, 7,7-dioxide, monohydrochloride, (4*S*-trans)-.

(4*S*,6*S*)-4-(Ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide, monohydrochloride [130693-82-2].

» Dorzolamide Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $C_{10}H_{16}N_2O_4S_3 \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light, and store at 15° to 30°.

### USP Reference standards <11>—

USP Dorzolamide Hydrochloride RS

USP Dorzolamide Hydrochloride Related Compound A RS (4*R*,6*R*)-4-(Ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide-7,7-dioxide, monohydrochloride.

$C_{10}H_{16}N_2O_4S_3 \cdot HCl$  360.91

### Identification—

**A:** Infrared Absorption <197M>.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** It meets the requirements for *Chloride* <191>.

**Water**, *Method I* <921>: not more than 0.5%, using 0.4 g.

**Residue on ignition** (281): not more than 0.1%, an ignition temperature of 600° being used.

**Heavy metals**, *Method II* <231>: 0.001%.

### Limit of dorzolamide hydrochloride related compound A—

**Mobile phase**—Prepare a filtered and degassed mixture of *tert*-butyl methyl ether, chromatographic *n*-heptane, acetonitrile, and water (63:35:2:0.2). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Transfer about 18 mg of USP Dorzolamide Hydrochloride RS and 2 mg of USP Dorzolamide Hydrochloride Related Compound A RS, each accu-

rately weighed, to a 15-mL centrifuge tube, dissolve in 4 mL of 0.5 N ammonium hydroxide, add 4 mL of ethyl acetate, and mix. Separate the ethyl acetate layer, and transfer to a 15-mL centrifuge tube. Add 4 mL of ethyl acetate to the aqueous layer, mix, separate the ethyl acetate layer, and combine it with the first extract. Evaporate the combined organic layers to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 3 mL of acetonitrile, add 3 drops of (*S*)-(-)- $\alpha$ -methylbenzyl isocyanate [NOTE—Discard the reagent if it is colored.], and allow to react for 5 minutes on a water bath maintained at 50°. Evaporate the mixture to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 10 mL of a mixture of *tert*-butyl methyl ether, glacial acetic acid, and acetonitrile (87:10:3).

**Test solution**—Transfer about 20 mg of Dorzolamide Hydrochloride, accurately weighed, to a 15-mL centrifuge tube, and proceed as directed for *System suitability solution* beginning with “dissolve in 4 mL”.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 1.0 for dorzolamide and 1.5 for dorzolamide hydrochloride related compound A; the resolution,  $R$ , between dorzolamide and dorzolamide hydrochloride related compound A is not less than 4.0; the column efficiency for the dorzolamide hydrochloride peak is not less than 4000 theoretical plates; the tailing factor is not more than 1.4.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *System suitability solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of dorzolamide hydrochloride related compound A in the portion of Dorzolamide Hydrochloride taken by the formula:

$$100r_1 / (r_1 + r_5)$$

in which  $r_1$  is the peak area of dorzolamide hydrochloride related compound A obtained from the *Test solution*; and  $r_5$  is the peak area of dorzolamide hydrochloride obtained from the *Test solution*: not more than 0.5% is found.

### Chromatographic purity—

**Phosphate buffer**, *Solution A*, *Solution B*, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Dorzolamide Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area of each individual impurity obtained from the *Test solution*; and  $r_s$  is the sum of all the peak areas obtained from the *Test solution*: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

### Assay—

**Phosphate buffer**—Dissolve 3.7 g of monobasic potassium phosphate in 1000 mL of water.

**Solution A**—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (94:6.5).

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve suitable quantities of USP Dorzolamide Hydrochloride RS in *Solution A* to obtain a solution having a known concentration of about 0.6 mg per mL.

**Assay preparation**—Transfer about 60 mg of Dorzolamide Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Solution A* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–15	100	0	isocratic
15–30	100→50	0→50	linear gradient
30–37	50→100	50→0	linear gradient
37–44	100	0	isocratic

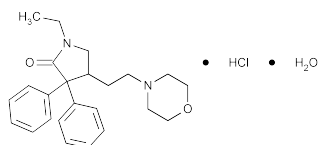
Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 6500 theoretical plates; the tailing factor is not less than 0.6 and not more than 1.2; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of C<sub>10</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>S<sub>3</sub> · HCl in the portion of Dorzolamide Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dorzolamide Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxapram Hydrochloride



C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> · HCl · H<sub>2</sub>O 432.98  
 2-Pyrrolidinone, 1-ethyl-4-[2-(4-morpholinyl)ethyl]-3,3-diphenyl-, monohydrochloride, monohydrate, (±)-  
 (±)-1-Ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone monohydrochloride monohydrate  
 [7081-53-0].  
 Anhydrous 414.98 [113-07-5].

» Doxapram Hydrochloride, dried at 105° for 2 hours, contains not less than 98.0 percent and not more than 100.5 percent of C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> · HCl.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Doxapram Hydrochloride RS

### Identification—

**A: Infrared Absorption** <197K>.

**B: Ultraviolet Absorption** <197U>—

*Solution*: 400 µg per mL.

*Medium*: water.

Absorptivities at 258 nm, calculated on the dried basis, do not differ by more than 3.0%.

**pH** <791>: between 3.5 and 5.0, in a solution (1 in 100).

**Loss on drying** <731>—Dry it at 105° for 2 hours: it loses between 3.0% and 4.5% of its weight.

**Residue on ignition** <281>: not more than 0.3%.

**Heavy metals, Method II** <231>: 0.002%.

### Chromatographic purity—

**Dragendorff reagent**—Dissolve 17 g of bismuth subnitrate and 200 g of tartaric acid in 800 mL of water (*Solution A*). Dissolve 160 g of potassium iodide in 400 mL of water (*Solution B*). Mix *Solution A* and *Solution B*. To 25 mL of this stock solution add 50 g of tartaric acid and 250 mL of water, and mix.

**Test preparation**—Dissolve 57 mg of Doxapram Hydrochloride in 0.5 mL of 0.1 N sodium hydroxide, add 1.0 mL of chloroform, and shake.

**Standard preparation A**—Dissolve 57 mg of USP Doxapram Hydrochloride RS in 0.5 mL of 0.1 N sodium hydroxide, add 1.0 mL of chloroform, and shake.

**Standard preparation B**—Dissolve 11.4 mg of USP Doxapram Hydrochloride RS in 0.5 mL of 0.1 N sodium hydroxide, add 100 mL of chloroform, and shake.

**Procedure**—Apply 10-µL portions of the chloroform solutions obtained from the *Test preparation* and the *Standard preparations* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a chromatographic chamber lined with paper and equilibrated with a solvent system consisting of a mixture of isopropyl alcohol and 1 N ammonium hydroxide (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Dragendorff reagent* in order to visualize the spots: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from *Standard preparation A*, and no spot, other than the principal spot, in the chromatogram of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation B* (0.2%).

**Assay**—Dissolve about 800 mg of Doxapram Hydrochloride, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS and 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.50 mg of C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> · HCl.

## Doxapram Hydrochloride Injection

» Doxapram Hydrochloride Injection is a sterile solution of Doxapram Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> · HCl · H<sub>2</sub>O.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Doxapram Hydrochloride RS

USP Endotoxin RS

**Identification**—

**A:** The chromatogram obtained from the *Assay preparation* in the *Assay* exhibits a major peak for doxapram, the retention time of which corresponds to that of the doxapram peak in the chromatogram of the *Standard preparation*.

**B:** Transfer a volume of Injection, equivalent to about 50 mg of doxapram hydrochloride hydrate, to a separator containing 5 mL of water. Add 1 mL of a saturated solution of sodium chloride to the separator, insert the stopper, and mix. Add 5 mL of 2.5 N sodium hydroxide, and extract with three 15-mL portions of chloroform. Pass each extract through a pledget of glass wool, combine the filtrates in a 50-mL volumetric flask, dilute with chloroform to volume, and mix. Evaporate to dryness about 5 mL of this solution. Dissolve the residue in 0.01 N sulfuric acid, dilute with the same solvent to 100 mL, and mix: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as a solution similarly prepared, about 50 mg of USP Doxapram Hydrochloride RS, instead of Doxapram Hydrochloride Injection, being used.

**Bacterial endotoxins** (85)—It contains not more than 3.3 USP Endotoxin Units per mg of doxapram hydrochloride.

**pH** (791): between 3.5 and 5.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—Dissolve 2.8 g of monobasic potassium phosphate in 1 L of water, adjust with 50% phosphoric acid or 1 N potassium hydroxide to a pH of  $3.0 \pm 0.1$ , and filter through a 0.5- $\mu$ m or finer porosity filter. Prepare a suitable mixture of this solution and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of diphenhydramine hydrochloride in water containing about 1.5 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Doxapram Hydrochloride RS in water to obtain a solution having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of doxapram hydrochloride monohydrate, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with water to volume, and mix.

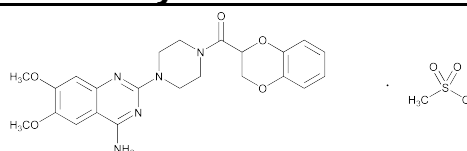
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm  $\times$  15-cm column containing 5- $\mu$ m packing L10, and is maintained at 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the relative retention times are about 1.0 for doxapram and 1.2 for diphenhydramine, the resolution, *R*, between the doxapram and diphenhydramine peaks is not less than 3.0; the tailing factor for the peaks is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of doxapram hydrochloride hydrate

( $C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$ ) in each mL of the Injection taken by the formula:

$$(432.98 / 414.98)(500C / V)(R_U / R_S)$$

in which 432.98 and 414.98 are the molecular weights of doxapram hydrochloride monohydrate and anhydrous doxapram hydrochloride, respectively, *C* is the concentration, in mg per mL, of USP Doxapram Hydrochloride RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses of doxapram and diphenhydramine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Doxazosin Mesylate**

$C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$  547.58

Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl)carbonyl]-, monomethanesulfonate.

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl)piperazine monomethanesulfonate [77883-43-3].

» Doxazosin Mesylate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, and store below 30°.

**USP Reference standards** (11)—

USP Doxazosin Mesylate RS

USP Doxazosin Related Compound A RS

N-1,4-Benzodioxane-2-carbonyl piperazine.

 $C_{13}H_{16}N_2O_3$  248.28

USP Doxazosin Related Compound B RS

6,7-Dimethoxyquinazoline-2,4-dione.

 $C_{10}H_{10}N_2O_4$  222.20

USP Doxazosin Related Compound C RS

2-Chloro-4-amino-6,7-dimethoxyquinazoline.

 $C_{10}H_{10}ClN_3O_2$  239.66

USP Doxazosin Related Compound D RS

1,4-Benzodioxane-2-carboxylic acid.

 $C_9H_8O_5$  196.16

USP Doxazosin Related Compound E RS

2,4-Dichloro-6,7-dimethoxyquinazoline.

 $C_{10}H_8Cl_2N_2O_2$  259.09

USP Doxazosin Related Compound F RS

N,N'-Bis(1,4-benzodioxane-2-carbonyl)piperazine.

 $C_{22}H_{22}N_2O_6$  410.42

USP Terazosin Related Compound A RS

1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.

 $C_{14}H_{19}N_5O_2 \cdot 2HCl$  362.25

USP Terazosin Related Compound C RS

1,4-Bis(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.

 $C_{24}H_{28}N_8O_4 \cdot 2HCl$  565.45**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the

chromatogram of the *Standard preparation*, as obtained in the Assay.

**Loss on drying** (731)—Dry it in vacuum at 105° for 4 hours: it loses not more than 2.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 20 µg per g.

**Related compounds—**

*Solvent A, Solvent D, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Solvent B*—Use acetonitrile.

*Solvent C*—Use water.

*Mobile phase*—Use variable mixtures of *Solvent A, Solvent B, and Solvent C* as directed for *Chromatographic system* in the Assay. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve accurately weighed quantities of USP Doxazosin Mesylate RS, USP Doxazosin Related Compound A RS, USP Doxazosin Related Compound B RS, USP Doxazosin Related Compound C RS, USP Doxazosin Related Compound D RS, USP Doxazosin Related Compound E RS, USP Doxazosin Related Compound F RS, USP Terazosin Related Compound A RS, and USP Terazosin Related Compound C RS in approximately 2 mL of *Solvent D*; and dilute quantitatively, and stepwise if necessary, with *Solvent C* and *Solvent D* to obtain a solution having a known concentration of 0.0015 mg per mL of each of the Reference Standards. The final ratio of *Solvent C* to *Solvent D* is maintained at 9:1. Sonicate briefly to dissolve completely.

*Test solution*—Dissolve an accurately weighed quantity of Doxazosin Mesylate in approximately 2 mL of *Solvent D*, and dilute with *Solvent C* and *Solvent D* to obtain a solution having a known concentration of 0.6 mg per mL. The final ratio of *Solvent C* to *Solvent D* is maintained at 9:1. Sonicate briefly to dissolve completely.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion of Doxazosin Mesylate taken by the formula:

$$100(C_S / C_T)(r_i / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of each Reference Standard in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Doxazosin Mesylate in the *Test solution*;  $r_i$  is the peak response for each individual impurity obtained from the *Test solution*; and  $r_s$  is the peak response for each individual impurity obtained from the *Standard solution*: not more than 0.3% of terazosin related compound A is found; not more than 0.25% of any other identified individual impurity is found; not more than 0.10% of any other unidentified impurity is found; and not more than 1.0% of total impurities is found. Calculate the percentages of doxazosin related compound G and doxazosin related compound H [NOTE—The doxazosin related compound G is the mesylate salt and has the same retention time as that of the terazosin related compound A. The doxazosin related compound H is the mesylate salt and has the same retention time as that of the doxazosin related compound C.] in the portion of Doxazosin Mesylate taken by the formula:

$$(100/F)(C_S / C_T)(r_i / r_s)$$

in which the response factor,  $F$ , is 0.735 for doxazosin related compound G and 0.769 for doxazosin related compound H;  $C_S$  is the concentration, in mg per mL, of USP Doxazosin Mesylate RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Doxazosin Mesylate in the *Test solution*;  $r_i$  is the peak response of doxazosin related compound G or doxazosin related compound H in the *Test*

*solution*; and  $r_s$  is the peak response of USP Doxazosin Mesylate RS in the *Standard solution*.

**Assay—**

*Solvent A*—Dissolve 5 g of phosphoric acid (84%–86%) in 100 mL of water.

*Solvent B*—Use acetonitrile.

*Solvent C*—Use water.

*Solvent D*—Prepare a mixture of 100 mL of *Solvent B* and 2 g of phosphoric acid (84%–86%).

*Mobile phase*—Use variable mixtures of degassed *Solvent A, Solvent B, and Solvent C*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve accurately weighed quantities of USP Doxazosin Related Compound A RS and USP Doxazosin Related Compound B RS in approximately 2.5 mL of *Solvent D*. Further dilute this solution quantitatively, and stepwise if necessary, with *Solvent C* and *Solvent D* to obtain a final solution having a known concentration of 12 µg per mL of each of the related compounds. The final ratio of *Solvent C* to *Solvent D* is maintained at 9:1. Sonicate briefly to dissolve completely.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Doxazosin Mesylate RS in approximately 2 mL of *Solvent D*, and dilute with *Solvent C* and *Solvent D* to obtain a solution having a known concentration of 0.6 mg per mL. The final ratio of *Solvent C* to *Solvent D* is maintained at 9:1. Sonicate briefly to dissolve completely.

*Assay preparation*—Dissolve an accurately weighed quantity of Doxazosin Mesylate in approximately 2 mL of *Solvent D*, and dilute with *Solvent C* and *Solvent D* to obtain a solution having a concentration of 0.6 mg per mL, based on the labeled quantity of doxazosin mesylate. The final ratio of *Solvent C* to *Solvent D* is maintained at 9:1. Sonicate briefly to dissolve completely.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 0.8 mL per minute, and the column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (min)	Solvent			Elution
	A (%)	Solvent B (%)	Solvent C (%)	
0–10	20	10→22	70→58	linear gradient
10–35	20	22→50	58→30	linear gradient
35–40	20	50	30	equilibration

[NOTE—Between sample injections, the system is re-equilibrated for at least 7 minutes, or until a stable baseline is obtained, representing the starting composition.]

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between doxazosin related compound A and doxazosin related compound B is not less than 4.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the doxazosin mesylate peaks. Calculate the percentage of  $C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$  in the portion of Doxazosin Mesylate taken by the formula:

$$100(C_S / C_T) (r_u / r_s)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Doxazosin Mesylate RS in the *Standard preparation*;  $C_T$  is the concentration, in mg per mL, of Doxazosin Mesylate in the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxazosin Tablets

» Doxazosin Tablets contain an amount of doxazosin mesylate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of doxazosin ( $C_{23}H_{25}N_5O_5$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Doxazosin Mesylate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of doxazosin mesylate ( $C_{23}H_{25}N_5O_5 \cdot CH_4SO_3$ ) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 246 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Doxazosin Mesylate RS in the same *Medium*.

**Tolerances**—Not less than 70%(Q) of the labeled amount of  $C_{23}H_{25}N_5O_5 \cdot CH_4SO_3$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Buffer solution**—Transfer 3.4 g of monobasic potassium phosphate into a 1-L flask, and add 800 mL of water and 4.0 mL of triethylamine to dissolve. Adjust with phosphoric acid to a pH of 4.5, and dilute with water to volume.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (11 : 9). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**: A mixture of methanol and 0.1 N hydrochloric acid (9 : 1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Doxazosin Mesylate RS in *Diluent*, and dilute quantitatively and stepwise if necessary, with *Diluent* to obtain a solution having a final concentration of about 49 µg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute, and the column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for doxazosin is not less than 2.0; the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Assay preparation**—Transfer 10 Tablets, whole or ground, into a 250-mL volumetric flask, add 10 mL of water, and sonicate until the Tablets are disintegrated. Add 150 mL of *Diluent*, sonicate for 30 minutes, dilute with *Diluent* to volume, and mix. Quantitatively dilute a portion of the supernatant with *Diluent* to obtain a solution having a concentration of about 0.04 mg of doxazosin per mL.

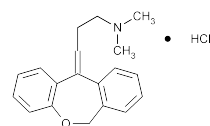
**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the doxazosin peaks. Calculate the

quantity, in mg, of doxazosin ( $C_{23}H_{25}N_5O_5$ ) in the portion of Tablets taken by the formula:

$$(451.48/547.58)CD(r_U / r_S)$$

in which 451.48 and 547.58 are the molecular weights of doxazosin and doxazosin mesylate, respectively; C is the concentration, in mg per mL, of USP Doxazosin Mesylate RS in the *Standard preparation*; D is the dilution volume, in mL, considering the initial 250-mL flask and any subsequent dilution used to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxepin Hydrochloride



$C_{19}H_{21}NO \cdot HCl$  315.84

1-Propanamine, 3-dibenz[*b,e*]oxepin-11(6*H*)ylidene-*N,N*-dimethyl-, hydrochloride.

*N,N*-Dimethyldibenz[*b,e*]oxepin- $\Delta^{11(6H),7}$ -propylamine hydrochloride [1229-29-4; 4698-39-9((*E*)-isomer); 25127-31-5((*Z*)-isomer)].

» Doxepin Hydrochloride, an (*E*) and (*Z*) geometric isomer mixture, contains the equivalent of not less than 98.0 percent and not more than 102.0 percent of doxepin hydrochloride ( $C_{19}H_{21}NO \cdot HCl$ ), calculated on the dried basis. It contains not less than 13.6 percent and not more than 18.1 percent of the (*Z*)-isomer, and not less than 81.4 percent and not more than 88.2 percent of the (*E*)-isomer.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Doxepin Hydrochloride RS

USP Doxepin Related Compound A RS

Dibenzo[*b,e*]oxepin-11(6*H*)-one.

$C_{14}H_{10}O_2$  210.23

USP Doxepin Related Compound B RS

11(*RS*)-(3-(Dimethylamino)propyl)-6,11-dihydrodibenzo[*b,e*]oxepin-11-ol.

$C_{19}H_{23}NO_2$  297.39

USP Doxepin Related Compound C RS

(*E,Z*)-3-(Dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)-*N*-methylpropan-1-amine.

$C_{18}H_{19}NO \cdot HCl$  301.81

**Identification**—

**A**: *Infrared Absorption* (197K).

**B**: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C**: A solution (1 in 100) in a mixture of water and alcohol (1:1) meets the requirements of the test for *Chloride* (191) in amine hydrochlorides.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours; it loses not more than 0.5% of its weight.



**Residue on ignition** (281): not more than 0.2%.

**Heavy metals**, Method II (231): 0.002%.

**Related compounds—**

*Diluted phosphoric acid*—Prepare a mixture of water and phosphoric acid (10:1), and mix well.

*Buffer*—Dissolve 1.42 g of dibasic sodium phosphate in 1 L of water, adjust with *Diluted phosphoric acid* to a pH of 7.7, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol, *Buffer*, and acetonitrile (50:30:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of *Mobile phase* and 2 N sodium hydroxide (1000:2).

*Standard solution*—Dissolve accurately weighed quantities of USP Doxepin Hydrochloride RS, USP Doxepin Related Compound A RS, USP Doxepin Related Compound B RS, and USP Doxepin Related Compound C RS in *Diluent* to obtain a solution having a known concentration of about 0.001 mg of doxepin hydrochloride, doxepin related compound A, and doxepin related compound B each per mL, and 0.002 mg per mL of doxepin related compound C. [NOTE—Sonication for about 1 minute may be used to aid the initial dissolution of the compounds.]

*Test solution*—Dissolve an accurately weighed quantity of Doxepin Hydrochloride in *Diluent* to obtain a final solution having a known concentration of about 1 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 30°. Chromatograph about 20 μL of the *Standard solution*, and record the peak areas as directed for *Procedure*: the resolution, *R*, between doxepin related compound A and doxepin related compound C is not less than 1.5; the resolution between doxepin related compound C and doxepin related compound B is not less than 1.5; and the signal-to-noise ratio for all the peaks is not less than 10. [NOTE—Use the approximate relative retention times given in *Table 1* for the purpose of peak identification. The doxepin related compound C peak will be the largest peak in the *Standard solution* chromatogram.]

**Table 1**

Name	Relative Retention Time (RRT)	Limit (%)
Doxepin related compound A	0.48	0.10
Doxepin related compound C	0.55	0.20
Doxepin related compound B	0.63	0.10
Doxepin hydrochloride	1.0	—
Unknown impurity	—	0.10 each

*Procedure*—Inject a volume (about 20 μL) of the *Test solution* into the chromatograph, record the chromatogram for up to 2.2 times the retention time of doxepin, and measure the peak responses. Calculate the percentage of each individual doxepin related compound in the portion of Doxepin Hydrochloride taken by the formula:

$$100(r_U / r_S)(C_S / C_T)$$

in which  $r_U$  is the individual peak response for each doxepin related compound obtained from the *Test solution*;  $r_S$  is the response of the corresponding peak in the *Standard solution*;  $C_S$  is the concentration, in mg per mL, of each doxepin related compound in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of Doxepin Hydrochloride in

the *Test solution*. The related substance limits are listed in *Table 1*. [NOTE—Discard any peak with a relative retention time less than 0.25. This method is not intended to resolve the *E*- and *Z*-isomers of doxepin hydrochloride. Minor variations in the mobile phase composition could result in a shoulder in the trailing edge of doxepin. In cases where there may be separation, both the *E*- and *Z*-isomers should be used in the appropriate calculations.] Use the response of the doxepin peak obtained from the *Standard solution* and the concentration of doxepin hydrochloride in the *Standard solution* to calculate the percentage of unknown individual impurities.

**Assay—**

*Mobile phase*—Prepare a mixture of 0.2 M monobasic sodium phosphate buffer and methanol (7:3), adjust with 2 N phosphoric acid to a pH of 2.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Doxepin Hydrochloride RS in *Mobile phase*, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a known concentration of about 100 μg per mL.

*Assay preparation*—Transfer about 50 mg of Doxepin Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

*Chromatographic system*—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 12.5-cm column, heated to 50°, that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution between the (*E*)- and (*Z*)-isomers is not less than 1.5, the tailing factor for each analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{19}H_{21}NO \cdot HCl$  in the portion of Doxepin Hydrochloride taken by the formula:

$$0.5C[(r_{U(Z)} + r_{U(E)}) / (r_{S(Z)} + r_{S(E)})]$$

in which  $C$  is the concentration, in μg per mL, of USP Doxepin Hydrochloride RS in the *Standard preparation*, and  $r_{U(Z)}$  and  $r_{U(E)}$  are the respective peak responses of the (*Z*)- and (*E*)-isomers obtained from the *Assay preparation*, and  $r_{S(Z)}$  and  $r_{S(E)}$  are the respective peak responses of the (*Z*)- and (*E*)-isomers obtained from the *Standard preparation*. Calculate the percentage of the (*Z*)-isomer in the *Assay preparation* taken by the formula:

$$(r_{U(Z)} / r_{S(Z)})(W_S / W_T)(P_Z)$$

in which  $W_S$  is the weight, in mg, of USP Doxepin Hydrochloride RS in the *Standard preparation*,  $W_T$  is the weight, in mg, in the portion of Doxepin Hydrochloride taken, and  $P_Z$  is the labeled percentage of (*Z*)-isomer in USP Doxepin Hydrochloride RS. Similarly calculate the percentage of (*E*)-isomer in the *Assay preparation* taken by the formula:

$$(r_{U(E)} / r_{S(E)})(W_S / W_T)(P_E)$$

in which  $P_E$  is the labeled percentage of (*E*)-isomer in USP Doxepin Hydrochloride RS.

## Doxepin Hydrochloride Capsules

» Doxepin Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of doxepin ( $C_{19}H_{21}NO$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Doxepin Hydrochloride RS

**Identification**—The retention times of the major peaks for (E)- and (Z)-isomers in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{19}H_{21}NO$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 292 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Doxepin Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{19}H_{21}NO$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements, the following procedure being used where the test for *Content Uniformity* is required.

**Diluting solution**—Prepare a mixture containing 500 mL of methanol and 500 mL of 0.05 M monobasic sodium phosphate, and filter. Adjust with 2 N sodium hydroxide to a pH of 6.7.

**Standard preparation**—Transfer 20 mg of USP Doxepin Hydrochloride RS, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and filter.

**Test preparation**—Transfer the contents of 1 Capsule into an appropriate volumetric flask, add *Diluting solution* to about 80% of the volume of the flask, and shake the flask by mechanical means for about 30 minutes. Dilute with *Diluting solution* to volume. Make further dilutions, if necessary, to obtain a solution having a known concentration of 0.1 mg per mL of doxepin hydrochloride. Individually test 9 more Capsules using the above procedure.

**Procedure**—Determine the amount of active ingredient in each unit of the *Test preparation* from UV absorbances at the wavelength of maximum absorbance at about 292 nm using 0.5-cm cells in comparison with the *Standard preparation*.

**Water, Method I** (921): not more than 9.0%, determined on the contents of 1 Capsule.

**Assay**—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Doxepin Hydrochloride*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Weigh the contents and determine the average weight per Capsule. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of doxepin hydrochloride, to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and shake by mechanical means for 30 minutes. Dilute with *Mobile phase* to volume, mix, and filter. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Doxepin Hydrochloride*. Calculate the quantity, in mg, of  $C_{19}H_{21}NO \cdot HCl$  in the portion of Capsules taken by the formula:

$$0.5C[(r_{U(Z)} + r_{U(E)}) / (r_{S(Z)} + r_{S(E)})]$$

in which the terms are as defined therein.

## Doxepin Hydrochloride Oral Solution

» Doxepin Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of doxepin ( $C_{19}H_{21}NO$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that each dose is to be diluted with water or other suitable fluid to approximately 120 mL, just prior to administration.

**USP Reference standards** (11)—

USP Doxepin Hydrochloride RS

**Identification**—

**Mobile phase**—Add 0.2 mL of diethylamine to a solution containing 250 mL of chloroform and 750 mL of acetonitrile in a vacuum flask. Prior to use, degas the contents of the flask by stirring vigorously with a magnetic stirrer, while applying vacuum, for 10 minutes.

**Procedure**—Transfer 5.0 mL of the Oral Solution to a 60-mL separator, add 1 mL of sodium hydroxide solution (1 in 25), 1 g of sodium chloride, and 5.0 mL of ethyl acetate, and shake the mixture vigorously for 1 minute. Allow the phases to separate, transfer 1.0 mL of the clear upper phase to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer about 22 mg of USP Doxepin Hydrochloride RS to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Inject 4-μL portions of both solutions into a high-pressure liquid chromatograph (see *Chromatography* (621)) fitted with a 50-cm × 2-mm column packed with silica microspheres and equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. Adjust the operating parameters to obtain a flow rate of about 24 mL per hour. The chromatogram of the test solution exhibits two peaks having retention times that are identical with those obtained with the *Standard solution*.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 7.0, the test specimen being allowed to remain in contact with the electrodes for 15 minutes prior to the measurement.

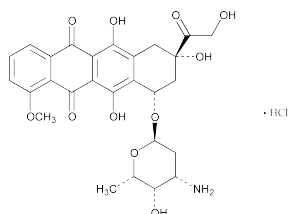
**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to 100 mg of doxepin, to a 100-mL volumetric flask, dilute with dilute hydrochloric acid (1 in 120) to volume, and mix. Dilute 4.0 mL of this solution with the same solvent to 50.0 mL. Transfer 15.0 mL of the resulting solution to a 125-mL separator, and extract with two 20-mL portions of ether. Dilute 10.0 mL of the extracted aqueous phase with dilute hydrochloric acid (1 in 120) to 25.0 mL. Prepare a *Standard solution* from a suitable quantity of USP Doxepin Hydrochloride RS, by quantitative and stepwise dilution with dilute hydrochloric acid (1 in 120) to obtain a solution having a known concentration of about 1.1 mg per

mL. Take a 4-mL aliquot of the Standard solution through the above-described procedure, beginning with "Dilute 4.0 mL of this solution." Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of doxepin ( $C_{19}H_{21}NO$ ) in each mL of the Oral Solution taken by the formula:

$$0.885(0.1 C/V)(A_U / A_S)$$

in which 0.885 is the ratio of the molecular weight of doxepin to that of doxepin hydrochloride;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Doxepin Hydrochloride RS in the Standard solution;  $V$  is the volume, in mL, of Oral Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Oral Solution and the Standard solution, respectively.

## Doxorubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$  579.98

5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxylacetyl)-1-methoxy-, hydrochloride (8*S*-*cis*)-, (8*S*,10*S*)-10-[3-Amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexopyranosyl)-oxy-8-glycoloyl]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride [25316-40-9].

» Doxorubicin Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{27}H_{29}NO_{11} \cdot HCl$ , calculated on the anhydrous, solvent-free basis.

**Caution**—Great care should be taken to prevent inhaling particles of doxorubicin hydrochloride and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature, except where it is labeled as amorphous, in which case it should be stored in the freezer.

**Labeling**—The amorphous form is so labeled.

### USP Reference standards (11)—

USP Doxorubicin Hydrochloride RS

**Identification**—The retention time of the doxorubicin peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Crystallinity** (695): meets the requirements, except that where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions.

**pH** (791): between 4.0 and 5.5, in a solution containing 5 mg per mL.

**Water, Method I** (921): not more than 4.0%.

**Chromatographic purity**—Proceed as directed in the Assay, except to use, instead of the Assay preparation, a test

solution prepared by dissolving Doxorubicin Hydrochloride in Mobile phase to obtain a solution containing about 0.5 mg per mL. From the chromatogram of the test solution, calculate the percentage of impurities taken by the formula:

$$100S/(S + r)$$

in which  $S$  is the sum of the responses of the minor component peaks; and  $r$  is the response of the major doxorubicin peak: the total of any impurities detected is not more than 2.0%.

**Limit of solvent residues** (as acetone and alcohol)—

**Standard solution**—Transfer to a 100-mL volumetric flask about 200 mg of acetone, 300 mg of dehydrated alcohol, and 1000 mg of dioxane, each accurately weighed, and mix. Dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 0.2 mg of acetone, 0.3 mg of  $C_2H_5OH$ , and 1 mg of dioxane per mL.

**Solvent**—Transfer about 100 mg of dioxane, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Dissolve about 200 mg of Doxorubicin Hydrochloride in 3.0 mL (3.0 g) of Solvent.

**Chromatographic system** (see Chromatography (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  2-m column packed with 8% to 10% liquid phase G16 and 2% potassium hydroxide on 100- to 120-mesh support S1A. The column temperature is maintained at about 60°, and helium is used as the carrier gas. Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 minutes. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.2 for acetone, 0.5 for alcohol, and 1.0 for dioxane; the resolution,  $R$ , between adjacent peaks is not less than 2.0; the tailing factor for the alcohol peak is not more than 1.5; and the relative standard deviations of the ratios of the peak responses of the acetone and dioxane peaks and of the alcohol and dioxane peaks for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 1  $\mu\text{L}$ ) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage, by weight, of acetone ( $CH_3COCH_3$ ) and alcohol ( $C_2H_5OH$ ), respectively, in the portion of Doxorubicin Hydrochloride taken by the same formula:

$$100(C_A / C_D)(D_U / W_U)(R_U / R_S)$$

in which  $C_A$  is the concentration, in mg per mL, of acetone or alcohol in the Standard solution;  $C_D$  is the concentration, in mg per mL, of dioxane in the Standard solution;  $D_U$  is the total quantity, in mg, of dioxane in the Test solution;  $W_U$  is the quantity, in mg, of Doxorubicin Hydrochloride taken to prepare the Test solution; and  $R_U$  and  $R_S$  are the area ratios of the analyte peak (acetone or alcohol) to the dioxane peak obtained from the Test solution and the Standard solution, respectively: not more than 0.5% of acetone is found; and the total of acetone and alcohol is not greater than 2.5%. Use the combined percentage of acetone and alcohol obtained to calculate the result obtained as directed in the Assay on the solvent-free basis.

### Assay—

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 g of sodium lauryl sulfate in 1000 mL of this solution, adjust with 2 N sodium hydroxide to a pH of  $3.6 \pm 0.1$ , and degas. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Resolution solution**—Dissolve about 10 mg of Doxorubicin Hydrochloride in 5 mL of water, add 5 mL of phosphoric acid, and allow to stand for about 30 minutes. Adjust with 2 N sodium hydroxide (about 37 mL) to a pH of  $2.6 \pm 0.1$ , add 15 mL of acetonitrile and 10 mL of methanol, mix, and filter. [NOTE—Portions of this solution may be frozen until needed, then thawed, and mixed before use.]

**Standard preparation**—Dissolve an accurately weighed quantity of USP Doxorubicin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer about 20 mg of Doxorubicin Hydrochloride, accurately weighed, to a 200-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L13. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the doxorubicin peak is not less than 0.7 and not more than 1.2; the column efficiency, determined from the doxorubicin peak, is not less than 2250 theoretical plates; and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for doxorubicinone and 1.0 for doxorubicin; and the resolution,  $R$ , between doxorubicinone and doxorubicin is not less than 5.5.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{27}H_{29}NO_{11} \cdot HCl$  in the Doxorubicin Hydrochloride taken by the formula:

$$0.2CP(r_U / r_S)$$

in which  $C$  is the concentration, in mg, of USP Doxorubicin Hydrochloride RS in each mL of the *Standard preparation*;  $P$  is the content, in  $\mu$ g per mg, of  $C_{27}H_{29}NO_{11} \cdot HCl$  in the USP Doxorubicin Hydrochloride RS; and  $r_U$  and  $r_S$  are the responses of the doxorubicin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxorubicin Hydrochloride Injection

» Doxorubicin Hydrochloride Injection is a sterile solution of Doxorubicin Hydrochloride in Sterile Water for Injection made isoosmotic with Sodium Chloride, Dextrose, or other suitable added substances. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{27}H_{29}NO_{11} \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store in a refrigerator. Injection may be packaged in multiple-dose containers not exceeding 100 mL in volume.

**USP Reference standards** <11>—  
USP Doxorubicin Hydrochloride RS  
USP Endotoxin RS

**Identification**—When chromatographed as directed in the *Assay*, the *Assay preparation* exhibits a major peak for doxorubicin, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**Bacterial endotoxins** <85>—Use a test solution prepared by diluting Doxorubicin Hydrochloride Injection with Sterile Water for Injection to obtain a concentration of 1.1 mg of doxorubicin hydrochloride per mL: the specimen under test contains not more than 2.2 USP Endotoxin Units per mg of doxorubicin hydrochloride.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the entire contents of all the containers being collected aseptically.

**pH** <791>: between 2.5 and 4.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

*Mobile phase*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Doxorubicin Hydrochloride*.

**Assay preparation**—Dilute an accurately measured volume of Injection, equivalent to not less than 2 mg of doxorubicin hydrochloride, quantitatively with *Mobile phase* to obtain a solution containing about 0.1 mg of doxorubicin hydrochloride per mL, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Doxorubicin Hydrochloride*. Calculate the quantity, in mg, of  $C_{27}H_{29}NO_{11} \cdot HCl$  in each mL of the Injection taken by the formula:

$$(CP / 1000)(L / D)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg per mL, of doxorubicin hydrochloride in the Doxorubicin Hydrochloride Injection taken,  $D$  is the concentration, in mg per mL, of doxorubicin hydrochloride in the *Assay preparation*, on the basis of the labeled quantity in the volume of Injection taken and the extent of dilution, and the other terms are as defined therein.

## Doxorubicin Hydrochloride for Injection

» Doxorubicin Hydrochloride for Injection is a sterile mixture of Doxorubicin Hydrochloride and Lactose. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{27}H_{29}NO_{11} \cdot HCl$ .

**Caution**—Great care should be taken to prevent inhaling particles of Doxorubicin Hydrochloride and exposing the skin to it.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>, except that multiple-dose containers may provide for the withdrawal of not more than 100 mL when constituted as directed in the labeling.

**USP Reference standards** <11>—  
USP Doxorubicin Hydrochloride RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Bacterial endotoxins** <85>—Use a solution of Doxorubicin Hydrochloride for Injection containing 1.1 mg of doxorubicin hydrochloride per mL: the specimen under test contains not more than 2.2 USP Endotoxin Units per mg of doxorubicin hydrochloride.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the entire contents of all the con-

tainers being collected aseptically with the aid of 200 mL of Fluid A before filtering.

**pH** (791): between 4.5 and 6.5, in the solution constituted as directed in the labeling, except that water is used as the diluent.

**Water, Method I** (921): not more than 4.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

**Other requirements**—It responds to the *Identification* test under *Doxorubicin Hydrochloride*, and meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling* under *Injections* (1).

#### Assay—

*Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Doxorubicin Hydrochloride*.

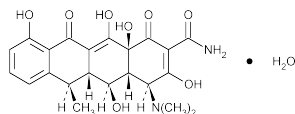
*Assay preparation*—Dilute the contents of 1 container quantitatively with *Mobile phase* to obtain a solution containing about 0.1 mg of doxorubicin hydrochloride per mL.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Doxorubicin Hydrochloride*. Calculate the quantity, in mg, of  $C_{27}H_{29}NO_{11} \cdot HCl$  in the container of Doxorubicin Hydrochloride for Injection taken by the formula:

$$(CP / 1000)(L / D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Doxorubicin Hydrochloride RS in the *Standard preparation*, *P* is the content, in µg per mg, of  $C_{27}H_{29}NO_{11} \cdot HCl$  in the USP Doxorubicin Hydrochloride RS, *L* is the labeled quantity of doxorubicin hydrochloride in the container, *D* is the concentration, in mg per mL, of doxorubicin hydrochloride in the *Assay preparation* on the basis of the labeled quantity in the container and the extent of dilution, and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of the doxorubicin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline



$C_{22}H_{24}N_2O_8 \cdot H_2O$  462.45

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, [4S-(4α,4aα,5α,5aα,6α,12α)]-, monohydrate.

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-de monohydrate [17086-28-1].

Anhydrous 444.44 [564-25-0].

» Doxycycline has a potency equivalent to not less than 880 µg and not more than 980 µg of  $C_{22}H_{24}N_2O_8$  per mg.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Doxycycline Hyclate RS

USP Methacycline Hydrochloride RS

**Identification**—Dissolve a suitable quantity in methanol to obtain a *Test Solution* containing 1 mg of doxycycline per mL, and proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 5.0 and 6.5, in an aqueous suspension containing 10 mg per mL.

**Water, Method I** (921): between 3.6% and 4.6%.

#### Related compounds—

*Mobile phase and Diluent*—Prepare as directed in the Assay under *Doxycycline Hyclate*.

*System suitability solution*—Prepare as directed for the *Resolution solution* in the Assay under *Doxycycline Hyclate*.

*Methacycline standard stock solution, Standard solution 1, Standard solution 2, and Chromatographic system*—Prepare as directed for the *Related compounds* test under *Doxycycline Hyclate*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the Assay.

*Procedure*—Proceed as directed for the *Related compounds* test under *Doxycycline Hyclate*. Calculate the percentage of methacycline in the portion of Doxycycline taken by the formula:

$$5000(C_M / W)(r_U / r_M)$$

in which *C<sub>M</sub>* is the concentration, in mg per mL, of USP Methacycline Hydrochloride RS in *Standard solution 2*; *W* is the weight, in mg, of Doxycycline taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>M</sub>* are the methacycline peak responses obtained from the *Test solution* and *Standard solution 2*, respectively. Not more than 2% of methacycline is found. Calculate the percentage of each related compound, other than methacycline, in the portion of Doxycycline taken by the formula:

$$5000(C_S / W)(r_i / r_S)$$

in which *C<sub>S</sub>* is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in *Standard solution 2*; *W* is the weight, in mg, of Doxycycline taken to prepare the *Test solution*; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test solution*; and *r<sub>S</sub>* is the doxycycline peak response obtained from *Standard solution 2*. Not more than 0.5% of any impurity eluting before methacycline is found; not more than 2% of 6-epidoxycycline is found; and not more than 0.5% of any impurity eluting after the main doxycycline peak is found.

#### Assay—

*Mobile phase, Diluent, Resolution solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Doxycycline Hyclate*.

**NOTE**—Throughout the following sections, protect the *Standard preparation* and the *Assay preparation* from light.

*Assay preparation*—Transfer about 55 mg of Doxycycline, accurately weighed, to a 50-mL volumetric flask, add 12 mL of 0.1 N hydrochloric acid, swirl to dissolve, dilute with *Diluent* to volume, and mix. Filter through a filter of 0.5 µm or finer porosity.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in each mg of the Doxycycline taken by the formula:

$$50(CP / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation*, *P* is the potency, in µg of doxycycline per mg, of USP Doxycycline Hyclate RS, *W* is the weight, in mg, of Doxycycline taken to prepare the *Assay preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline Capsules

» Doxycycline Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Doxycycline Hyclate RS

**Identification**—

A: Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing 1 mg of doxycycline per mL, and filter. Using the filtrate as the *Test solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2*: 75 rpm.

*Time*: 60 minutes.

*Procedure*—Determine the amount of  $C_{22}H_{24}N_2O_8$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 268 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Doxycycline Hyclate RS in the same *Medium*.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $C_{22}H_{24}N_2O_8$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.5%.

**Assay**—

*Mobile phase, Diluent, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

NOTE—Throughout the following sections, protect the *Standard preparation*, the *Assay preparation*, and the stock solutions used in their preparation, from light.

*Assay preparation*—Remove, as completely as possible, the contents of not less than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask. Add 20 mL of 0.1 N hydrochloric acid, sonicate for 5 minutes, shake for 15 minutes, dilute with *Diluent* to volume, and mix. Filter a portion of this solution through a filter of 0.5  $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in the portion of Capsules taken by the formula:

$$0.1 CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation*, P is the designated potency, in  $\mu$ g of doxycycline per mg, of USP Doxycycline Hyclate RS, and  $r_U$  and  $r_S$  are the doxycycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline for Injection

» Doxycycline for Injection contains an amount of Doxycycline Hyclate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), protected from light.

**USP Reference standards** (11)—

USP Doxycycline Hyclate RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—Dissolve a suitable quantity in methanol to obtain a solution containing the equivalent of 1 mg of doxycycline per mL, and filter. Using the filtrate as the *Test solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

**Bacterial endotoxins** (85)—It contains not more than 1.14 USP Endotoxin Units per mg of doxycycline.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined, Fluid D* being used instead of *Fluid A*.

**pH** (791): between 1.8 and 3.3, in the solution constituted as directed in the labeling.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: the article containing added substances loses not more than 2.0% of its weight; the article containing no added substances loses not more than 4.0% of its weight.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay**—

*Mobile phase, Diluent, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Doxycycline for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Diluent* to obtain a solution containing the equivalent of about 1000  $\mu$ g of doxycycline per mL.

*Assay preparation 2* (where the label states the quantity of doxycycline equivalent in a given volume of constituted solution)—Constitute Doxycycline for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Diluent* to obtain a solution containing the equivalent of about 1000  $\mu$ g of doxycycline per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Doxycycline Hyclate*. Calculate the quantity, in mg, of doxycycline ( $C_{22}H_{24}N_2O_8$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L / D)(CP)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of doxycycline in the container, or in the volume of constituted solution taken, D is the concentration, in  $\mu$ g of doxycycline per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution, and the other terms are as defined therein.

## Doxycycline for Oral Suspension

» Doxycycline for Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{24}N_2O_8$  when constituted as directed. It contains one or more suitable buffers, colors, diluents, flavors, and preservatives.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Doxycycline Hyclate RS

**Identification**—To a quantity of Doxycycline for Oral Suspension (powder), equivalent to about 50 mg of doxycycline, add 50 mL of methanol, shake, and allow to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 6.5, in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 3.0%.

**Assay**—

*Mobile phase, Diluent, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Doxycycline Hyclate*.

*Assay preparation*—Constitute Doxycycline for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add 50 mL of 0.1 N hydrochloric acid, and shake by mechanical means for about 15 minutes. Dilute with *Diluent* to volume, and mix. Filter through filter paper, discarding the first 10 mL of the filtrate, then filter through a filter of 0.5- $\mu$ m or finer porosity.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Doxycycline Hyclate*. Calculate the quantity, in mg of doxycycline ( $C_{22}H_{24}N_2O_8$ ), in each mL of the constituted suspension taken by the formula:

$$0.1(CP / V)(r_U / r_S)$$

in which *V* is the volume, in mL, of the constituted suspension taken to prepare the *Assay preparation*, and the other terms are as defined therein.

**Add the following:**

## ▲Doxycycline Tablets

### DEFINITION

Doxycycline Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** A solution containing 3.2 g/L of monobasic potassium phosphate, 0.87 g/L of sodium hydroxide, 0.59 g/L of tetrabutylammonium hydrogen sulfate, and 0.47 g/L of disodium edetate

**Mobile phase:** To 850 mL of *Buffer* add 60 g of tertiary butyl alcohol, and dilute with water to 1000 mL. Adjust with 1 N sodium hydroxide to a pH of 8.0.

**Diluent A:** 0.1 N hydrochloric acid

**Diluent B:** 0.01 N hydrochloric acid

**System suitability solution:** 1.2 mg/mL of USP Doxycycline Hyclate RS and 1 mg/mL of USP Doxycycline Related Compound A RS in a mixture of *Diluent A* and *Diluent B* (20:80), prepared as follows. Dissolve first in *Diluent A* using 20% of the final volume, and then dilute with *Diluent B* to volume. Sonicate as necessary to dissolve.

**Standard solution:** 1.2 mg/mL of USP Doxycycline Hyclate RS in a mixture of *Diluent A* and *Diluent B* (20:80), prepared as follows. Dissolve first in *Diluent A* using 20% of the final volume, and then dilute with *Diluent B* to volume. Sonicate as necessary to dissolve. Protect this solution from light using low-actinic glassware.

**Sample solution:** 1 mg/mL of doxycycline in *Diluent* from NLT 20 Tablets, finely crushed, in a mixture of *Diluent A* and *Diluent B* (20:80) prepared as follows. Suspend a suitable portion of crushed Tablets in *Diluent A* using 20% of the final volume, sonicate as necessary, and dilute with *Diluent B* to volume. Pass through a suitable filter. Protect this solution from light using low-actinic glassware.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L21

**Column temperature:** 60°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

The relative retention times for doxycycline related compound A and doxycycline are about 0.7 and 1.0, respectively.

### Suitability requirements

**Resolution:** NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

**Tailing factor:** NMT 2.0 for the doxycycline peak, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in the portion of Doxycycline Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

$P$  = potency of doxycycline in USP Doxycycline Hyclate RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001  $\text{mg}/\mu\text{g}$

Acceptance criteria: 90.0%–120.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: 0.01  $\text{mg}/\text{mL}$  of doxycycline from USP Doxycycline Hyclate RS in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute a portion of the filtrate with Medium to a concentration that is similar to that of the Standard solution.

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV

Analytical wavelength: 268 nm

Cell: 1 cm

Blank: Medium

### Analysis

Samples: Standard solution and Sample solution

Determine the percentage of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times P \times 100$$

$A_U$  = absorbance of the Sample solution

$A_S$  = absorbance of the Standard solution

$C_S$  = concentration of USP Doxycycline Hyclate RS in the Standard solution ( $\text{mg}/\text{mL}$ )

$L$  = label claim ( $\text{mg}/\text{Tablet}$ )

$V$  = volume of Medium, 900 mL

$P$  = potency of doxycycline in USP Doxycycline Hyclate RS ( $\mu\text{g}/\text{mg}$ )

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) is dissolved.

### UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

### ORGANIC IMPURITIES

Diluent A, Diluent B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

### Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Doxycycline Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of doxycycline from the Sample solution

$F$  = relative response factor as listed in Table 1

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxytetracycline <sup>a</sup>	0.3	—	—
4-Epidoxycycline <sup>b</sup>	0.4	0.8	1.5
Methacycline <sup>a,c</sup>	0.6	—	—
Doxycycline related compound A (6-epidoxycycline) <sup>a,d</sup>	0.7	—	—
Doxycycline	1.0	—	—
Any individual unspecified impurity	—	1.0	0.3
Total impurities	—	—	2.5

<sup>a</sup> These are synthetic process impurities, which are controlled in the drug substance. They are listed here for reference only and are not to be reported. Total impurities does not include these peaks.

<sup>b</sup> (4*R*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

<sup>c</sup> (4*S*,4*aR*,5*S*,5*aR*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

<sup>d</sup> (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

### USP REFERENCE STANDARDS (11)

USP Doxycycline Hyclate RS

USP Doxycycline Related Compound A RS

(4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

$\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$  444.43▲<sup>USP36</sup>

## Doxycycline Calcium Oral Suspension

» Doxycycline Calcium Oral Suspension is prepared from Doxycycline Hyclate, and contains one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Doxycycline Hyclate RS

**Identification**—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of doxycycline, add 50 mL of methanol, shake, and allow to settle. Using the clear supernatant as the Test Solution, proceed as directed for Method II under Identification—Tetracyclines (193).

### Uniformity of dosage units (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.



**Deliverable volume** (698): meets the requirements.

**pH** (791): between 6.5 and 8.0.

**Assay—**

*Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Doxycycline Hyclate.

*Edetate diluent*—Transfer 5.2 g of edetate disodium to a 500-mL volumetric flask, add about 400 mL of *Mobile phase*, sonicate for about 10 minutes or until dissolved, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Constitute Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the constituted oral suspension, freshly mixed and free from air bubbles, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add 50 mL of *Edetate diluent*, sonicate for 15 minutes, and then shake by mechanical means for about 15 minutes. Dilute with *Edetate diluent* to volume, and mix. Pass through filter paper, discarding the first 10 mL of the filtrate, then pass through a filter having a 0.5- $\mu$ m or finer porosity.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Doxycycline Hyclate. Calculate the quantity, in mg, of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in each mL of the Oral Suspension taken by the formula:

$$0.1(CP / V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation*; and the other terms are as defined therein.

## Doxycycline Hyclate

( $C_{22}H_{24}N_2O_8 \cdot HCl$ )<sub>2</sub> ·  $C_2H_6O \cdot H_2O$  1025.87

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, compd. with ethanol (2:1), monohydrate, [4S-(4 $\alpha$ ,4a $\alpha$ ,5 $\alpha$ ,5a $\alpha$ ,6 $\alpha$ ,12 $\alpha$ )]-.  
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride, compound with ethyl alcohol (2:1), monohydrate [24390-14-5].

» Doxycycline Hyclate has a potency equivalent to not less than 800  $\mu$ g and not more than 920  $\mu$ g of doxycycline ( $C_{22}H_{24}N_2O_8$ ) per mg.

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Doxycycline Hyclate RS

USP Endotoxin RS

USP Methacycline Hydrochloride RS

**Identification, Infrared Absorption** (197K)

**Crystallinity** (695): meets the requirements.

**pH** (791): between 2.0 and 3.0, in a solution containing 10 mg of doxycycline per mL.

**Water, Method I** (921): between 1.4% and 2.8%.

**Related compounds—**

*Mobile phase and Diluent*—Prepare as directed in the Assay.

*System suitability solution*—Prepare as directed for *Resolution solution* in the Assay.

*Methacycline standard stock solution*—Dissolve an accurately weighed quantity of USP Methacycline Hydrochloride RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 1.2 mg per mL.

*Standard solution 1*—Prepare as directed for the *Standard preparation* in the Assay.

*Standard solution 2*—Transfer 2.0 mL of *Standard solution 1* and 2.0 mL of the *Methacycline standard stock solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.024 mg each of USP Doxycycline Hyclate RS and USP Methacycline Hydrochloride RS per mL.

*Test solution*—Use the *Assay preparation*, prepared as directed in the Assay.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the Assay. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for 4-epidoxycycline (the main degradation product), 0.6 for methacycline, 0.7 for 6-epidoxycycline, and 1.0 for doxycycline; the resolution,  $R$ , between 4-epidoxycycline and doxycycline is not less than 3.0; and the tailing factor is not more than 2.0. Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of *Standard solution 2* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is 1.7 times the retention time of doxycycline, and measure the peak areas. Calculate the percentage of methacycline in the portion of Doxycycline Hyclate taken by the formula:

$$10,000(C_M / W)(r_U / r_M)$$

in which  $C_M$  is the concentration, in mg per mL, of USP Methacycline Hydrochloride RS in *Standard solution 2*;  $W$  is the weight, in mg, of Doxycycline Hyclate taken to prepare the *Test solution*; and  $r_U$  and  $r_M$  are the methacycline peak responses obtained from the *Test solution* and *Standard solution 2*, respectively. Not more than 2% of methacycline is found. Calculate the percentage of each related compound, other than methacycline, in the portion of Doxycycline Hyclate taken by the formula:

$$10,000(C_S / W)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in *Standard solution 2*;  $W$  is the weight, in mg, of Doxycycline Hyclate taken to prepare the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the doxycycline peak response obtained from *Standard solution 2*. Not more than 0.5% of any impurity eluting before methacycline is found; not more than 2% of 6-epidoxycycline is found; and not more than 0.5% of any impurity eluting after the main doxycycline peak is found.

**Other requirements**—Where the label states that Doxycycline Hyclate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under Doxycycline for Injection. Where the label states that Doxycycline Hyclate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under Doxycycline for Injection.

**Assay—**

*Mobile phase*—Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add about 850 mL of

water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of  $8.0 \pm 0.1$ . Pass this solution through a filter having a porosity of 0.5  $\mu\text{m}$  or finer, and degas before using. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)). Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

**Diluent**—Use 0.01 N hydrochloric acid.

**Resolution solution**—Prepare a solution of USP Doxycycline Hyclate RS in *Diluent* containing about 6 mg of doxycycline per mL. Transfer 5 mL of this solution to a 25-mL volumetric flask, heat on a steam bath for 60 minutes, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5  $\mu\text{m}$  or finer, and use the filtrate as the *Resolution solution*. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days. [NOTE—Throughout the following sections, protect the *Standard preparation* and the *Assay preparation* from light.]

**Standard preparation**—Transfer about 12 mg of USP Doxycycline Hyclate RS, accurately weighed, to a 10-mL volumetric flask, add about 6 mL of *Diluent*, sonicate for about 5 minutes or until dissolved, dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 120 mg of Doxycycline Hyclate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Pass through a membrane filter having a porosity of 0.5  $\mu\text{m}$  or finer.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 270 nm detector, and a 4.6-mm  $\times$  25-cm column that contains packing L21 and is maintained at  $60 \pm 1^\circ$ . The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for 4-epidoxycycline (the main degradation product), 0.7 for 6-epidoxycycline, and 1.0 for doxycycline, the resolution, *R*, between the 4-epidoxycycline peak and the doxycycline peak is not less than 3.0, and the tailing factor for the doxycycline peak is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is 1.7 times the retention time of doxycycline, and measure the responses for the major peaks. Calculate the potency, in  $\mu\text{g}$  of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) per mg, of the Doxycycline Hyclate taken by the formula:

$$100(\text{CP} / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation*; *P* is the designated potency, in  $\mu\text{g}$  of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) per mg, of USP Doxycycline Hyclate RS; *W* is the quantity, in mg, of Doxycycline Hyclate taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline Hyclate Capsules

» Doxycycline Hyclate Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Doxycycline Hyclate RS

**Identification**—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing the equivalent of 1 mg of doxycycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 75 rpm, the distance between the blade and the inside bottom of the flask being maintained at  $4.5 \pm 0.5$  cm during the test.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Doxycycline Hyclate RS in the same medium.

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of  $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 8.5%.

**Assay**—

*Mobile phase*, *Diluent*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

**Assay preparation**—Remove, as completely as possible, the contents of not less than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add about 75 mL of *Diluent*, sonicate for 5 minutes, shake for 15 minutes, dilute with *Diluent* to volume, and mix. Filter through a membrane filter of 0.5  $\mu\text{m}$  or finer porosity.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Doxycycline Hyclate*. Calculate the quantity, in mg, of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) in the portion of Capsules taken by the formula:

$$0.1 \text{CP}(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation*, *P* is the designated potency, in  $\mu\text{g}$  of doxycycline per mg, of USP Doxycycline Hyclate RS, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline Hyclate Delayed-Release Capsules

» Doxycycline Hyclate Delayed-Release Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—The label indicates that the contents of the Capsules are enteric-coated.

**USP Reference standards** (11)—  
USP Doxycycline Hyclate RS

**Identification**—Shake a suitable quantity of finely powdered Capsule contents with methanol to obtain a solution containing the equivalent of 1 mg of doxycycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

**Dissolution** (711)—Proceed as directed for *Procedure* for *Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

ACID STAGE—[NOTE—Conduct the test by transferring the contents of each Capsule to the individual basket units of the apparatus.]

Medium: 0.06 N hydrochloric acid; 900 mL.

Apparatus 1: 50 rpm.

Time: 20 minutes.

Diluting solvent: 0.1 N hydrochloric acid.

*Procedure*—Determine the amount of  $C_{22}H_{24}N_2O_8$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 345 nm on filtered portions of the solution under test, suitably diluted with *Diluting solvent*, in comparison with a Standard solution having a known concentration of about 0.01 mg of USP Doxycycline Hyclate RS per mL in *Diluting solvent*.

*Tolerances*—*Level 1* (6 Capsules tested): No individual value exceeds 50% dissolved. *Level 2* (6 Capsules tested): Not more than 2 individual values of 12 tested are greater than 50% dissolved.

BUFFER STAGE—[NOTE—Conduct this stage of testing on separate specimens, selecting Capsules that were not previously subjected to *Acid stage* testing and transferring the contents of each Capsule to the individual basket units of the apparatus.]

Medium: pH 5.5 neutralized phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

Apparatus 1: 50 rpm.

Time: 30 minutes.

Diluting solvent: 0.1 N hydrochloric acid.

*Procedure*—Determine the amount of  $C_{22}H_{24}N_2O_8$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 345 nm on filtered portions of the solution under test, suitably diluted with *Diluting solvent*, in comparison with a Standard solution having a known concentration of about 0.01 mg of USP Doxycycline Hyclate RS per mL in *Diluting solvent*.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $C_{22}H_{24}N_2O_8$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.0%.

### Assay—

*Mobile phase*, *Diluent*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add about 75 mL of *Diluent*, sonicate for 5 minutes, shake for 15 minutes, dilute with *Diluent* to volume, and mix. Pass through a membrane filter having a 0.5- $\mu$ m or finer porosity.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Doxycycline Hyclate*. Calculate the quantity, in mg, of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in the portion of Capsules taken by the formula:

$$0.1 CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g of doxycycline per mg, of USP Doxycycline Hyclate RS; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Doxycycline Hyclate Delayed-Release Tablets

### DEFINITION

Doxycycline Hyclate Delayed-Release Tablets contain an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ).

### IDENTIFICATION

A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### Change to read:

#### • PROCEDURE

**Mobile phase:** Transfer 0.77 g of ammonium acetate, 0.75 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and dissolve. Add 70 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with acetic acid or ammonium hydroxide to a pH of  $9.00 \pm 0.05$ .

**Standard solution:** 1.16 mg/mL of doxycycline hyclate in methanol and water (1:9). Transfer USP Doxycycline Hyclate RS to a suitable volumetric flask, and add methanol to 10% of the final volume. Sonicate for 5 min or until dissolved. Dilute with water to volume. Protect the *Standard solution* from light. • Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu$ g/mg of doxycycline in USP Doxycycline Hyclate RS. • (RB 1-Jun-2012)

**Sample solution:** Equivalent to 1 mg/mL of doxycycline in a mixture of methanol and water (1:9) from NLT 10 Tablets, crushed. Prepare the solution as follows. Weigh and crush NMT 2 Tablets at a time in a suitable mortar. Transfer a weighed portion of the powder to a suitable volumetric flask, add methanol to 10% of the final vol-

ume, and sonicate. Dilute with water to volume, sonicating as necessary. Pass through a suitable filter. Protect the *Sample solution* from light.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 25-cm; packing L21

**Column temperature:** 52 ± 2°

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

**Run time:** 1.7 times the retention time of the doxycycline peak

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% from six replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

#### Change to read:

#### • DISSOLUTION <711>

**Test 1:** Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure* <711>.

#### Acid stage

**Medium:** 0.06 N hydrochloric acid; 900 mL, degassed with helium

**Apparatus 1:** 50 rpm

**Time:** 20 min

**Standard solution:** 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_S$ , in mg/mL of doxycycline, using the designated potency, in µg/mg of doxycycline in USP Doxycycline Hyclate RS. [NOTE—Sonicate if necessary to dissolve.]

**Sample solution:** Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

**Detector:** UV 346 nm

**Cell:** 0.1-cm quartz

**Blank:** *Medium*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

#### Tolerances

**Level 1 (6 Tablets tested):** No individual value is more than 30% of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) dissolved in 20 min.

**Level 2 (6 Tablets tested):** NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) in 20 min.

#### Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL, degassed

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Standard solution:** 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_S$ , in mg/mL of doxycycline, using the designated potency, in µg/mg of doxycycline in USP Doxycycline Hyclate RS. [NOTE—Sonicate if necessary to dissolve.]

**Sample solution:** Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

**Analysis:** Determine the percentage of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) dissolved by the procedure described for the *Acid stage*.

**Tolerances:** NLT 85% (Q) of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure* <711>.

#### Acid stage

**Medium, Apparatus 1, Time, Blank, and Analysis:** Proceed as directed for *Acid stage* in *Test 1*.

**Standard solution:** (L/900) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_S$ , in mg/mL of doxycycline, using the designated potency, in µg/mg of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector:** UV 345 nm

**Cell:** See *Table 1*.

Table 1

Tablet Strength (mg/Tablet)	Cell Size (cm)
75	0.5
100	0.5
150	0.2

#### Tolerances

**Level 1 (6 Tablets tested):** No individual value is more than 50% of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) dissolved in 20 min.

**Level 2 (6 Tablets tested):** NMT 2 individual values of the 12 tested are greater than 50% of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) in 20 min.

#### Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 1000 mL, degassed

**Apparatus 1 and Analysis:** Proceed as directed for *Buffer stage* in *Test 1*.

**Time:** 45 min

**Standard solution:** (L/1000) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector and Cell:** Proceed as directed for *Acid stage* in *Test 2*.

**Tolerances:** NLT 70% (Q) of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*. Proceed as directed for *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B, Procedure (711)*.

#### Acid stage

**Apparatus 1 and Time** • (RB 1-Jun-2012): Proceed as directed for *Acid stage* in *Test 1*.

**Medium:** 0.06 N hydrochloric acid; 900 mL

**Standard solution:** • Prepare the solutions as directed in *Table 2* from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS.

**Table 2**

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.17

• (RB 1-Jun-2012)

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector:** UV 345 nm

**Cell:** 0.2 cm

• **Blank:** *Medium*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL • (RB 1-Jun-2012)

**Tolerances:** See *Table 3*.

• **Table 3**

Level	Number of Tablets Tested	Tolerances	
		Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
$A_1$	6	No individual value exceeds 50% at 20 min.	No individual value exceeds 30% at 20 min.
$A_2$	6	Average of 12 units ( $A_1 + A_2$ ) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 12 units ( $A_1 + A_2$ ) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.
$A_3$	12	Average of 24 units ( $A_1 + A_2 + A_3$ ) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 24 units ( $A_1 + A_2 + A_3$ ) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.

• (RB 1-Jun-2012)

#### Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 1000 mL

**Apparatus 1:** 50 rpm

**Time:** 60 min

**Standard solution:** • Prepare the solutions as directed in *Table 4* from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS.

**Table 4**

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.15

• (RB 1-Jun-2012)

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector:** UV 345 nm

**Cell:** 0.2 cm

**Blank:** *Medium*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of doxycycline ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 1000 mL  
**Tolerances:** • See *Table 5*.

**Table 5**

Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
NLT 80% (Q) of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) is dissolved.	NLT 70% (Q) of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) is dissolved.

**Test 4:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*. Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, Method B, Procedure (711).

**Acid stage**

**Medium:** 0.06 N hydrochloric acid; 900 mL, degassed

**Apparatus 1:** 50 rpm

**Time:** 20 min

**Standard solution:** 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS.

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector:** UV 345 nm

**Cell:** 0.2-cm quartz

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances**

**Level 1 (6 Tablets tested):** No individual value is more than 30% of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) dissolved in 20 min.

**Level 2 (6 Tablets tested):** NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) in 20 min.

**Buffer stage**

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 1000 mL, degassed

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Standard solution:** 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*

**Sample solution:** Pass portions of the solution under test through a suitable filter. Calculate the concentration,  $C_s$ , in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS.

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 1000 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of doxycycline ( $C_{22}H_{24}N_2O_8$ ) is dissolved. • (RB 1-Jun-2012)

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****Change to read:**• **ORGANIC IMPURITIES**

**Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1.16 mg/mL of doxycycline hyclate in methanol and water (1:9). Transfer USP Doxycycline Hyclate RS to a suitable volumetric flask, and add methanol to 10% of the final volume. Sonicate for 5 min or until dissolved. Dilute with water to volume. Protect the solution from light. • Calculate the concentration, in mg/mL of doxycycline, using the designated potency, in  $\mu\text{g}/\text{mg}$  of doxycycline in USP Doxycycline Hyclate RS. • (RB 1-Jun-2012)

**Standard solution:** 0.02 mg/mL of doxycycline from the *Standard stock solution*. Protect the solution from light.

**Sensitivity solution:** 1  $\mu\text{g}/\text{mL}$  of doxycycline from the *Standard solution*. Protect the solution from light.

**System suitability stock solution:** 0.04 mg/mL each of USP Oxytetracycline Hydrochloride RS, USP Methacycline Hydrochloride RS, and USP Doxycycline Related Compound A RS. Protect the solution from light.

**System suitability solution:** Transfer 5 mL of the *Standard stock solution* into a 25-mL volumetric flask. Heat on a steam bath for 60 min, and gently evaporate to dryness on a hot plate (partial degradation of doxycycline to 4-epidoxycycline). Add 3 mL of the *System suitability stock solution* to the flask, and dilute with water to volume. Pass through a suitable filter. Protect the solution from light.

**System suitability**

**Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution*

**Suitability requirements**

**Signal-to-noise ratio:** NLT 10 for doxycycline, *Sensitivity solution*

**Resolution:** NLT 1.5 between doxycycline and 6-epidoxycycline, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Sample solution* and *Standard solution*  
 Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of doxycycline from the *Standard solution*  
 $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of doxycycline in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Table 6*)  
**Acceptance criteria:** See *Table 6*.

**Table 6**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxytetracycline	0.3	1.0	0.5
4-Epidoxycycline <sup>a</sup>	0.4	1.0	1.0
Methacycline	0.6	1.0	2.0
6-Epidoxycycline (doxycycline related compound A) <sup>b</sup>	0.7	0.86	2.0
Doxycycline	1.0	—	—

<sup>a</sup> (4*R*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide monohydrate.

<sup>b</sup> (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(Dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide monohydrate.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Doxycycline Hyclate RS
  - USP Doxycycline Related Compound A RS
  - 6-Epidoxycycline, or (4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-(dimethylamino)-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide.
  - C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub> 444.43
  - USP Methacycline Hydrochloride RS
  - USP Oxytetracycline Hydrochloride RS

## Doxycycline Hyclate Tablets

**DEFINITION**

Doxycycline Hyclate Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>).

**IDENTIFICATION**• **PROCEDURE**

**Sample solution:** Shake a suitable quantity of finely ground Tablets with methanol to obtain a solution containing the equivalent of 1 mg/mL of doxycycline, and filter. Use the filtrate as the *Sample solution*.

**Analysis:** Proceed as directed under *Identification—Tetracyclines* (193), *Method II*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

**Diluent:** 0.01 N hydrochloric acid

[NOTE—Throughout the following sections, protect the *Standard solution* and the *Sample solution* from light.]

**Standard solution:** 1.2 mg/mL of USP Doxycycline Hyclate RS in Diluent. [NOTE—Sonicate as necessary to dissolve.]

**System suitability solution:** Prepare 6 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Diluent*. Transfer 5 mL of this solution to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, dilute with *Diluent* to volume, and filter. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

**Sample solution:** Transfer an equivalent to 100 mg of doxycycline, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask, add 75 mL of *Diluent*, sonicate for 5 min, shake for 15 min, dilute with *Diluent* to volume, and mix. Pass through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 25-cm; packing L21

**Column temperature:** 60 ± 1°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for 4-epidoxycycline (the main degradation product), 6-epidoxycycline, and doxycycline are about 0.4, 0.7, and 1.0, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution* is dissolved.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms for a period of time that is 1.7 times the retention time of doxycycline.

Calculate the percentage of C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (P/1000) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

$P$  = potency of doxycycline, converted from µg/mg to mg/mg

**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS**

- **DISSOLUTION** (711) [NOTE—Use low-actinic glassware to prepare the solutions.]

**Test 1**

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm, the distance between the blade and the inside bottom of the flask being maintained at 4.5 ± 0.5 cm during the test

**Time:** 90 min

**Standard solution:** USP Doxycycline Hyclate RS in *Medium*

**Sample solution:** Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

**Spectrometric conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** UV-Vis**Analytical wavelength:** 276 nm**Tolerances:** NLT 85% (Q) of the labeled amount of  $C_{22}H_{24}N_2O_8$  is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** Water; 900 mL**Apparatus 2:** 50 rpm, the distance between the blade and the inside bottom of the vessel being maintained at  $4.5 \pm 0.5$  cm during the test**Time:** 30 min**Standard solution:** 22 µg/mL of doxycycline in *Medium*, prepared using USP Doxycycline Hyclate RS**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.**Spectrometric conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** UV-Vis**Analytical wavelength:** 276 nm**Cell:** 0.5 cm**Blank:** *Medium*

Calculate the percentage of doxycycline dissolved in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of doxycycline in the *Standard solution* (mg/mL) $L$  = Tablet label claim (mg) $V$  = volume of *Medium*, 900 mL**Tolerances:** NLT 85% (Q) of the labeled amount of doxycycline is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method I* (921): NMT 5.0%

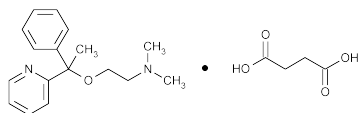
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
USP Doxycycline Hyclate RS

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**Doxylamine Succinate**


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 $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  388.46Ethanamine, *N,N*-dimethyl-2-[1-phenyl-

1-(2-pyridinyl)ethoxy]-, butanedioate (1:1).

2-[α-[2-(Dimethylamino)ethoxy]-α-methylbenzyl]pyridine succinate (1:1) [562-10-7].

» Doxylamine Succinate contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Doxylamine Succinate RS

**Identification**—**A:** *Ultraviolet absorption* (197U)—*Solution:* 20 µg per mL.*Medium:* 0.1 N hydrochloric acid.

Absorptivities at 262 nm, calculated on the dried basis, do not differ by more than 3.0%.

**B:** It meets the requirements under *Identification—Organic Nitrogenous Bases* (181).

**C:** Dissolve about 500 mg in 5 mL of water, and add a slight excess of 6 N ammonium hydroxide. Extract the liberated doxylamine with several portions of ether, discard the ether extracts, and evaporate the aqueous solution on a steam bath to dryness. Add 2 mL of 3 N hydrochloric acid, and again evaporate on the steam bath to dryness. Cool, add about 10 mL of ether, allow to stand for a few minutes, and decant the clear supernatant. Evaporate the ether solution to dryness, and dry the residue at 105° for 30 minutes; the succinic acid so obtained melts between 184° and 188°, the procedure for *Class I* being used (see *Melting Range or Temperature* (741)).

**Melting range**, *Class I* (741): between 103° and 108°, but the range between beginning and end of melting does not exceed 3°.

**Loss on drying** (731)—Dry it in vacuum over phosphorus pentoxide for 5 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Volatile related compounds**—Dissolve 650 mg in 20 mL of 0.1 N hydrochloric acid in a separator. Render the solution alkaline with 2.5 N sodium hydroxide, and immediately extract with four 25-mL portions of ether, filtering each extract through an ether-saturated pledget of cotton. Evaporate the combined ether extracts on a water bath with the aid of a current of air to dryness at a temperature not exceeding 50°, and dissolve the residue in 5 mL of alcohol. Inject about 1 µL of this solution into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a flame-ionization detector. Under typical conditions, the instrument contains a 2-m × 4-mm glass column containing 5% packing G16 and 5% packing G12 on 60- to 80-mesh S1A. The column is maintained at about 212°, the injection port and detector block are maintained at about 250°, and dry helium is used as the carrier gas. The total relative area of all extraneous peaks (except that of the solvent peak) does not exceed 2.0%, and the relative area of any individual extraneous peak does not exceed 1.0%.

**Assay**—Dissolve about 500 mg of Doxylamine Succinate, accurately weighed, in 80 mL of glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.42 mg of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ .

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**Doxylamine Succinate Oral Solution**


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» Doxylamine Succinate Oral Solution contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Doxylamine Succinate RS



**Identification**—Use a volume of Oral Solution equivalent to about 50 mg of doxylamine succinate, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with “Transfer the liquid to a separator.” The Oral Solution meets the requirements of the test.

**Assay**—Proceed with Oral Solution as directed under *Salts of Organic Nitrogenous Bases* (501), determining the absorbance at the wavelength of maximum absorbance at about 262 nm. Calculate the quantity, in mg, of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ) in each mL of the Oral Solution taken by the formula:

$$(0.05C / V)(A_U / A_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Doxylamine Succinate RS in the *Standard Preparation*; and  $V$  is the volume, in mL, of Oral Solution taken.

## Doxylamine Succinate Tablets

» Doxylamine Succinate Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Doxylamine Succinate RS

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 262 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Doxylamine Succinate RS in the same *Medium*.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask containing 65 mL of 0.1 N hydrochloric acid. Shake frequently during a 10-minute period, dilute with 0.1 N hydrochloric acid to volume, and mix. Allow the insoluble material to settle, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with 0.1 N hydrochloric acid to provide a solution containing approximately 25  $\mu\text{g}$  of doxylamine succinate per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Doxylamine Succinate RS in the same medium having a known concentration of about 25  $\mu\text{g}$  per mL in 1-cm cells at the wavelength of maximum absorbance at about 262 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of doxylamine succinate in the Tablet,  $C$  is the concentration, in  $\mu\text{g}$  per

mL, of USP Doxylamine Succinate RS in the Standard solution,  $D$  is the concentration, in  $\mu\text{g}$  per mL, of doxylamine succinate in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution, and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

### Assay—

**Mobile phase**—Transfer 340 mg of monobasic potassium phosphate, 150 mg of triethylamine hydrochloride, and 150 mg of sodium lauryl sulfate to a 100-mL volumetric flask, add 63 mL of water, and mix. Dilute with acetonitrile to volume, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Doxylamine Succinate RS in 0.1 M hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 M hydrochloric acid to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an amount of powder, equivalent to about 25 mg of doxylamine succinate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with 0.1 M hydrochloric acid to volume, and mix.

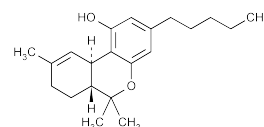
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 262-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Doxylamine Succinate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dronabinol



$C_{21}H_{30}O_2$  314.46

6*H*-Dibenzo[*b,d*]pyran-1-ol, 6*a*,7,8,10*a*-tetrahydro-6,6,

9-trimethyl-3-pentyl-, (6*aR-trans*)-.

(6*aR*,10*aR*)-6*a*,7,8,10*a*-Tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol [1972-08-3].

» Dronabinol is  $\Delta^9$ -tetrahydrocannabinol. It contains not less than 95.0 percent of  $C_{21}H_{30}O_2$ .

**Packaging and storage**—Preserve in tight, light-resistant glass containers in inert atmosphere. Store as per labeling instructions.

**USP Reference standards** (11)—USP  $\Delta^9$ -Tetrahydrocannabinol RSUSP *exo*-Tetrahydrocannabinol RS(6aR, 10aR)-6,6-Dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol  
C<sub>21</sub>H<sub>30</sub>O<sub>2</sub> 314.46**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** *Visualizing agent*—Transfer about 100 mg of Fast Blue B salt to a suitable flask containing about 100 mL of methanol, stir for about 5 minutes, and allow to settle. Decant the clear liquid into the sprayer reservoir. [NOTE—Prepare fresh daily.]

*Identification solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Apply separately 10  $\mu$ L each of the *Identification solution* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the plate in a chromatographic chamber that has been equilibrated (for about 2 minutes) with vapors from a solvent mixture of *n*-hexane and methylene chloride (1:1) until the solvent front has moved about 10 cm. Remove the plate from the developing chamber, quickly mark the solvent front, and allow the plate to dry at room temperature for about 5 minutes. Spray the plate with the *Visualizing agent* until it is uniformly damp (not saturated). Heat the plate at about 80° until the spots are developed: the color and *R<sub>F</sub>* value of the spots from the *Test solution* correspond to those obtained from the *Identification solution*.

**Related compounds**—[NOTE—Minimize exposure to air and light for the *Standard solution* and the *Test solution*. Analyze all samples within 24 hours.]

*Mobile phase*, *System suitability solution*, and *Standard preparation*—Proceed as directed in the *Assay*.

*Standard solution*—Dilute an accurately measured volume of the *Standard preparation* quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.004 mg per mL.

*Sensitivity solution*—Quantitatively dilute an accurately measured volume of the *Standard solution* with dehydrated alcohol to obtain a solution having a concentration of about 0.2  $\mu$ g per mL.

*Test solution*—Use the *Assay preparation*.

*Chromatographic system*—Proceed as directed in the *Assay*. In addition, chromatograph the *Sensitivity solution*, and calculate the signal-to-noise ratio, *S/N*, by the formula:

$$(2H)/h$$

in which *H* is the measured height of the peak, and *h* is the amplitude of the average measured baseline noise; the signal-to-noise ratio is not less than 10.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Limit (%)
Cannabinol	0.78	2.7	1.5
$\Delta^9$ -Tetrahydrocannabinol	1.00	1.0	—
<i>Exo</i> -tetrahydrocannabinol <sup>1</sup>	1.07	0.92	0.5
$\Delta^8$ -Tetrahydrocannabinol	1.18	0.90	2.0
Any other individual impurity	—	1.0	1.0

<sup>1</sup>(6aR, 10aR)-6,6-Dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol.

Calculate the percentage of each impurity in the portion of Dronabinol taken by the formula:

$$100(1/F)(CV/W)(r_U / r_S)$$

in which *F* is the relative response factor for each impurity (see *Table 1*); *C* is the concentration, in mg per mL, of  $\Delta^9$ -tetrahydrocannabinol in the *Standard solution*; *V* is the volume, in mL, of the *Test solution*; *W* is the weight, in mg, of Dronabinol taken to prepare the *Test solution*; *r<sub>U</sub>* is the peak area response of each impurity in the *Test solution*; and *r<sub>S</sub>* is the peak area response of  $\Delta^9$ -tetrahydrocannabinol in the *Standard solution*. In addition to not exceeding the limits in *Table 1*, not more than 5.0% of total impurities is found.

**Assay**—[NOTE—Minimize exposure to air and light for the *Standard preparation* and the *Assay preparation*. Analyze all samples within 24 hours.]

*Mobile phase*—Prepare a filtered and degassed mixture of methanol, water, tetrahydrofuran, and acetonitrile (45:25:20:10), making adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Transfer accurately measured volumes of USP  $\Delta^9$ -Tetrahydrocannabinol RS and USP *Exo*-tetrahydrocannabinol RS to a suitable volumetric flask, and dilute with dehydrated alcohol to prepare a solution that contains about 200  $\mu$ g of  $\Delta^9$ -tetrahydrocannabinol and about 10  $\mu$ g of *exo*-tetrahydrocannabinol per mL.

*Standard preparation*—Quantitatively dilute an accurately measured volume of USP  $\Delta^9$ -Tetrahydrocannabinol RS with dehydrated alcohol to obtain a solution having a known concentration of about 0.2 mg per mL.

*Assay preparation*—Transfer about 20 mg of Dronabinol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with dehydrated alcohol to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 228-nm detector and a 4.6-mm  $\times$  15-cm analytical column that contains 4- $\mu$ m packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 20°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between  $\Delta^9$ -tetrahydrocannabinol and *exo*-tetrahydrocannabinol is not less than 1.5; and the tailing factor of  $\Delta^9$ -tetrahydrocannabinol is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks. Calculate the quan-

tity, in mg, of  $C_{21}H_{30}O_2$  in the portion of Dronabinol taken by the formula:

$$CV(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of  $\Delta^9$ -tetrahydrocannabinol in the *Standard preparation*;  $V$  is the volume, in mL, of the *Assay preparation*; and  $r_U$  and  $r_S$  are the  $\Delta^9$ -tetrahydrocannabinol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dronabinol Capsules

### DEFINITION

Dronabinol Capsules contain dronabinol in Sesame Oil. Dronabinol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of dronabinol ( $C_{21}H_{30}O_2$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol, tetrahydrofuran, and water (71:5:24)

**System suitability stock solution:** 1.0 mg/mL of  $\Delta^8$ -tetrahydrocannabinol in methanol

**System suitability solution:** 0.5 mg/mL of USP  $\Delta^9$ -Tetrahydrocannabinol RS and 0.5 mg/mL of  $\Delta^8$ -tetrahydrocannabinol. Mix equal volumes of USP  $\Delta^9$ -Tetrahydrocannabinol RS and *System suitability stock solution*.

**Standard solution:** 0.2 mg/mL of USP  $\Delta^9$ -Tetrahydrocannabinol RS in dehydrated alcohol

**Sample solution:** Equivalent to 0.2 mg/mL of dronabinol, from Capsule contents (NLT 20) in dehydrated alcohol

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Guard column:** 4.6-mm  $\times$  30-mm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution* [NOTE—The relative retention times for  $\Delta^9$ -tetrahydrocannabinol and  $\Delta^8$ -tetrahydrocannabinol are about 1.0 and 1.14, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between dronabinol and  $\Delta^8$ -tetrahydrocannabinol, *System suitability solution*

**Tailing factor:** NMT 2.0 of  $\Delta^9$ -tetrahydrocannabinol, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{21}H_{30}O_2$  in the portion of Capsules taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of dronabinol from the *Sample solution*

$r_S$  = peak response of dronabinol from the *Standard solution*

$C_S$  = concentration of USP  $\Delta^9$ -Tetrahydrocannabinol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of dronabinol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Analysis:** Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances:** The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in NLT 15 but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in NLT 15 min but NMT 30 min.

- UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

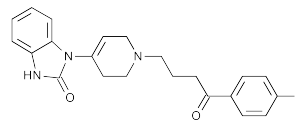
### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, in a cool place.

#### USP REFERENCE STANDARDS <11>

USP  $\Delta^9$ -Tetrahydrocannabinol RS

## Droperidol



$C_{22}H_{22}FN_3O_2$  379.43

2H-Benzimidazol-2-one, 1-[1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-1H-benzimidazol-2-one [548-73-2].

» Droperidol, dried in vacuum at 70° for 4 hours, contains not less than 98.0 percent and not more than 102.0 percent of  $C_{22}H_{22}FN_3O_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, under nitrogen, in a cool place.

#### USP Reference standards <11>

USP Droperidol RS

#### Identification—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 15  $\mu$ g per mL.

*Medium:* 0.1 N hydrochloric acid in isopropyl alcohol (1 in 10).

**Loss on drying** <731>—Dry it in vacuum at 70° for 4 hours; it loses not more than 5.0% of its weight.

**Residue on ignition** <281>: not more than 0.2%.

**Heavy metals, Method II** <231>: 0.002%.

**Limit of 4,4'-bis[1,2,3,6-tetrahydro-4-(2-oxo-1-benzimidazol-1-yl)pyridyl]butyrophene**—Dissolve about 30.0 mg of the sample in 70 mL of isopropyl alcohol in a 100-mL volumetric flask. Add 10.0 mL of 0.1 N hydrochloric acid, dilute with isopropyl alcohol to volume, and mix. The absorptivity of the solution in 1-cm cells at the wavelength of maximum absorbance at about 330 nm, with a suitable spectrophotometer, using a 1 in 10 solution of

0.1 N hydrochloric acid in isopropyl alcohol as the blank, is not more than 0.7 (equivalent to limit of 1.5%).

**Assay**—Dissolve about 240 mg of Droperidol, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.94 mg of  $C_{22}H_{22}FN_3O_2$ .

## Droperidol Injection

» Droperidol Injection is a sterile solution of Droperidol in Water for Injection, prepared with the aid of Lactic Acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{22}FN_3O_2$ , as the lactate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Droperidol RS

USP Endotoxin RS

**Identification**—

**A:** Dissolve about 25 mg of USP Droperidol RS in 10 mL of water containing 0.3 mL of 50% acetic acid in a separator to obtain a solution containing about 2.5 mg per mL. Transfer a volume of Injection, equivalent to about 25 mg of droperidol, to a second separator. Separately add 2 mL of ammonia TS to each separator, and mix. Extract each solution with two 10-mL portions of chloroform, collecting the chloroform extracts from the solutions in separate 50-mL beakers. Apply separately 20  $\mu$ L of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a chamber with an unequilibrated solvent system consisting of a mixture of ethyl acetate, chloroform, methanol, and acetate buffer (0.2 M sodium acetate adjusted with 50% acetic acid to a pH of 4.7) (54:23:18:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dinitrophenylhydrazine TS, then examine under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 35.7 USP Endotoxin Units per mg of droperidol.

**pH** (791): between 3.0 and 3.8.

**Chromatographic purity**—

*Mobile phase*, *System suitability preparation*, *Standard stock preparation*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Test preparation*—Use the *Assay preparation*.

*Procedure*—Inject a volume (about 100  $\mu$ L) of the *Test preparation* into the chromatograph, record the chromato-

gram of twice the retention time of droperidol, and measure the peak responses. Calculate the percentage of each impurity in the portion of Droperidol taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the sum of the responses of all the peaks: the sum of all impurities is not more than 2.0%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Borate buffer*—Dissolve 31 g of boric acid in about 800 mL of water. Slowly add sodium hydroxide solution (1 in 5) in small quantities until all of the boric acid is dissolved and the pH is constant at 7.0. Quantitatively transfer the solution to a glass-stoppered, 1000-mL volumetric flask, dilute with water to volume, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol, water, and *Borate buffer* (700:280:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard stock preparation*—Dissolve an accurately weighed quantity of USP Droperidol RS in methanol to obtain a solution having a known concentration of about 1 mg per mL.

*Standard preparation*—Transfer 5.0 mL of *Standard stock preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*System suitability preparation*—Prepare a solution of 4'-fluoroacetophenone in methanol containing about 1 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Standard stock preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of droperidol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

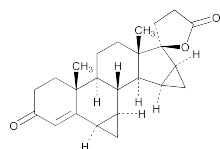
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between 4'-fluoroacetophenone and droperidol is not less than 5.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of droperidol ( $C_{22}H_{22}FN_3O_2$ ) in each mL of the Injection taken by the formula:

$$100(C / V)(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Droperidol RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Drospirenone



$C_{24}H_{30}O_3$  366.49  
(6R,7R,8R,9S,10R,13S,14S,15S,16S,17S)-1,3',4',6,6a,7,8,9,10,11,12,13,14,15,15a,16-Hexadecahydro-10,13-dimethylspiro-[17H-dicyclopropa[6,7:15,16]cyclopenta[a]phenanthrene-17,2'(5'H)-furan]-3,5'(2H)-dione; 17-Hydroxy-6 $\beta$ ,7 $\beta$ :15 $\beta$ ,16 $\beta$ -dimethylene-3-oxo-17  $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone [67392-87-4].

### DEFINITION

Drospirenone contains NLT 98.0% and NMT 102.0% of  $C_{24}H_{30}O_3$ , calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197M>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### Change to read:

- PROCEDURE**  
Solution A: Water  
Solution B: Acetonitrile  
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	63	37
2.0	63	37
16.0	52	48
23.0	52	48
31.0	20	80
39.0	20	80
39.1	63	37
49.0	63	37

**Diluent:** Acetonitrile and water (1:1)

**System suitability solution:** 60  $\mu$ g/mL of USP Drospirenone RS and 60  $\mu$ g/mL of USP Drospirenone Related Compound A RS in *Diluent*

**Standard solution:** 0.6 mg/mL of USP Drospirenone RS in *Diluent*

**Sample solution:** 0.6 mg/mL of Drospirenone in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 245 nm

**Column:** 4.6-mm  $\times$  25-cm; 3- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 5.0 between drospirenone and drospirenone related compound A, *System suitability solution*

**Tailing factor:** Between 0.8 and 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

- Calculate the percentage of drospirenone (ERR 1-May-2012) ( $C_{24}H_{30}O_3$ ) in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Drospirenone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of drospirenone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** <281>: NMT 0.1%
- HEAVY METALS**, *Method II* <231>: 20 ppm

#### Change to read:

### Organic Impurities

- PROCEDURE 1: LIMIT OF 1,2-DIMETHOXYETHANE AND DIISOPROPYL ETHER** (if present)

**Standard solution:** 0.1 mg/mL of 1,2-dimethoxyethane and 0.05 mg/mL of diisopropyl ether in dimethylformamide

**Sample solution:** 50 mg/mL of Drospirenone in dimethylformamide

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm  $\times$  30-m; 1.4- $\mu$ m coating of phase G43

**Temperature**

**Injector:** 160°

**Detector:** 250°

**Column:** See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	5	70	0
70	30	220	0

**Carrier gas:** Helium

**Flow rate:** 32  $\pm$  8 cm/s. [NOTE— For pressure-controlled systems, a column pressure of about 130 kPa is necessary.]

**Injector type:** Headspace

**Sample volume:** 2.0 mL/vial

**Vial treatment:** Maintain at 80° for 60 min before injection.

#### System suitability

**Sample:** *Standard solution*

[NOTE— The relative retention times for diisopropyl ether and 1,2-dimethoxyethane are about 0.6 and 1.0, respectively.]

**Suitability requirements****Relative standard deviation:** NMT 4.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 1,2-dimethoxyethane and diisopropyl ether in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of 1,2-dimethoxyethane or diisopropyl ether from the *Sample solution* $r_S$  = peak response of 1,2-dimethoxyethane or diisopropyl ether from the *Standard solution* $C_S$  = concentration of 1,2-dimethoxyethane or diisopropyl ether in the *Standard solution* (mg/mL) $C_U$  = concentration of Drospirenone in the *Sample solution* (mg/mL)**Acceptance criteria****Individual impurities:** NMT 0.2% of 1,2-dimethoxyethane and NMT 0.1% of diisopropyl ether• **PROCEDURE 2****Solution A:** Water**Solution B:** Acetonitrile**Mobile phase:** See *Table 3*.**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	63	37
2.0	63	37
16.0	52	48
23.0	52	48
31.0	20	80
39.0	20	80
39.1	63	37
49.0	63	37

**Diluent:** Acetonitrile and water (1:1)**System suitability solution:** 60 µg/mL of USP Drospirenone RS and 60 µg/mL of USP Drospirenone Related Compound A RS in *Diluent***Standard solution:** 0.6 µg/mL of USP Drospirenone RS in *Diluent***Sample solution:** 0.6 mg/mL of Drospirenone in *Diluent***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 195 nm and 245 nm**Column:** 4.6-mm × 25-cm; 3-µm packing L1**Column temperature:** 35 ± 5°**Flow rate:** 1 mL/min**Injection size:** 10 µL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 5.0 between drospirenone and drospirenone related compound A, *System suitability solution***Tailing factor:** Between 0.8 and 1.5, *Standard solution***Relative standard deviation:** • NMT 15.0%, • (ERR 1-Jul-2012) *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of drospirenone from the *Standard solution* $C_S$  = concentration of USP Drospirenone RS in the *Standard solution* (µg/mL) $C_U$  = concentration of Drospirenone in the *Sample solution* (µg/mL) $F$  = relative response factor for each individual impurity (see *Table 4*)

[NOTE—The percentage of hydroxydrospirenone is calculated at 195 nm.]

**Acceptance criteria**

[NOTE—Disregard any peaks that are less than 0.05% of the drospirenone peak.]

**Individual impurities:** See *Table 4*.**Table 4**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Hydroxymethyl drospirenone at 245 nm <sup>a</sup>	0.43	1.9	0.1
5-Hydroxydrospirenone at 195 nm <sup>b</sup>	0.57	0.57	0.1
17-Keto drospirenone at 245 nm <sup>c</sup>	0.77	1.2	0.1
Drospirenone at 245 and 195 nm	1.00	—	—
Drospirenone 6-ene at 245 nm <sup>d</sup>	1.04	0.30	0.1
Drospirenone related compound A at 245 nm <sup>e</sup>	1.11	1.0	0.1
6,7-Epidrospirenone at 245 nm <sup>f</sup>	1.14	1.3	0.1
6,7-Desmethylene drospirenone at 245 nm <sup>g</sup>	1.18	2.2	0.1
15-Methyl drospirenone at 245 nm <sup>h</sup>	1.34	0.99	0.1
7-Chloromethyl drospirenone at 245 nm <sup>i</sup>	1.38	1.6	0.1
7-Chloromethyl 17-epidrospirenone at 245 nm <sup>j</sup>	1.51	1.9	0.1
7-Hydroxymethyl 3,5(6)-diene drospirenone at 245 nm <sup>k</sup>	1.55	1.4	0.1

<sup>a</sup> 17-Hydroxy-7β-hydroxymethyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>b</sup> 5β,17β-Dihydroxy-6β,7β:15β,16β-dimethylene-17α-pregnan-21-carboxylic acid, γ-lactone.<sup>c</sup> 6β,7β:15β,16β-Dimethyleneandrosta-4-ene-3,17-dione.<sup>d</sup> 17-Hydroxy-15β,16β-methylene-3-oxo-17α-pregn-4,6-diene-21-carboxylic acid, γ-lactone.<sup>e</sup> 17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone; 17-epidrospirenone.<sup>f</sup> 17-Hydroxy-6α,7α:15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>g</sup> 17-Hydroxy-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>h</sup> 17-Hydroxy-15β-methyl-6β,7β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>i</sup> 17-Hydroxy-7β-chloromethyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>j</sup> 17-Hydroxy-7β-chloromethyl-15β,16β-methylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.<sup>k</sup> 17-Hydroxy-7β-hydroxymethyl-15β,16β-methylene-17α-pregn-3,5(6)-diene-21-carboxylic acid, γ-lactone.

Table 4 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified impurity at 245 nm	—	1.0	0.1
Total impurities	—	—	0.4

<sup>a</sup> 17-Hydroxy-7 $\beta$ -hydroxymethyl-15 $\beta$ ,16 $\beta$ -methylene-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>b</sup> 5 $\beta$ ,17 $\beta$ -Dihydroxy-6 $\beta$ ,7 $\beta$ :15 $\beta$ ,16 $\beta$ -dimethylene-17 $\alpha$ -pregnan-21-carboxylic acid,  $\gamma$ -lactone.

<sup>c</sup> 6 $\beta$ ,7 $\beta$ :15 $\beta$ ,16 $\beta$ -Dimethyleneandrost-4-ene-3,17-dione.

<sup>d</sup> 17-Hydroxy-15 $\beta$ ,16 $\beta$ -methylene-3-oxo-17 $\alpha$ -pregn-4,6-diene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>e</sup> 17-Hydroxy-6 $\beta$ ,7 $\beta$ :15 $\beta$ ,16 $\beta$ -dimethylene-3-oxo-17 $\beta$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone; 17-epidrospirenone.

<sup>f</sup> 17-Hydroxy-6 $\alpha$ ,7 $\alpha$ :15 $\beta$ ,16 $\beta$ -dimethylene-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>g</sup> 17-Hydroxy-15 $\beta$ ,16 $\beta$ -methylene-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>h</sup> 17-Hydroxy-15 $\beta$ -methyl-6 $\beta$ ,7 $\beta$ -methylene-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>i</sup> 17-Hydroxy-7 $\beta$ -chloromethyl-15 $\beta$ ,16 $\beta$ -methylene-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>j</sup> 17-Hydroxy-7 $\beta$ -chloromethyl-15 $\beta$ ,16 $\beta$ -methylene-3-oxo-17 $\beta$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.

<sup>k</sup> 17-Hydroxy-7 $\beta$ -hydroxymethyl-15 $\beta$ ,16 $\beta$ -methylene-17 $\alpha$ -pregn-3,5(6)-diene-21-carboxylic acid,  $\gamma$ -lactone.

## SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (781S)**  
Sample solution: 10 mg/mL in methanol  
Acceptance criteria:  $-187^{\circ}$  to  $-193^{\circ}$  at  $20^{\circ}$  on the anhydrous and solvent-free basis
- MELTING RANGE OR TEMPERATURE, Class 1 (741):**  
 $198^{\circ}$ – $203^{\circ}$ . [NOTE—Dry over silica gel for NLT 24 h before testing.]
- WATER DETERMINATION, Method I (921):** NMT 0.2%

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Drospirenone RS  
USP Drospirenone Related Compound A RS  
17-Hydroxy-6 $\beta$ ,7 $\beta$ :15 $\beta$ ,16 $\beta$ -dimethylene-3-oxo-17 $\beta$ -pregn-4-ene-21-carboxylic acid,  $\gamma$ -lactone.  
 $C_{24}H_{30}O_3$  366.49

## Drospirenone and Ethinyl Estradiol Tablets

### DEFINITION

Drospirenone and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of drospirenone ( $C_{24}H_{30}O_3$ ) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ( $C_{20}H_{24}O_2$ ).

### IDENTIFICATION

- A.** The retention times of the drospirenone and ethinyl estradiol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 132 g of dibasic ammonium phosphate in 0.8 L of water, adjust with phosphoric acid to a pH of 6.8, and dilute to 1 L.

**Solution B:** *Solution A* and water (1:24)

**Mobile phase:** Acetonitrile and *Solution B* (1:1). Adjust with phosphoric acid to a pH of 6.8.

**Standard solution:** ( $L/25$ ) mg/mL of USP Drospirenone RS and of USP Ethinyl Estradiol RS in *Mobile phase*, where  $L$  is the Tablet label claim, in mg/Tablet, of each compound

**Sample solution:** Transfer 10 Tablets to a 250-mL volumetric flask, add 230 mL of *Mobile phase*, and sonicate with intermittent shaking for NLT 10 min, or until the Tablets are completely dispersed. Equilibrate to room temperature. Dilute with *Mobile phase* to volume, and centrifuge the sample until a clear supernatant is obtained. Use the supernatant.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector 1:** UV 270 nm for drospirenone

**Detector 2:** Fluorescence, excitation wavelength at 285 nm, emission wavelength at 315 nm for ethinyl estradiol. [NOTE—*Detector 1* and *Detector 2* are connected in series.]

**Column:** 4.0-mm  $\times$  12.5-cm; 3- $\mu$ m packing L1

**Column temperature:**  $25 \pm 3^{\circ}$

**Flow rate:** 1.2 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** Between 0.8 and 1.8 for both drospirenone and ethinyl estradiol

**Relative standard deviation:** NMT 2.0% for both drospirenone and ethinyl estradiol

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of drospirenone ( $C_{24}H_{30}O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of drospirenone from the *Sample solution*

$r_S$  = peak response of drospirenone from the *Standard solution*

$C_S$  = concentration of USP Drospirenone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of drospirenone in the *Sample solution* (mg/mL)

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethinyl estradiol ( $C_{20}H_{24}O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of ethinyl estradiol from the *Sample solution*

$r_S$  = peak response of ethinyl estradiol from the *Standard solution*

$C_S$  = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of ethinyl estradiol; 90.0%–110.0% of drospirenone

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Test 1:** For drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol, or 3 mg of drospirenone and 0.02 mg of ethinyl estradiol

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** ( $L/900$ ) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Medium*, where  $L$  is the Tablet label claim of each compound. A volume of methanol not exceeding 2% of the final

total volume of solution may be used to aid in dissolving these compounds.

**Sample solution:** Pass a portion of the solution under test through a suitable cellulose filter of 0.45- $\mu$ m pore size, discarding the first 10 mL.

**Mobile phase:** Acetonitrile and water (40:60)

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 210 nm and detection at 315 nm, or with excitation at 281 nm and detection at 305 nm

**Column:** 4.6-mm  $\times$  6-cm; 3- $\mu$ m packing L1

**Column temperature:** 22°

**Flow rate:** 1 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 for both drospirenone and ethinyl estradiol

**Tailing factor:** Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol

**Relative standard deviation:** NMT 3% for both drospirenone and ethinyl estradiol

**Samples:** *Standard solution* and *Sample solution*

[NOTE—In *Medium*, drospirenone is partially converted into 17-epidrospirenone, which has a relative retention time of approximately 1.2 relative to drospirenone. The amount of drospirenone dissolved is calculated from the sum of drospirenone and 17-epidrospirenone.]

Calculate the percentage of drospirenone and ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 85% (Q) of the labeled amount of drospirenone and NLT 75% (Q) of the labeled amount of ethinyl estradiol is dissolved.

**Test 2:** For drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol. If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** ( $L/900$ ) mg/mL of USP Drospirenone RS and of USP Ethinyl Estradiol RS in *Medium*, where  $L$  is the Tablet label claim of each compound.

**Sample solution:** Centrifuge a portion of the solution under test at 3500 rpm for 15 min, and use the supernatant.

**Mobile phase:** Acetonitrile, methanol, and water (40:5:55)

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 280 nm and detection at 310 nm

**Column:** 4.6-mm  $\times$  10-cm; 3- $\mu$ m packing L1

**Temperature**

**Column:** 30°

**Autosampler:** 5°

**Flow rate:** 1 mL/min

**Injection size:** 200  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2 for both drospirenone and ethinyl estradiol

**Relative standard deviation:** NMT 3% for both drospirenone and ethinyl estradiol

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of drospirenone and ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of drospirenone and NLT 85% (Q) of the labeled amount of ethinyl estradiol is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A:** Acetonitrile, methanol, and water (26:19:55)

**Solution B:** Acetonitrile, methanol, and water (76:19:5)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Flow (mL/min)	Solution A (%)	Solution B (%)
0	0.5	90	10
40	0.5	90	10
53	0.5	0	100
59	1.0	0	100
60	0.5	90	10
70	0.5	90	10

**System suitability stock solution:** ( $L_1 \times 18/100$ )  $\mu$ g/mL of USP Drospirenone RS in *Solution A*, where  $L_1$  is the label claim ( $\mu$ g/Tablet) of drospirenone

**System suitability solution:** Transfer 1.0 mL of the *System suitability stock solution* into a 10-mL volumetric flask, add 1.0 mL of 0.1 N HCl, and heat for 30 min in a 40° water bath. Immediately add 1 mL of 0.1 N NaOH and allow to reach room temperature. Dilute with *Solution A* to volume to obtain a solution containing drospirenone and 17-epidrospirenone. [NOTE—NaOH must be added immediately after heating for the reaction to proceed properly. The drospirenone to 17-epidrospirenone ratio must be between 3:1 and 7:1.]

**Standard solution:** ( $L_1 \times 15/1000$ )  $\mu$ g/mL of USP Drospirenone RS, ( $L_2 \times 30/1000$ )  $\mu$ g/mL of USP Ethinyl Estradiol RS, and ( $L_2 \times 30/1000$ )  $\mu$ g/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where  $L_1$  and  $L_2$  are the label claim ( $\mu$ g/Tablet) of drospirenone and ethinyl estradiol, respectively

**Sensitivity solution:** ( $L_1 \times 15/10,000$ )  $\mu$ g/mL of USP Drospirenone RS, ( $L_2 \times 30/10,000$ )  $\mu$ g/mL of USP Ethinyl Estradiol RS, and ( $L_2 \times 30/10,000$ )  $\mu$ g/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where  $L_1$  and  $L_2$  are the label claim ( $\mu$ g/Tablet) of dro-



spirenone and ethinyl estradiol, respectively, prepared from the *Standard solution*

**Sample solution:** Transfer 15 Tablets to a 10-mL glass-stoppered test tube, and add 5.0 mL of *Solution A*. Shake vigorously, sonicate for NLT 5 min, and place in an ice bath for NLT 10 min. Centrifuge the sample at least until an almost clear supernatant is obtained. Filter the supernatant, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector 1:** UV 222 nm

**Detector 2:** Fluorescence, excitation wavelength at 215 nm, emission wavelength at 315 nm. Monitor the signal at 344 nm between 37 and 42 min. [NOTE—*Detector 1* and *Detector 2* are connected in series. Use the response at 344 nm to quantify ethinyl estradiol related compound B.]

**Column:** 3.0-mm × 30-cm; 3-μm packing L1 followed, in series, by a 4.6-mm × 10-cm; chromolith packing L1

**Column temperature:** 40°

**Flow rate:** See *Table 1*.

**Injection size:** 20 μL

#### System suitability

**Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution*

#### Suitability requirements

**Tailing Factor:** Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol, *Standard solution*

**Resolution:** NLT 2.0 between drospirenone and

17-epidrospirenone, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for both drospirenone and ethinyl estradiol, *Standard solution*

**Signal-to-noise ratio:** NLT 10 for drospirenone and ethinyl estradiol related compound B and NLT 7.0 for ethinyl estradiol, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Identify the ethinyl estradiol degradation products using the relative retention times given in *Table 2*. Calculate the percentage of each ethinyl estradiol degradation product and unspecified degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each ethinyl estradiol degradation product from the *Sample solution*

$r_S$  = peak response of ethinyl estradiol from the *Standard solution*

$C_S$  = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of ethinyl estradiol in the *Sample solution* (μg/mL)

$F$  = relative response factor for each degradation product (see *Table 2*)

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of ethinyl estradiol related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of ethinyl estradiol related compound B from the *Sample solution*

$r_S$  = peak response of ethinyl estradiol related compound B from the *Standard solution*

$C_S$  = concentration of USP Ethinyl Estradiol Related Compound B RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of ethinyl estradiol in the *Sample solution* (μg/mL)

**Samples:** *Standard solution* and *Sample solution*

Identify the drospirenone degradation products using the relative retention times given in *Table 3*. Calculate the percentage of each drospirenone degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each drospirenone degradation product from the *Sample solution*

$r_S$  = peak response of drospirenone from the *Standard solution*

$C_S$  = concentration of USP Drospirenone RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of drospirenone in the *Sample solution* (μg/mL)

$F$  = relative response factor for each degradation product (see *Table 3*)

#### Acceptance criteria

[NOTE—Report only degradation products greater than 0.1%.]

**Individual degradation products:** See *Table 2* for ethinyl estradiol and *Table 3* for drospirenone.

**Total degradation products:** NMT 3.5%

**Table 2**

Name	Detection Mode	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%) <sup>a</sup>	Acceptance Criteria, NMT (%) <sup>b</sup>
6α-Hydroxy ethinyl estradiol <sup>c</sup>	Fl (215 nm/315 nm) <sup>g</sup>	0.25	0.73	0.3	0.3
6β-Hydroxy ethinyl estradiol <sup>d</sup>	Fl (215 nm/315 nm)	0.27	0.64	0.3	0.3
6-Keto ethinyl estradiol <sup>e</sup>	UV 222 nm	0.41	2.3	1.5	0.5
Ethinyl estradiol related compound B <sup>f</sup>	Fl (215 nm/344 nm)	0.88	—	1.0	1.0
Ethinyl estradiol	Fl (215 nm/315 nm)	1.0	—	—	—
Any unspecified degradation product	Fl (215 nm/315 nm) and UV 222 <sup>h</sup>	—	1.0	0.3	0.5
Total degradation product	—	—	—	3.0	2.5

<sup>a</sup> Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.

<sup>b</sup> Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.

<sup>c</sup> 19-Nor-6α,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.

<sup>d</sup> 19-Nor-6β,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.

<sup>e</sup> 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol-6-one.

<sup>f</sup> Δ<sup>9</sup>,11-Ethinyl estradiol. 19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.

<sup>g</sup> Fl = Fluorescence.

<sup>h</sup> Determine unknown impurities using both modes of detection. Report the values from the detection mode that yield higher impurity levels.

Table 3

Name	Detection Mode (λ nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%) <sup>a</sup>	Acceptance Criteria, NMT (%) <sup>b</sup>
Drospirenone	UV 222	0.75	—	—	—
17-Epidrospirenone <sup>c</sup>	UV 222	0.83	1.0	0.3	0.3
Ethinyl estradiol	UV 222	1.0	—	—	—
Any unspecified degradation product	UV 222	—	1.0	0.3	0.5
Total degradation product	—	—	—	0.5	1.0

<sup>a</sup> Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.

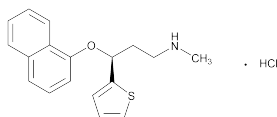
<sup>b</sup> Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.

<sup>c</sup> 17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Drospirenone RS
  - USP Ethinyl Estradiol RS
  - USP Ethinyl Estradiol Related Compound B RS
  - 19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.
  - C<sub>20</sub>H<sub>22</sub>O<sub>2</sub> 294.39

## Duloxetine Hydrochloride



C<sub>18</sub>H<sub>19</sub>NOS · HCl 333.88  
 2-Thiophenepropylamine, *N*-methyl-γ-(1-naphthalenyloxy)-, hydrochloride, (*S*)-;  
 (+)-(*S*)-*N*-Methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [136434-34-9].

## DEFINITION

Duloxetine Hydrochloride contains NLT 97.0% and NMT 102.0% of C<sub>18</sub>H<sub>19</sub>NOS · HCl, calculated on the dried basis.

## IDENTIFICATION

### Change to read:

- **A. INFRARED ABSORPTION** (197K) • (ERR 1-Jul-2012)
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the duloxetine *S*-isomer from the *System suitability solution* in the test for *Limit of Duloxetine Related Compound A*.

### Change to read:

- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)
  - **Sample solution:** 5 mg/mL in methanol
  - **Acceptance criteria:** Meets the requirements • (ERR 1-Jul-2012)

## ASSAY

### PROCEDURE

Protect solutions of duloxetine from light.  
**Buffer:** 2.9 g/L of phosphoric acid in water. Adjust with sodium hydroxide solution to a pH of 2.5. To each L of this solution add 10.3 g of sodium 1-hexanesulfonate monohydrate, and dissolve.

**Mobile phase:** Acetonitrile, *n*-propanol, and *Buffer* (13:17:70)

**Diluent:** Acetonitrile and water (25:75)

**System suitability solution:** 0.2 mg/mL of USP Duloxetine Hydrochloride RS in *Mobile phase*. Heat the solution to at least 40° for a minimum of 1 h. [NOTE—The resulting solution contains duloxetine impurity B, duloxetine impurity C, duloxetine impurity D, duloxetine impurity E, and duloxetine related compound F.]

**Standard solution:** 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

**Sample solution:** 0.1 mg/mL of Duloxetine Hydrochloride in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L7

**Column temperature:** 40 ± 3°

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

**Run time:** 2 times the retention time of duloxetine

### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

### Suitability requirements

**Resolution:** NLT 1.5 between duloxetine and duloxetine related compound F peaks

**Tailing factor:** NMT 1.5 for the duloxetine peak

**Relative standard deviation:** NMT 1.0% for the duloxetine peak

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of duloxetine hydrochloride (C<sub>18</sub>H<sub>19</sub>NOS · HCl) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Duloxetine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

## IMPURITIES

• **HEAVY METALS, Method II** (231): NMT 10 ppm

• **RESIDUE ON IGNITION** (281): NMT 0.2%

### ORGANIC IMPURITIES

Protect solutions of duloxetine from light.

**Buffer, Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the *Assay*.

**Sensitivity solution:** 0.2 μg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

**Sample solution:** 0.2 mg/mL of Duloxetine Hydrochloride in *Diluent*

**Chromatographic system:** Proceed as directed in the Assay

**Run time:** 2.4 times the retention time of duloxetine

**System suitability**

**Samples:** *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 1* for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between duloxetine impurity C and duloxetine impurity D; NLT 1.5 between duloxetine and duloxetine related compound F, *System suitability solution*

**Tailing factor:** NMT 1.5 for the duloxetine peak, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for the duloxetine peak, *System suitability solution*

**Signal-to-noise ratio:** NLT 20 for the duloxetine peak, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of any individual impurity in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of the responses of all the peaks from the *Sample solution*

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Duloxetine impurity B <sup>a,g</sup>	0.15	0.36	—
Duloxetine impurity C <sup>b,g</sup>	0.43	1.0	—
Duloxetine impurity D <sup>c,g</sup>	0.48	1.8	—
Duloxetine impurity E <sup>d,g</sup>	0.74	1.0	—
Duloxetine	1.0	—	—
Duloxetine related compound F <sup>e</sup>	1.1	1.0	0.5
Duloxetine impurity G <sup>f,g</sup>	1.4	0.51	—
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.6

<sup>a</sup> 3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol.

<sup>b</sup> 4-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.

<sup>c</sup> Naphthalen-1-ol.

<sup>d</sup> 1-(3-(Methylamino)-1-(thiophen-2-yl)propyl)naphthalen-2-ol.

<sup>e</sup> (S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine.

<sup>f</sup> 1-Fluoronaphthalene.

<sup>g</sup> Controlled at *Any individual unspecified impurity* level.

**• LIMIT OF DULOXETINE RELATED COMPOUND A**

**Mobile phase:** Hexane and isopropyl alcohol (83:17). To 1 L of this mixture add 2 mL of diethylamine.

**System suitability solution:** 0.1 mg/mL each of USP Duloxetine Hydrochloride RS and USP Duloxetine Related Compound A RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**Sensitivity solution:** 0.1 µg/mL of USP Duloxetine Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Duloxetine Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L40

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**Run time:** 2 times the retention time of duloxetine

**System suitability**

**Samples:** *Sensitivity solution* and *System suitability solution*

[NOTE—The relative retention times for duloxetine and duloxetine related compound A are 1.0 and 1.3, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.5 between duloxetine and duloxetine related compound A, *System suitability solution*

**Tailing:** Between 0.8 and 1.5 each for duloxetine and duloxetine related compound A peaks, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for the duloxetine peak, *System suitability solution*

**Signal-to-noise ratio:** NLT 3, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of duloxetine related compound A in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for duloxetine related compound A from the *Sample solution*

$r_T$  = sum of the responses of duloxetine and duloxetine related compound A peaks from the *Sample solution*

**Acceptance criteria:** NMT 0.5%

**SPECIFIC TESTS**

- LOSS ON DRYING** <731>: Dry at 105° for 3 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Protect from light. Store at room temperature.
- USP REFERENCE STANDARDS** <11>
  - USP Duloxetine Hydrochloride RS
  - USP Duloxetine Related Compound A RS
  - (R)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine hydrochloride.
  - C<sub>18</sub>H<sub>19</sub>NOS · HCl 333.88

## Duloxetine Delayed-Release Capsules

**DEFINITION**

Duloxetine Delayed-Release Capsules contain an amount of Duloxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of duloxetine (C<sub>18</sub>H<sub>19</sub>NOS).

**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197S>

**Spectral range:** 1650 cm<sup>-1</sup> to 900 cm<sup>-1</sup>

**Standard:** 1 mg/mL of USP Duloxetine Hydrochloride RS in methylene chloride. Shake the contents, and sonicate for 1 min. Transfer 15 mL of filtrate into a separatory funnel, and add 15 mL of pH 7.5 phosphate buffer. Collect the organic layer, and evaporate to dryness. Redis-

solve the residue with a few drops of methylene chloride, and transfer to a KBr or NaCl plate. Allow it to dry.

**Sample:** 1 mg/mL of duloxetine, from the contents of NLT 10 Capsules in methylene chloride. Proceed as directed for the *Standard*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

Protect solutions of duloxetine from light.

**Buffer A:** 3.4 g/L of monobasic potassium phosphate in water. To 1 L of this solution add 15 mL of triethylamine, and adjust with phosphoric acid to a pH of 5.5.

**Buffer B:** 0.2 g/L of monobasic ammonium phosphate and 4.5 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 8.0.

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer A* (323:90:587)

**Diluent:** Methanol and *Buffer B* (50:50)

**System suitability solution:** 0.1 mg/mL USP Duloxetine Hydrochloride RS, 0.05 mg/mL of 1-naphthol, 0.01 mg/mL of USP Duloxetine Related Compound F RS, and 0.025 mg/mL of USP Duloxetine Related Compound H RS, in *Diluent*. [NOTE—Add 1 mL of methanol before diluting to volume to assist with dissolving contents. Duloxetine related compound H is used for peak identification purposes in this solution.]

**Standard solution:** 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

**Sample solution:** Nominally 0.1 mg/mL of duloxetine from the contents of NLT 5 Capsules, in *Diluent*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

[NOTE—It is recommended to preheat the *Mobile phase* to 45°.]

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 7.5-cm; 3- or 3.5-μm packing L7

**Column temperature:** 45°

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

**Run time:** 6 times the retention time of duloxetine

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* under *Organic Impurities* for relative retention times.]

### Suitability requirements

**Resolution:** NLT 1.6 between duloxetine and duloxetine related compound F; NLT 2 between 1-naphthol and duloxetine related compound H, *System suitability solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of duloxetine (C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of duloxetine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of duloxetine free base, 297.42

$M_{r2}$  = molecular weight of duloxetine hydrochloride, 333.88

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION <711>

**Acid stage medium:** 0.1 N hydrochloric acid; 1000 mL  
**Time:** 2 h

**Buffer stage medium:** pH 6.8 phosphate buffer; 1000 mL

**Time:** 60 min for Capsules containing 20% w/w pellets; 90 min for Capsules containing 32% w/w pellets

**Apparatus 1:** 100 rpm

**Buffer A and Mobile phase:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.28 mg/mL of USP Duloxetine Hydrochloride RS in *Buffer stage medium*. Use a small amount of methanol, not exceeding 2% of the final volume, to dissolve duloxetine.

**Acid stage standard solution:** 2.3 μg/mL of duloxetine hydrochloride, from the *Standard stock solution* diluted with *Buffer stage medium*

**Buffer stage standard solution:** 23 μg/mL of duloxetine hydrochloride, from the *Standard stock solution* diluted with *Buffer stage medium*

**Sample solution:** After 2 h in the *Acid stage medium*, pass a portion of the solution under test through a suitable filter. Transfer the basket containing the pellets to the vessel containing the *Buffer stage medium*. After the appropriate time in the *Buffer stage medium*, pass a portion of the solution under test through a suitable filter.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 7.5-cm; 3-μm packing L7

**Column temperature:** 45°

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

### System suitability

**Sample:** *Acid stage standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Acid stage standard solution*, *Buffer stage standard solution*, and *Sample solution*

Calculate the percentage of duloxetine released in the *Acid stage medium* ( $P_A$ ):

$$P_A =$$

$$\{(r_U/r_S) + [(r_{2U}/r_S) \times 1/F]\} \times C_S/L \times V \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of duloxetine from the *Sample solution*

$r_S$  = peak response of duloxetine from the *Acid stage standard solution*

$r_{2U}$  = peak response for 1-naphthol from the *Sample solution*

$F$  = relative response factor for 1-naphthol, 0.49  
 $C_S$  = concentration of duloxetine hydrochloride in the *Acid stage standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$V$  = volume of *Medium*, 1000 mL

$M_{r1}$  = molecular weight of duloxetine free base, 297.42

$M_{r2}$  = molecular weight of duloxetine hydrochloride, 333.88

Calculate the percentage of duloxetine released in the *Buffer stage medium*:

$$\text{Result} = [(r_U/r_S) \times C_S/L \times V \times (M_{r1}/M_{r2}) \times 100] + P_A$$

- $r_U$  = peak response of duloxetine from the *Sample solution*  
 $r_S$  = peak response of duloxetine from the *Buffer stage standard solution*  
 $C_S$  = concentration of duloxetine hydrochloride in the *Buffer stage standard solution* (mg/mL)  
 $L$  = label claim (mg/Capsule)  
 $V$  = volume of *Medium*, 1000 mL  
 $M_{r1}$  = molecular weight of duloxetine free base, 297.42  
 $M_{r2}$  = molecular weight of duloxetine hydrochloride, 333.88  
 $P_A$  = percentage of duloxetine released in the *Acid stage medium*

**Tolerances**

**Acid stage:** No individual unit releases more than 10% of the labeled amount of duloxetine in 2 h.

**Buffer stage**

**For Capsules containing 20% w/w pellets:** NLT 75% (Q) of the labeled amount of duloxetine is dissolved in 60 min.

**For Capsules labeled to contain 32% w/w pellets:** NLT 75% (Q) of the labeled amount of duloxetine is dissolved in 90 min.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

Protect solutions of duloxetine from light.

**Buffer A, Buffer B, Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- $r_U$  = peak response of each impurity from *Sample solution*  
 $r_T$  = sum of the responses of all the peaks from *Sample solution*

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Duloxetine	1.0	—
Duloxetine related compound F <sup>a,d</sup>	1.1	—
1-Naphthol <sup>b,d</sup>	1.5	—
Duloxetine related compound H <sup>c</sup>	2.2	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.4

<sup>a</sup> (S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine hydrochloride.

<sup>b</sup> Naphthalen-1-ol.

<sup>c</sup> (S)-4-{Methyl[3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propyl]amino}-4-oxobutanoic acid.

<sup>d</sup> For system suitability purposes only.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11):**

USP Duloxetine Hydrochloride RS

USP Duloxetine Related Compound F RS

(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine hydrochloride.

C<sub>18</sub>H<sub>19</sub>NOS · HCl 333.88

USP Duloxetine Related Compound H RS

(S)-4-{Methyl[3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propyl]amino}-4-oxobutanoic acid.

C<sub>22</sub>H<sub>23</sub>NO<sub>4</sub>S 397.49

**Absorbable Dusting Powder**

» Absorbable Dusting Powder is an absorbable powder prepared by processing cornstarch and intended for use as a lubricant for surgical gloves. It contains not more than 2.0 percent of magnesium oxide.

**Packaging and storage—**Preserve in well-closed containers. It may be preserved in sealed paper packets.

**Identification—**A 1 in 10 suspension is colored purplish blue to deep blue by iodine TS.

**Stability to autoclaving—**Transfer about 2 g to a suitable paper packet, and seal or close the packet with a double fold. Wrap the paper packet in muslin, transfer to an autoclave, heat to 121° for 30 minutes, and cool: the powder is not caked, and any lumps are easily crushed between the fingers.

**Sedimentation—**Boil 100 mL of a 1 in 10 suspension in water for 20 minutes. Cool, transfer to a 100-mL graduated cylinder, dilute with water to volume, and allow to stand undisturbed for 24 hours: the volume occupied by the settled Powder is between 45 mL and 75 mL.

**pH (791):** between 10.0 and 10.8, in a 1 in 10 suspension.

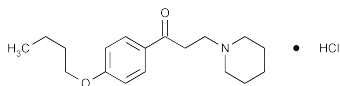
**Loss on drying (731)—**Dry about 2 g, accurately weighed, at 105° to constant weight: it loses not more than 12% of its weight.

**Residue on ignition (281)—**Heat about 1 g, accurately weighed, in a covered platinum crucible until most of the carbon is burned away, but do not ignite the sample. Remove the cover, and ignite to constant weight: not more than 3.0% of residue remains.

**Heavy metals, Method II (231):** 0.001%.

**Assay for magnesium oxide—**Weigh accurately about 2.5 g, and transfer to a beaker. Add 25 mL of water and 2 mL of 3 N hydrochloric acid, and stir the mixture for 5 minutes. Add 5 mL of hydroxylamine hydrochloride solution (1 in 10), 15 mL of ammonia-ammonium chloride TS, 5 mL of potassium cyanide solution (1 in 10), and 5 drops of eriochrome black TS, mix, and titrate with 0.05 M edetate disodium VS until the solution becomes distinctly blue in color. Each mL of 0.05 M edetate disodium is equivalent to 2.015 mg of MgO.

## Dyclonine Hydrochloride



$C_{18}H_{27}NO_2 \cdot HCl$  325.87

1-Propanone, 1-(4-butoxyphenyl)-3-(1-piperidinyl)-hydrochloride.

4'-Butoxy-3-piperidinopropiophenone hydrochloride [536-43-6].

» Dyclonine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{27}NO_2 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Dyclonine Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** Add 2 mL of silver nitrate TS to 10 mL of Dyclonine Hydrochloride solution (1 in 100): a white precipitate is formed. Add 2 mL of nitric acid, centrifuge, and discard the supernatant. Wash the precipitate twice by adding 10 mL of 2 N nitric acid, centrifuging, and discarding the supernatant: the precipitate so obtained is soluble in 6 N ammonium hydroxide.

**Melting range** (741): between 173° and 178°.

**pH** (791): between 4.0 and 7.0, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Dyclonine Hydrochloride Gel*.

*Assay preparation*—Transfer about 50 mg of Dyclonine Hydrochloride, accurately weighed, to a 500-mL volumetric flask, dissolve in 0.001 N phosphoric acid, dilute with 0.001 N phosphoric acid to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{27}NO_2 \cdot HCl$  in the portion of Dyclonine Hydrochloride taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dyclonine Hydrochloride Gel

» Dyclonine Hydrochloride Gel contains not less than 90.0 percent and not more than 110.0 per-

cent of the labeled amount of  $C_{18}H_{27}NO_2 \cdot HCl$ . It may contain suitable stabilizers and antimicrobial agents.

**Packaging and storage**—Preserve in collapsible, opaque plastic tubes or in tight, light-resistant glass containers. [NOTE—Do not use aluminum or tin tubes.]

**USP Reference standards** (11)—

USP Dyclonine Hydrochloride RS

**Identification**—Shake a portion of Gel, equivalent to about 400 mg of dyclonine hydrochloride, with 25 mL of chloroform, and allow the layers to separate. Remove the chloroform layer, evaporate on a steam bath to dryness, and dry the residue at 105° for 1 hour: the dyclonine hydrochloride so obtained responds to the *Identification* tests under *Dyclonine Hydrochloride*.

**pH** (791): between 2.0 and 4.0.

**Assay**—

*Mobile phase*—Dissolve 0.20 g of monobasic potassium phosphate and 0.45 mL of *n*-heptylamine in about 350 mL of water. Adjust with phosphoric acid to a pH of 3.0, dilute with water to 400 mL, add 600 mL of acetonitrile, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dyclonine Hydrochloride RS in 0.001 N phosphoric acid to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer an accurately measured portion of Gel, equivalent to about 5.0 mg of dyclonine hydrochloride, to a 50-mL volumetric flask. Add 10 mL of 0.001 N phosphoric acid, and sonicate to dissolve the gel. Dilute with 0.001 N phosphoric acid to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  25-cm column that contains 5- $\mu$ m diameter packing L13. The flow rate is about 1.2 mL per minute. Adjust the flow rate, if necessary, so that the retention time of dyclonine hydrochloride is not less than 5 minutes. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0 and the relative standard deviation is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dyclonine hydrochloride ( $C_{18}H_{27}NO_2 \cdot HCl$ ) in the portion of Gel taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dyclonine Hydrochloride Topical Solution

» Dyclonine Hydrochloride Topical Solution is a sterile, aqueous solution of Dyclonine Hydrochloride. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{18}H_{27}NO_2 \cdot HCl$ . It may contain suitable stabilizers and antimicrobial agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Dyclonine Hydrochloride RS

**Identification**—To a volume of Topical Solution, equivalent to about 300 mg of dyclonine hydrochloride, add 15 mL of chloroform, shake, and allow the layers to separate. Remove a portion of the chloroform solution, evaporate on a steam bath to dryness, and dry the residue at 105° for 1 hour: the dyclonine hydrochloride so obtained responds to the *Identification* tests under *Dyclonine Hydrochloride*.

**Sterility** (71): meets the requirements.

**pH** (791): between 3.0 and 5.0.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Dyclonine Hydrochloride Gel*.

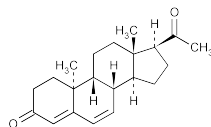
*Assay preparation*—Transfer an accurately measured volume of Topical Solution, equivalent to about 50 mg of dyclonine hydrochloride, to a 500-mL volumetric flask. Dilute with water to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{27}NO_2 \cdot HCl$  in each mL of the Topical Solution taken by the formula:

$$500(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of the Topical Solution taken, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dydrogesterone



$C_{21}H_{28}O_2$  312.45  
Pregna-4,6-diene-3,20-dione, (9 $\beta$ ,10 $\alpha$ )-.  
9 $\beta$ ,10 $\alpha$ -Pregna-4,6-diene-3,20-dione [152-62-5].

» Dydrogesterone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{21}H_{28}O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Dydrogesterone RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 6  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 285 nm, calculated on the dried basis, do not differ by more than 2.5%.

**Melting range** (741): between 167° and 171°.

**Specific rotation** (781S): between –442° and –462°.

*Test solution:* 10 mg per mL, in trichloroethane.

**Loss on drying** (731)—Dry it in vacuum at 50° for 1 hour: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Chromatographic purity**—

*Mobile phase, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Test solution*—Prepare a solution of Dydrogesterone in *Mobile phase* having a concentration of about 0.1 mg per mL.

*Procedure*—Inject about 20  $\mu$ L of the *Test solution* into the chromatograph, record the chromatograms for not less than 20 minutes, and measure the peak area responses. The sum of the areas of the secondary peaks, is not more than 2.0% of the total peak area.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of water, alcohol, and acetonitrile (530:260:210). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dydrogesterone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer about 100 mg of Dydrogesterone, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*System suitability preparation*—Transfer 10 mg of dydrogesterone to a 100-mL volumetric flask, add 30 mL of alcohol, and mix to dissolve the solid. Add 1 mL of 0.2 N sodium hydroxide, and heat the mixture at 85° for 10 minutes. Cool to room temperature, neutralize with 1 mL of 0.2 N hydrochloric acid, add 20 mL of acetonitrile, dilute with water to volume, and mix. This solution contains dydrogesterone and 17  $\alpha$ -dydrogesterone.

*Chromatographic system*—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains 3- $\mu$ m packing L1 and is maintained at 40°. The flow rate is about 1 mL per minute. Chromatograph 20  $\mu$ L of the *System suitability preparation*, and record the peak responses as directed under *Procedure*: the resolution between the dydrogesterone and 17  $\alpha$ -dydrogesterone is not less than 5, and the relative standard deviation of dydrogesterone peak responses from replicate injections is not more than 1.5%. The relative retention times are about 1.0 for dydrogesterone and about 1.3 for 17  $\alpha$ -dydrogesterone.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{28}O_2$  in the portion of Dydrogesterone taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dydrogesterone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses for dydrogesterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dydrogesterone Tablets

» Dydrogesterone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{28}O_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dydrogesterone RS

**Identification**—Extract a quantity of the powdered Tablets, containing about 60 mg of Dydrogesterone, with 20 mL of methanol, filter, and evaporate to dryness: the residue so obtained responds to *Identification* test A under *Dydrogesterone*.

**Dissolution** (711)—

*Medium*: 0.3% sodium lauryl sulfate; 500 mL.

*Apparatus 2*: 100 rpm.

*Time*: 60 minutes.

**Procedure**—Determine the amount of  $C_{21}H_{28}O_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 295 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Dydrogesterone RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{28}O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Dydrogesterone*.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, equivalent to about 20 mg of Dydrogesterone, to a 200-mL volumetric flask, add about 100 mL of *Mobile phase*, and sonicate for 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and determine the peak responses by area measurement. Calculate the quantity, in mg, of  $C_{21}H_{28}O_2$  in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Dydrogesterone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the Dydrogesterone peak area responses from the *Assay preparation* and the *Standard preparation*, respectively.

( $\pm$ )-7-(2,3-Dihydroxypropyl)theophylline [479-18-5].

» Dyphylline contains not less than 98.0 percent and not more than 102.0 percent of  $C_{10}H_{14}N_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dyphylline RS

**Identification**—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution*: 10  $\mu$ g per mL.

*Medium*: water.

**Melting range** (741): between 160° and 164°.

**pH** (791): between 5.0 and 7.5, in a solution (1 in 100).

**Loss on drying** (731)—Dry about 1 g, accurately weighed, at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.15%.

**Chloride** (221): A 1.0-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

**Sulfate** (221): A 1.0-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.010%).

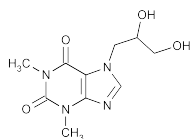
**Heavy metals, Method I** (231): 0.002%.

**Limit of theophylline**—Dissolve 2.0 g in 10 mL of hot water. Cool, add 10 drops of bromothymol blue TS and 5.0 mL of silver nitrate TS, and mix. Not more than 0.50 mL of 0.10 N sodium hydroxide is required to change the color of the solution to blue (0.5%).

**Related compounds**—Dissolve about 200 mg in a mixture of 30 volumes of methanol and 20 volumes of water in a 10-mL volumetric flask, dilute with the same mixture to volume, and mix (*Test solution*). Dilute 1 mL of the *Test solution* with methanol to 100 mL (*Diluted test solution*). Separately apply 10  $\mu$ L of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of binder-free chromatographic silica gel mixture. Develop the plate in a suitable chamber containing a mixture of chloroform, dehydrated alcohol, and ammonium hydroxide (90:10:1) until the solvent front has moved about 15 cm. Remove the plate, air-dry at room temperature, and examine under short-wavelength UV light: no spot in the chromatogram of the *Test solution* other than the principal spot is larger or more intense than the principal spot from the *Diluted test solution* (1.0%).

**Assay**—Dissolve about 200 mg of Dyphylline, accurately weighed, in 2 mL of formic acid, and carefully add, with stirring, 50 mL of acetic anhydride. Add 0.2 mL of Sudan IV TS, and titrate with 0.1 N perchloric acid VS to a deep violet endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 25.42 mg of  $C_{10}H_{14}N_4O_4$ .

## Dyphylline



$C_{10}H_{14}N_4O_4$  254.24

1*H*-Purine-2,6-dione, 7-(2,3-dihydroxypropyl)-3,7-dihydro-1,3-dimethyl-, ( $\pm$ )-.

## Dyphylline Injection

» Dyphylline Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{10}H_{14}N_4O_4$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. To avoid precipitation, store at a temperature of not below 15°, but avoid excessive heat.



**USP Reference standards** (11)—

USP Dyphylline RS

USP Endotoxin RS

**Labeling**—Label it to indicate that the Injection is not to be used if crystals have separated.**Identification**—**A:** Dilute about 5 mL with 20 mL of water, and add 1.0 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.**C:** Transfer a portion of Injection, equivalent to about 100 mg of dyphylline, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 2 mL to a 100-mL volumetric flask, dilute with water to volume, and mix: the UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of a solution of USP Dyphylline RS containing 10 µg per mL, concomitantly measured.**Bacterial endotoxins** (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of dyphylline.**pH** (791): between 5.0 and 8.0.**Other requirements**—It meets the requirements under *Injections* (1).**Assay**—*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Dyphylline Tablets*.*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of dyphylline, to a 500-mL volumetric flask, add water to volume, and mix.*Procedure*—Proceed as directed in the *Assay* under *Dyphylline Tablets*. Calculate the quantity, in mg, of  $C_{10}H_{14}N_4O_4$  in each mL of the Injection taken by the formula:

$$(0.5C / V)(r_U / r_S)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

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**Dyphylline Oral Solution**

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» Dyphylline Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dyphylline ( $C_{10}H_{14}N_4O_4$ ).**Packaging and storage**—Preserve in tight containers.**USP Reference standards** (11)—

USP Dyphylline RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Alcohol content**—*Standard preparation*—Pipet 5 mL of dehydrated alcohol and 5 mL of acetone into a 200-mL volumetric flask containing 50 mL of water, add water to volume, and mix. Pipet 10 mL of this solution into a 200-mL volumetric flask, add water to volume, and mix.*Test preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 5 mL of alcohol, to a 200-mL volumetric flask containing about 30 mL of water. Pipet 5 mL of acetone into the flask, add water to volume,

and mix. Pipet 10 mL of this solution into a 200-mL volumetric flask, add water to volume, and mix.

*Chromatographic system*—The gas chromatograph is equipped with a flame-ionization detector and contains a 75-cm × 4-mm column packed with 20% phase G20 on support S1AB, conditioned as directed (see *Chromatography* (621)). The column is maintained at a temperature of about 85°, and the injection port and detector block are maintained at about 175° and 225°, respectively. Nitrogen is used as the carrier gas at a flow rate of about 18 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, is not less than 2.0; the tailing factor of the alcohol peak is not greater than 1.5; and the relative standard deviation for replicate injections is not more than 2.0% in the ratio of the peak of alcohol to the peak of acetone.*Procedure*—Separately inject equal volumes (about 4 µL) of the *Test preparation* and the *Standard preparation*, in duplicate, into the gas chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of alcohol in the specimen taken by the formula:

$$(500 / V)(R_U / R_S)$$

in which *V* is the volume, in mL, of Oral Solution taken, and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of alcohol to that of acetone obtained from the *Assay preparation* and the *Standard preparation*, respectively: the alcohol content, obtained as the average of the calculated results, is between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .**Assay**—*Mobile phase and Chromatographic system*—Prepare as directed in the *Assay* under *Dyphylline Tablets*.*Standard preparation*—Dissolve an accurately weighed quantity of USP Dyphylline RS in *Mobile phase* to obtain a solution having a known concentration of about 500 µg per mL.*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg of dyphylline, to a 200-mL volumetric flask, add *Mobile phase* to volume, and mix.*Procedure*—Separately inject equal volumes (about 10 µL), of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dyphylline ( $C_{10}H_{14}N_4O_4$ ) in the volume of Oral Solution taken by the formula:

$$0.2C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Dyphylline RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the dyphylline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Dyphylline Tablets**

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» Dyphylline Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{10}H_{14}N_4O_4$ .**Packaging and storage**—Preserve in tight containers.**USP Reference standards** (11)—

USP Dyphylline RS

**Identification**—**A:** To a solution of 5 Tablets in 20 mL of water add 1 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

**C:** Weigh a portion of finely powdered Tablets, equivalent to about 100 mg of dyphylline, and transfer to a 200-mL volumetric flask. Add about 100 mL of water, shake by mechanical means for about 15 minutes, dilute with water to volume, and mix. Filter, and transfer 2 mL of the filtrate to a 100-mL volumetric flask, dilute with water to volume, and mix: the UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of a solution of USP Dyphylline RS containing 10 µg per mL, concomitantly measured.

#### **Dissolution** <711>—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{10}H_{14}N_4O_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Dyphylline RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{10}H_{14}N_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### **Assay**—

*Mobile phase*—Dissolve 1.4 g of monobasic potassium phosphate in 1350 mL of water in a 2-liter volumetric flask, add methanol to volume, and mix. Filter through a 0.5-µm porosity membrane filter. [NOTE—The composition may be varied to meet system suitability requirements.]

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dyphylline RS in water to obtain a solution having a known concentration of about 200 µg per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of dyphylline, to a 500-mL volumetric flask, add 200 mL of water, insert the stopper, and shake by mechanical means for 10 minutes. Add water to volume, and mix. Just prior to injection, filter about 5 mL through a 1.2-µm porosity membrane filter.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%. The tailing factor is not more than 2.0.

*Procedure*—Inject separately equal volumes, accurately measured (about 10 µL), of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_{10}H_{14}N_4O_4$  in the portion of Tablets taken by the formula:

$$0.5C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Dyphylline RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the dyphylline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## **Dyphylline and Guaifenesin Oral Solution**

» Dyphylline and Guaifenesin Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of dyphylline ( $C_{10}H_{14}N_4O_4$ ) and guaifenesin ( $C_{10}H_{14}O_4$ ).

**Packaging and storage**—Preserve in tight containers.

#### **USP Reference standards** <11>—

USP Dyphylline RS

USP Guaifenesin RS

#### **Identification**—

**A:** To an amount of the Oral Solution equivalent to about 100 mg of dyphylline, add water to make 20 mL, mix, and add 2.0 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.

**B:** Transfer a volume of Oral Solution, equivalent to about 100 mg of guaifenesin, to a 60-mL separator, add 10 mL of chloroform, shake for 30 seconds, and allow the layers to separate. Decant the lower (chloroform) layer through chloroform-washed cotton into a small beaker. Evaporate 1 mL of the extract, on a watch glass, on a steam bath to dryness. To the residue add 1 drop of formaldehyde TS and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

**C:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** <791>: between 5.0 and 7.0.

#### **Alcohol content**—

*Standard preparation, Test preparation, Chromatographic system, and Procedure*—Proceed as directed in *Alcohol content* under *Dyphylline Oral Solution*: the alcohol content is between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

#### **Assay**—

*Mobile phase*—Prepare a suitable filtered and degassed mixture of 0.01 M monobasic potassium phosphate and methanol (79:21). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve accurately weighed quantities of USP Dyphylline RS and USP Guaifenesin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.1 mg of guaifenesin and about 0.1 µg of dyphylline per mL,  $f$  being the ratio of the labeled amount of dyphylline to that of guaifenesin.

*Resolution solution*—Prepare a solution in *Mobile phase* containing in each mL about 0.1 mg each of dyphylline and guaifenesin and about 0.01 mg of guaiacol.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg of guaifenesin, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector, a guard column that contains packing L1, and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the guaiacol and guaifenesin peaks is not less than 1.8, and the resolution,  $R$ , between the guaiacol and dyphylline peaks is not less than 9.0. The relative retention times are about 0.25 for dyphylline

line, 0.7 for guaiacol, and 1.0 for guaifenesin. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0% for both dyphylline and guaifenesin.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of dyphylline ( $C_{10}H_{14}N_4O_4$ ) and guaifenesin ( $C_{10}H_{14}O_4$ ) in each mL of the Oral Solution taken by the formula:

$$1000C/V(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, V is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dyphylline and Guaifenesin Tablets

» Dyphylline and Guaifenesin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of dyphylline ( $C_{10}H_{14}N_4O_4$ ) and guaifenesin ( $C_{10}H_{14}O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dyphylline RS

USP Guaifenesin RS

**Identification**—

**A:** Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of dyphylline, with 20 mL of water, and transfer to a test tube. Add 2.0 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.

**B:** Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and on a watch glass evaporate 1 mL of the filtrate, on a steam bath, to dryness. To the residue add 1

drop of formaldehyde TS and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

**C:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amounts of dyphylline ( $C_{10}H_{14}N_4O_4$ ) and guaifenesin ( $C_{10}H_{14}O_4$ ) dissolved, using the procedure set forth in the *Assay*, making any necessary modifications.

*Tolerances*—Not less than 75% (Q) of the labeled amounts of  $C_{10}H_{14}N_4O_4$  and  $C_{10}H_{14}O_4$  are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase*, *Resolution solution*, and *Standard preparation*—Prepare as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of guaifenesin, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase*, sonicate for 30 minutes, and shake by mechanical means for an additional 30 minutes. Dilute with *Mobile phase* to volume, mix, and pass through a membrane filter having a 0.45- $\mu$ m or finer porosity, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

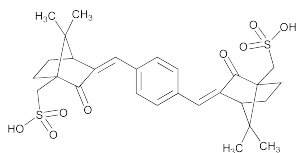
*Chromatographic system*—Prepare as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution*, except omit the use of the guard column.

*Procedure*—Proceed as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution*. Calculate the quantities, in mg, of dyphylline ( $C_{10}H_{14}N_4O_4$ ) and guaifenesin ( $C_{10}H_{14}O_4$ ) in the portion of Tablets taken by the formula:

$$1000C(r_U / r_S)$$

in which the terms are as defined therein.

## Ecamsule Solution



$C_{28}H_{34}O_8S_2$  562.69

Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 3,3'-(1,4-phenylenedimethylidyne)bis[7,7-dimethyl-2-oxo]-, (±)-(3*E*,3'*E*)-3,3'-(*p*-Phenylenedimethylidyne)bis[2-oxo-10-bornanesulfonic acid] [92761-26-7].

» Ecamsule Solution is an aqueous solution of  $C_{28}H_{34}O_8S_2$ . It contains not less than 30.0 percent and not more than 34.0 percent, by weight, of ecamsule ( $C_{28}H_{34}O_8S_2$ ).

**Packaging and storage**—Preserve in tight containers. Protect from light, and store at room temperature.

### USP Reference standards (11)—

USP Ecamsule Solution RS

USP Ecamsule Related Compound A RS  
1,4-Phenylenedimethanol.

$C_8H_{10}O_2$  138.16

USP Ecamsule Related Compound B RS  
4-(Hydroxymethyl)benzoic acid.

$C_8H_8O_3$  152.15

USP Ecamsule Related Compound C RS  
Terephthalic acid.

$C_8H_6O_4$  166.13

USP Ecamsule Related Compound D RS  
((1*SR*,4*RS*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonic acid.

$C_{10}H_{16}O_4S$  232.30

USP Ecamsule Related Compound E RS  
Sodium ((1*SR*,4*SR*,*E*)-3-(4-(hydroxymethyl)benzylidene)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonate.

$C_{18}H_{21}NaO_5S$  372.41

USP Ecamsule Related Compound F RS  
4-((*E*)-((1*SR*,4*SR*)-7,7-Dimethyl-3-oxo-4-(sulfomethyl)bicyclo[2.2.1]heptan-2-ylidene)methyl)benzoic acid.

$C_{18}H_{20}O_6S$  364.41

USP Ecamsule Related Compound G RS  
4-((*E*)-((1*SR*,4*SR*)-7,7-Dimethyl-3-oxo-4-(sulfomethyl)bicyclo[2.2.1]heptan-2-ylidene)methyl)benzaldehyde, sodium salt.

$C_{18}H_{19}NaO_5S$  370.40

USP Ecamsule Triethanolamine RS  
Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 3,3'-(1,4-phenylenedimethylidyne)bis[7,7-dimethyl-2-oxo]-, di-triethanolamine salt (1:2).

$C_{28}H_{34}O_8S_2 \cdot (C_6H_{15}NO_3)_2$  861.07

**Labeling**—The label states that this article is not intended for direct administration to humans or animals.

### Identification—

**A: Infrared Absorption** (197A)—Place a drop of Ecamsule Solution on a diamond sampling surface and dry it with a stream of warm air. The IR absorption spectrum conforms to that of USP Ecamsule Solution RS, similarly obtained.

**B: Ultraviolet Absorption** (197U)—

**Solution**—Transfer 0.25 g of Ecamsule Solution to a 100-mL volumetric flask, and dilute with water to volume. Further dilute 2 mL of this solution with water to 100 mL.

The Solution exhibits absorption maximum between 342 and 346 nm.

**Limit of chloride**—Dissolve about 10 g of Ecamsule Solution, accurately weighed, in 70 mL of water. Titrate this solution with 0.01 N silver nitrate, determine the endpoint potentiometrically (see *Titrimetry* (541)), and calculate the percentage of chloride in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100(35.5)(VN/W)(100/A)$$

in which 35.5 is the atomic weight, in g per mole, of chloride; *V* is the volume, in mL, of silver nitrate used for titration; *N* is the concentration, in normality, of silver nitrate; *W* is the weight, in mg, of Ecamsule Solution taken for determination; and *A* is the assay, in percent, of Ecamsule Solution: not more than 0.3% of chloride is found.

### Limit of sodium—

**Diluent**—Transfer 5 mL of nitric acid in a 1000-mL volumetric flask containing about 500 mL of water, and dilute with water to volume.

**Test solution**—Transfer about 1 g of Ecamsule Solution, accurately weighed, to a 100-mL volumetric flask, and dilute with Diluent to volume.

**Standard solutions**—Dilute quantitatively, and stepwise if necessary, a commercially available sodium atomic absorption standard solution containing 1000 µg of sodium per mL with Diluent to obtain solutions having known concentrations of 1, 5, 10, and 20 µg per mL, respectively.

**Procedure** (see *Spectrophotometry and Light Scattering* (851))—Concomitantly determine the absorbances of the Standard solutions and the Test solution at the sodium emission line of 330 nm or 589 nm with a suitable atomic absorption spectrophotometer equipped with a sodium lamp and an air-acetylene flame, using Diluent as the blank. Determine the concentration of sodium, in µg per mL, in the Test solution using the calibration graph. Calculate the percentage of sodium in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100 \times 10^{-6}(CV/W)(100/A)$$

in which *C* is the concentration, in µg per mL, of sodium in the Test solution, the multiplier of  $10^{-6}$  is for conversion of µg per mL to g per mL; *V* is the volume, in mL, of Test solution; *W* is the weight, in g, of Ecamsule Solution taken for determination; and *A* is the assay, in percent, of Ecamsule Solution: not more than 0.3% of sodium is found.

### Related compounds—

**Test for related compounds A to F—**

**Solvent A**—Prepare a mixture of acetonitrile and 85% phosphoric acid (1000:1).

**Solvent B**—Prepare a mixture of water and 85% phosphoric acid (1000:1).

**Standard solution**—Dissolve an accurately weighed quantity of USP Ecamsule Related Compound A RS, USP Ecamsule Related Compound B RS, USP Ecamsule Related Compound C RS, USP Ecamsule Related Compound D RS, USP Ecamsule Related Compound E RS, and USP Ecamsule Related Compound F RS in water, sonicating if necessary, to obtain a solution having known concentrations as found in Table 1.

**Test solution**—Transfer about 100 mg, accurately weighed, of Ecamsule Solution to a 50-mL volumetric flask, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with either a programmable variable wavelength detector or two separate detectors capable of monitoring at 200 nm and 300 nm and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–25	20	80	isocratic
25–27	20→80	80→20	linear gradient
27–47	80	20	isocratic
47–50	80→20	20→80	linear gradient
50–65	20	80	equilibration

Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviations of the ecamsule related compound peaks for replicate injections are not more than 10.0%; and the resolution,  $R$ , between all adjacent peak pairs of ecamsule related compounds is not less than 1.5 measured at 200 nm.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, allow the chromatogram to run for about 20 minutes for the *Standard solution* and 60 minutes for the *Test solution*, record the chromatograms at 200 nm from 0 to 8 minutes and at 300 nm after 8 minutes, and measure the peak areas. Calculate the percentage of ecamsule related compounds A, B, C, D, and F in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100[100C_S / (C_U \times A)](r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the ecamsule related compound in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*;  $A$  is the assay, in percent, obtained from the *Assay*; and  $r_U$  and  $r_S$  are the peak areas of the ecamsule related compound obtained from the *Test solution* and *Standard solution*, respectively. Calculate the percentage of ecamsule related compound E in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100(350.43/372.41)[100C_S / (C_U \times A)](r_U / r_S)$$

in which 350.43 and 372.41 are the molecular weights of ecamsule related compound E (free acid) and USP Ecamsule Related Compound E RS (sodium salt), respectively;  $C_S$  is the concentration, in mg per mL, of ecamsule related compound E in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*;  $A$  is the assay, in percent, obtained from the *Assay*; and  $r_U$  and  $r_S$  are the peak areas of the ecamsule related compound E obtained from the *Test solution* and *Standard solution*, respectively. The limits are given in Table 1.

*Test for related compound G, Ecamsule exo-2-hydroxyecamsule, Ecamsule endo-2-hydroxyecamsule, and unspecified impurities*—

*Mobile phase*—Proceed as directed in the *Assay*.

*Standard solution 1*—Dissolve an accurately weighed quantity of USP Ecamsule Related Compound G RS in water to obtain a solution having a known concentration of about 0.005 mg per mL.

*Standard solution 2*—Use the *Standard preparation*, as described in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Chromatographic system*—Prepared as directed in the *Assay*. Use the liquid chromatograph equipped with a 310-nm detector in addition to using a 343-nm detector. [NOTE—Ecamsule related compound G is detected at 310 nm; and Ecamsule exo-2-hydroxyecamsule, Ecamsule endo-2-hydroxyecamsule, and unspecified impurities are detected at 343 nm.]

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of *Standard solution 1*, *Standard solution 2*, and the *Test solution* into a chromatograph, record the chromatograms for not less than 6 times the retention time of ecamsule trans-trans isomer, and measure the peak areas. Calculate the percentage of ecamsule related compound G in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100(348.41/370.40)[100C_S / (C_U \times A)](r_U / r_S)$$

in which 348.41 and 370.40 are the molecular weights of ecamsule related compound G and USP Ecamsule Related Compound G RS, respectively;  $C_S$  is the concentration, in mg per mL, of USP Ecamsule Related Compound G RS in *Standard solution 1*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*;  $A$  is the assay, in percent, obtained from the *Assay*; and  $r_U$  and  $r_S$  are the peak areas of ecamsule related compound G obtained from the *Test solution* and *Standard solution 1*, respectively. Calculate the percentage of Ecamsule exo-2-hydroxyecamsule and Ecamsule endo-2-hydroxyecamsule in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100(1/F)[100C_S / (C_U \times A)](r_i / r_S)$$

in which  $F$  is the relative response factor for each impurity obtained from Table 2;  $C_S$  is the concentration, in mg per mL, of USP Ecamsule Triethanolamine RS in *Standard solution 2*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*;  $A$  is the assay, in percent, obtained from the *Assay*;  $r_i$  is the peak area for each impurity obtained from the *Test solution*; and  $r_S$  is the sum of peak areas corresponding to the trans-trans and cis-trans isomers obtained from the *Standard solution*. Calculate the percentage of any unspecified impurity in the portion of  $C_{28}H_{34}O_8S_2$  taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak area for each unspecified impurity obtained from the *Test solution*; and  $r_S$  is the sum of all peak areas obtained from the *Test solution*. The limits are given in Table 2.

Table 1

Name	Concentration (mg/mL) in the Standard solution	Detection wavelength (nm)	RRT <sup>1</sup>	Limit (%)
Ecamsule related compound A	0.001	200	0.42	0.2
Ecamsule related compound B	0.001	200	0.54	0.2
Ecamsule related compound C	0.001	200	0.70	0.2
Ecamsule related compound D	0.008	200	1.00	1.3
Ecamsule related compound E (free acid)	0.004	300	2.52	0.7
Ecamsule related compound F	0.004	300	3.26	0.7

<sup>1</sup>Ecamsule elutes after 27 minutes and is a broad peak in the *Test for related compounds A to F*. The relative retention times of related compounds are measured with respect to ecamsule related compound D.

Table 2

Name	RRT <sup>3</sup>	F	Limit (%)
Ecamsule related compound G	0.9	—	0.2
Ecamsule exo-2-hydroxyecamsule <sup>1</sup>	1.4	0.6	0.2
Ecamsule endo-2-hydroxyecamsule <sup>2</sup>	1.6	0.6	0.3
Any single unspecified impurity	—	1.0	0.5

<sup>1</sup>[(1*SR*,2*R*,4*SR*,*E*)-3-(4-((*E*)-[(1*SR*,4*SR*)-7,7-dimethyl-3-oxo-4-(sulfomethyl)bicyclo[2.2.1]heptan-2-ylidene)methyl)benzylidene)-2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methanesulfonic acid [C<sub>28</sub>H<sub>36</sub>O<sub>8</sub>S<sub>2</sub>, 564.71].

<sup>2</sup>[(1*SR*,2*S*,4*SR*,*E*)-3-(4-((*E*)-[(1*SR*,4*SR*)-7,7-dimethyl-3-oxo-4-(sulfomethyl)bicyclo[2.2.1]heptan-2-ylidene)methyl)benzylidene)-2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methanesulfonic acid [C<sub>28</sub>H<sub>36</sub>O<sub>8</sub>S<sub>2</sub>, 564.71].

<sup>3</sup>The relative retention times are measured with respect to ecamsule trans-trans isomer.

**Total impurities**—Calculate the sum of the related compounds and unspecified impurities from the *Test for related compounds A to F* and the *Test for related compound G*, *Ecamsule exo-2-hydroxyecamsule*, *Ecamsule endo-2-hydroxyecamsule*, and *unspecified impurities*: not more than 5.0% of total impurities is found.

**Assay**—[NOTE—Prepare solutions immediately before use, and protect them from light in low-actinic glassware.]

**1% Triethylamine solution**—Prepare a mixture of water and triethylamine (100:1), and adjust with phosphoric acid to a pH of 7.

**Mobile phase**—Prepare a mixture of 1% Triethylamine solution and methanol (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ecamsule Triethanolamine RS in *Mobile phase*, sonicating if necessary, to obtain a solution having a known concentration of about 0.12 mg per mL of ecamsule triethanolamine.

**Assay preparation**—Transfer about 500 mg, accurately weighed, of Ecamsule Solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 343-nm detector and a 4.0-mm × 125-mm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for ecamsule trans-trans isomer and 2.9 for the ecamsule cis-trans isomer; the relative standard deviation of the sum of the ecamsule trans-trans and cis-trans peak areas for replicate injections is not more than 2.0%; and the number of theoretical plates of the peak corresponding to the trans-trans isomer is not less than 1430.

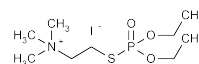
**Procedure**—Inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of C<sub>28</sub>H<sub>34</sub>O<sub>8</sub>S<sub>2</sub> in the portion of Ecamsule Solution taken by the formula:

$$100(562.69/861.07)(V/W) C (r_U / r_S)$$

in which 562.69 and 861.07 are the molecular weights of ecamsule and ecamsule triethanolamine, respectively; *V* is the volume, in mL, of the *Assay preparation*; *W* is the weight, in mg, of Ecamsule Solution used for the *Assay preparation*; *C* is the concentration, in mg per mL, of ecamsule triethanolamine in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the sum of peak areas corresponding to trans-trans and

cis-trans isomers obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Echothiophate Iodide



C<sub>9</sub>H<sub>23</sub>INO<sub>3</sub>PS 383.23

Ethanaminium, 2-[(diethoxyphosphinyl)thio]-*N,N,N*-trimethyl-, iodide.

(2-Mercaptoethyl)trimethylammonium iodide *S*-ester with *O*, *O*-diethyl phosphorothioate [513-10-0].

» Echothiophate Iodide contains not less than 95.0 percent and not more than 100.5 percent of C<sub>9</sub>H<sub>23</sub>INO<sub>3</sub>PS, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, preferably at a temperature below 0°.

### Identification—

**A:** Dissolve about 100 mg in 2 mL of water in a test tube, and add 1 mL of nitric acid: a brown precipitate of iodine is formed. Transfer 1 drop of this mixture to another test tube, add 1 mL of solvent hexane, and shake: the solvent hexane takes on a pink color.

**B:** Heat the remainder of the brown reaction mixture from *Identification test A* over a flame until a colorless solution remains (about 3 minutes). Cool, and dilute with water to about 10 mL: a 2-mL portion of this solution responds to the tests for *Phosphate* <191>.

**C:** A 2-mL portion of the solution obtained in *Identification test B* responds to the tests for *Sulfate* <191>.

**Loss on drying** <731>—Dry it in vacuum over phosphorus pentoxide at 50° for 3 hours: it loses not more than 1.0% of its weight.

**Assay**—[NOTE—In the preparation of all reagents, and throughout this procedure, wherever water is specified, use only water that has been distilled, boiled for 10 minutes, and cooled while protected from the atmosphere.]

**pH 12 phosphate buffer**—Transfer 5.44 g of anhydrous dibasic sodium phosphate to a 100-mL volumetric flask, and add a volume of 1 N sodium hydroxide VS that contains 36.5 mEq of sodium hydroxide. Add about 40 mL of water, shake to dissolve the sodium phosphate, dilute with water to volume, and mix.

**0.004 N Iodine**—Dilute 0.1 N iodine with water to 0.004 N, and standardize the solution on the day of use as follows. Weigh accurately about 150 mg of arsenic trioxide, and dissolve in 20 mL of 1 N sodium hydroxide, by warming if necessary, in a 500-mL volumetric flask. Dilute with 40 mL of water, add 2 drops of methyl orange TS, then add 3 N hydrochloric acid until the yellow color is changed to pink. Add 2 g of sodium bicarbonate, add water to volume, and mix. Transfer 5.0 mL of this solution to a titration vessel, and add 50 mL of pH 7 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*). Titrate with 0.004 N iodine, determining the endpoint potentiometrically, using platinum and silver-silver chloride electrodes. Calculate the normality. Each 197.8 μg of arsenic trioxide is equivalent to 1 mL of 0.004 N iodine.

**Procedure**—Dissolve about 125 mg of Echothiophate Iodide, accurately weighed, in about 50 mL of water in a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a titration vessel containing 30 mL of water. Add 10.0 mL of pH 12 phosphate buffer, mix, cover, and allow to stand for 20 minutes at 25 ± 3°. Add 2 mL of glacial acetic acid, rapidly and with mixing.

Titrate with 0.004 N iodine, determining the endpoint potentiometrically, using platinum and silver-silver chloride electrodes. Correct for the amount of free thiol sulfur by repeating the procedure but adding the glacial acetic acid first, then the pH 12 phosphate buffer, and titrating immediately. Subtract the volume of 0.004 N iodine used in the second titration from that used in the first. Each mL of 0.004 N iodine is equivalent to 1.533 mg of  $C_9H_{23}INO_3PS$ .

## Echothiophate Iodide for Ophthalmic Solution

» Echothiophate Iodide for Ophthalmic Solution is sterile Echothiophate Iodide. It may contain Mannitol or other suitable diluent. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of  $C_9H_{23}INO_3PS$ .

**Packaging and storage**—Preserve in tight containers, preferably of Type I glass, at controlled room temperature.

**Completeness of solution** (641)—The contents of 1 container dissolve in 10 mL of carbon dioxide-free water to yield a clear solution.

### Identification—

**A:** Dissolve a quantity, equivalent to about 12 mg of echothiophate iodide, in 10 mL of water. To 1 mL of this solution add 0.2 mL of 2 N hydrochloric acid and 0.2 mL of 30 percent hydrogen peroxide: the color turns brown. Add a few drops of solvent hexane, and shake: the solvent hexane acquires a pink color.

**B:** To 5 mL of the solution prepared for Identification test A add 0.5 mL of sodium hydroxide solution (1 in 2), heat at 50° for 2 minutes, cool to room temperature, then add 1 mL of sodium nitroferricyanide TS: a deep-red color is produced.

**Sterility** (71): meets the requirements.

**Water**—[NOTE—Dry all glassware used in the following procedure at 105° for 4 hours, and store in a desiccator or dry box. Perform as many operations as possible in a dry box.]

**Dry alcohol**—Wash about 150 g of 8- to 12-mesh type 3A molecular sieve with several portions of dehydrated alcohol to remove the fine particles. Place the washed molecular sieve in a shallow borosilicate glass tray, heat in an oven at 350° for 2 hours, and cool in a dry box. Transfer the dry molecular sieve to a 1-liter conical flask, add about 700 mL of dehydrated alcohol, insert the stopper, mix, and allow to stand for not less than 48 hours before using.

**Internal standard solution**—[NOTE—Prepare this solution fresh daily.] Place 0.17 mL of methanol in a 100-mL volumetric flask, add Dry alcohol to volume, and mix.

**Standard preparations**—[NOTE—Prepare these solutions fresh daily.] Into three 25-mL volumetric flasks, each containing about 15 mL of Internal standard solution, transfer 5  $\mu$ L, 40  $\mu$ L, and 75  $\mu$ L of water, respectively. Dilute with Internal standard solution to volume, and mix.

**Test preparation**—Carefully remove the protective retainer and cap from 5 vials of Echothiophate Iodide for Ophthalmic Solution without removing the elastomeric septum closure. Discard the separated parts, and weigh accurately each closed vial and contents. Inject through the septum of each vial 400  $\mu$ L of Internal standard solution,

accurately measured, using a suitable gas-tight syringe, and allow to stand for 1 hour, swirling occasionally to dissolve the residue. After 1 hour, using a gas-tight syringe, remove 300  $\mu$ L of solution from each vial, transfer to a dry small-volume sample-collecting vial equipped with a sampling valve system,\* and mix the combined solutions.

**Chromatographic system** (see Chromatography (621))—The chromatograph is equipped with a thermal conductivity detector and a 2-mm  $\times$  1.8-m silylated glass column packed with 80- to 100-mesh surface-silanized packing S3. The column is maintained at a temperature of about 115°, the injection port and detector block are maintained at temperatures of about 200° and 225°, respectively, and dry helium is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph a sufficient number of injections of a Standard preparation, and record the peak responses as directed for Procedure: the resolution factor between the water and methanol peaks is not less than 2.0; and the relative standard deviation is not more than 5.0%.

**Procedure**—Inject a portion (3  $\mu$ L to 4  $\mu$ L) of each Standard preparation into the chromatograph, record the chromatogram, and measure the responses for the first (water) and second (methanol) major peaks obtained for each. Plot the ratios of the peak responses of water to methanol versus the concentration, in mg per mL, of water in each Standard preparation. [NOTE—If the plot is not linear, discard it, and repeat the chromatography on additional portions of the Standard preparations.] Similarly inject a portion of the Test preparation, record the chromatogram, and measure the responses for the two major peaks. By comparison with the linear standard plot, determine the concentration, in mg per mL, of water in the Test preparation as that corresponding to the ratio of the peak responses of water to methanol from the Test preparation. Remove the elastomeric septum closure from each test vial, discard the contents, and rinse each vial and closure with several portions of methanol. Dry the vials and the closures in a stream of dry nitrogen, weigh accurately, and subtract this weight from that of the closed vials and contents obtained as directed under Test preparation. Calculate the water content, in percentage, taken by the formula:

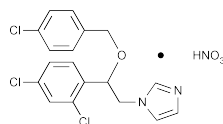
$$100CV / W$$

in which C is the concentration, in mg per mL, of water in the Test preparation, V is the volume, in mL, of Internal standard solution added to each test specimen vial as directed under Test preparation, and W is the average weight, in mg, of the test specimens in the vials: not more than 2.0% is found.

**Assay**—[NOTE—In the preparation of all reagents, and throughout this procedure, wherever water is specified, use only water that has been distilled, boiled for 10 minutes, and cooled while protected from the atmosphere.] Dissolve the contents of a counted number of vials of Echothiophate Iodide for Ophthalmic Solution, equivalent to not less than 30 mg of echothiophate iodide, by adding 5.0 mL of water to each vial. Combine the solutions, and mix. Dilute a portion of the mixture, equivalent to about 12 mg of echothiophate iodide, with water to 40 mL, and proceed as directed for Procedure in the Assay under Echothiophate Iodide, beginning with "Add 10.0 mL of pH 12 phosphate buffer."

\* Suitable sample-collecting vials and sampling valve systems are available as catalog Nos. 13098 and 13099 3-mL and 5-mL vials, and catalog No. 10135 valve, from Pierce Chemical Co., Box 117, Rockford, IL 61105.

## Econazole Nitrate



$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$  444.70

1*H*-Imidazole, 1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-, mononitrate, (±)-

(±)-1-[2,4-Dichloro-β-[(*p*-chlorobenzyl)oxy]phenethyl]-imidazole mononitrate [68797-31-9].

» Econazole Nitrate contains not less than 98.5 percent and not more than 101.0 percent of  $C_{18}H_{15}Cl_3N_2O \cdot HNO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**USP Reference standards** (11)—  
USP Econazole Nitrate RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 800 μg per mL.

*Medium:* 0.1 N hydrochloric acid in methanol (1 in 10).

**C:** Shake 10 mg with 5 mL of water, and cool the resulting suspension in ice. Keeping the suspension cool, add 0.4 mL of potassium chloride solution (1 in 10), 0.1 mL of diphenylamine TS, and dropwise, with shaking, 5 mL of sulfuric acid: an intense blue color develops.

**Melting range** (741): between 162° and 166°, with decomposition.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

### Chromatographic purity—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Econazole Nitrate RS in methanol to obtain a solution containing 75 μg per mL.

*Test preparation*—Dissolve an accurately weighed quantity of Econazole Nitrate in methanol to obtain a solution containing 20 mg per mL.

*Procedure*—Apply separately 20 μL of the *Test preparation* and 20 μL of the *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of 1,4-dioxane, toluene, and 13.5 M ammonium hydroxide (60:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate in a current of air. Expose the plate to iodine vapors for 1 hour, and air-dry until the iodine has dissipated. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with that of the principal spot in the chromatogram of the *Standard preparation*: no individual secondary spots in the chromatogram of the *Test preparation* are larger or more intense than the principal spot produced by the *Standard preparation*, corresponding to not more than 0.375%, and the total of all such secondary spots observed is not more than 2.0%.

**Assay**—Dissolve about 400 mg of Econazole Nitrate, accurately weighed, in 50 mL of glacial acetic acid, and titrate

with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-calomel electrode system (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 44.47 mg of  $C_{18}H_{15}Cl_3N_2O \cdot HNO_3$ .

## Edetate Calcium Disodium

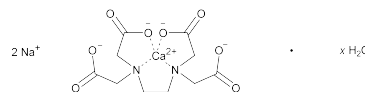
### Pharmacopeial Discussion Group Sign-Off Document

Attributes	EP	JP	USP
Definition	+	+	+
Identification B	+	+	+
Identification C	+	+	+
pH	+	+	+
Purity (1) Chloride	+	+	+
Purity (2) Disodium edetate	+	+	+
Water	+	+	+
Assay	+	+	+

**Legend:** + will adopt and implement; – will not stipulate.

**Nonharmonized attributes:** Clarity and/or color of solution, Heavy metals, Identification by IR absorption, Limit of nitrilotriacetic acid, Storage.

**Specific local attributes:** Description (JP), Iron (EP).



$C_{10}H_{12}CaN_2Na_2O_8 \cdot xH_2O$

374.27

Calciate (2-), [[*N,N'*-1,2-ethanediylbis[*N*-(carboxymethyl)glycinato]](4-)-*N,N',O,O',O'',O'''*]-, disodium, hydrate, (OC-6-21)-;

Disodium[(ethylenedinitrilo)tetraacetato]calciate(2-) hydrate [23411-34-9].

Anhydrous [62-33-9].

### DEFINITION

Edetate Calcium Disodium contains NLT 98.0% and NMT 102.0% of  $C_{10}H_{12}CaN_2Na_2O_8$  (374.27), calculated on the anhydrous basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197M)

• **B. PROCEDURE**

*Sample:* 2 g

*Analysis:* Dissolve the *Sample* in 10 mL of water, add 6 mL of lead (II) nitrate solution (33 in 1000), shake, and add 3 mL of potassium iodide TS: no yellow precipitate is formed. Make this solution alkaline by the addition of diluted ammonia solution (7 in 50), and add 3 mL of ammonium oxalate TS.

**Acceptance criteria:** A white precipitate is formed.

• **C. PROCEDURE**

*Sample:* 0.5 g

*Analysis:* Dissolve 0.5 g in 10 mL of water, and add 10 mL of potassium pyroantimonate TS.

**Acceptance criteria:** A white, crystalline precipitate is formed. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

### ASSAY

• **PROCEDURE**

*Sample:* 500 mg of Edetate Calcium Disodium

*Analysis:* Transfer the *Sample* into a 200-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. Transfer exactly 20 mL of this solution to 80 mL of water, and adjust with dilute nitric acid to a pH of 2–3.



Add two drops of xylenol orange TS, and titrate with 0.01 M bismuth nitrate VS until the color of the solution changes from yellow to red. Each mL of 0.01 M bismuth nitrate VS is equivalent to 3.743 mg of  $C_{10}H_{12}CaN_2Na_2O_8$ .

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

## IMPURITIES

### Inorganic Impurities

#### • CHLORIDE AND SULFATE, Chloride <221>

**Sample solution:** To 0.70 g of Edetate Calcium Disodium add 20 mL of water and 30 mL of diluted nitric acid. Allow to stand for 30 min, and filter. To 10 mL of the filtrate add water to make 50 mL.

**Control solution:** Prepare with 0.40 mL of 0.01 M hydrochloric acid VS.

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** NMT 0.10%

#### • HEAVY METALS, Method II <231>: NMT 20 ppm

### Organic Impurities

#### • PROCEDURE 1: DISODIUM EDETATE

**Sample:** 1.00 g of Edetate Calcium Disodium

**Analysis:** Dissolve the *Sample* in 50 mL of water. Add 5 mL of ammonia–ammonium chloride buffer TS, and 40 mg of eriochrome black T–sodium chloride indicator. Titrate with 0.01 M magnesium chloride VS until the color of the solution changes from blue to red-violet.

**Acceptance criteria:** NMT 3.0 mL of 0.01 M magnesium chloride VS is consumed (NMT 1.0%).

#### • PROCEDURE 2: LIMIT OF NITRILOTRIACETIC ACID

**Mobile phase:** Add 10 mL of 1.0 M tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of  $7.5 \pm 0.1$ . Transfer the solution to a 1000-mL volumetric flask, add 90 mL of methanol, dilute with water to volume, pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

**Cupric nitrate solution:** 10 mg/mL of cupric nitrate ( $Cu(NO_3)_2$ ) in water

**Standard stock solution:** Transfer 100 mg of nitrilotriacetic acid to a 10-mL volumetric flask, add 0.5 mL of ammonium hydroxide, and mix. Dilute with water to volume, and mix.

**Standard solution:** Transfer 1.0 g of Edetate Calcium Disodium to a 100-mL volumetric flask, add 100  $\mu$ L of *Standard stock solution*, dilute with *Cupric nitrate solution* to volume, and mix. Sonicate, if necessary, to achieve complete solution.

**System suitability solution:** Transfer 10 mg of Edetate Calcium Disodium to a 100-mL volumetric flask, add 100  $\mu$ L of *Standard stock solution*, dilute with *Cupric nitrate solution* to volume, and mix. Sonicate, if necessary, to dissolve.

**Sample solution:** 10 mg/mL of Edetate Calcium Disodium in *Cupric nitrate solution*. Sonicate, if necessary, to achieve complete solution.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L7

**Flow rate:** 2 mL/min

**Injection size:** 50  $\mu$ L

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE— The relative retention times for nitrilotriacetic acid, copper, and edetate are about 0.35, 0.65, and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3 between nitrilotriacetic acid and copper, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Measure the responses for the major peaks.

**Acceptance criteria:** The peak response of nitrilotriacetic acid from the *Sample solution* does not exceed the difference between the nitrilotriacetic acid peak responses obtained from the *Standard solution* and the *Sample solution* (0.1%).

## SPECIFIC TESTS

### • pH <791>

**Sample solution:** A solution (1 in 5)

**Acceptance criteria:** 6.5–8.0

### • WATER DETERMINATION, Method I <921>

**Sample:** 0.2 g

**Acceptance criteria:** 5.0%–13.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.

• **USP REFERENCE STANDARDS <11>**  
USP Edetate Calcium Disodium RS

## Edetate Calcium Disodium Injection

» Edetate Calcium Disodium Injection is a sterile solution of Edetate Calcium Disodium in Water for Injection. It contains, in each mL, not less than 180 mg and not more than 220 mg of  $C_{10}H_{12}CaN_2Na_2O_8$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards <11>**—

USP Edetate Calcium Disodium RS

USP Endotoxin RS

### Identification—

**A:** Transfer a volume of Injection, equivalent to about 1 g of edetate calcium disodium, to an evaporating dish, and evaporate on a steam bath to dryness: the residue responds to *Identification test A* under *Edetate Calcium Disodium*.

**B:** It responds to *Identification test B* under *Edetate Calcium Disodium*.

**Bacterial endotoxins <85>**—It contains not more than 0.01 USP Endotoxin Unit per mg of edetate calcium disodium.

**pH <791>:** between 6.5 and 8.0.

**Particulate matter <788>:** meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Dilute an accurately measured volume of Injection, equivalent to about 1 g of edetate calcium disodium with water to about 75 mL. Add 25 mL of 1 N acetic acid and 1 mL of diphenylcarbazone TS, mix, and titrate slowly with 0.1 M mercuric nitrate VS to the appearance of the first purplish color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M mercuric nitrate is equivalent to 37.43 mg of  $C_{10}H_{12}CaN_2Na_2O_8$ .

## Edetate Disodium

$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$  372.24

$C_{10}H_{14}N_2Na_2O_8$  336.21

Glycine, *N,N'*-1,2-ethanediyldis[*N*-(carboxymethyl)-, disodium salt, dihydrate;

Disodium (ethylenedinitrilo)tetraacetate dihydrate [6381-92-6].

Anhydrous [139-33-3].

### DEFINITION

Edetate Disodium contains NLT 99.0% and NMT 101.0% of edetate disodium ( $C_{10}H_{14}N_2Na_2O_8$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION <197K>

Sample: Undried

Acceptance criteria: Meets the requirements

#### • B.

Sample: 50 mg

Analysis: To 5 mL of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. To the deep red solution add the *Sample*.

Acceptance criteria: The red color is discharged, leaving a yellowish solution.

#### • C. IDENTIFICATION TESTS—GENERAL, Sodium <191>: It meets the requirements of the flame test.

### ASSAY

#### • PROCEDURE

Sample solution: Dissolve 5 g of Edetate Disodium in about 100 mL of water contained in a 250-mL volumetric flask. Add water to volume.

#### Titrimetric system

(See *Titrimetry* <541>.)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Place 200 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 h and cooled in a desiccator, into a 400-mL beaker. Add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and without removing the latter, add 2 mL of 3 N hydrochloric acid from a pipet. Swirl the contents of the beaker, and dissolve the calcium carbonate. With water, wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute with water to 100 mL. While stirring the solution, preferably with a magnetic stirrer, add 30 mL of the *Sample solution* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 0.30 g of hydroxy naphthol blue, and continue the titration with the *Sample solution* to a blue endpoint.

Calculate the percentage of edetate disodium ( $C_{10}H_{14}N_2Na_2O_8$ ) in the portion of Edetate Disodium taken:

$$\text{Result} = (V_T/V_U) \times W \times (M_{r1}/M_{r2}) \times 100$$

$V_T$  = total volume of the *Sample solution* (mL)

$V_U$  = volume of the *Sample solution* consumed in the titration (mL)

$W$  = weight of calcium carbonate (mg)

$M_{r1}$  = molecular weight of edetate disodium, 336.21

$M_{r2}$  = molecular weight of calcium carbonate, 100.09

Acceptance criteria: 99.0%–101.0% on the dried basis

### IMPURITIES

#### • HEAVY METALS, Method II <231>: NMT 50 ppm

#### • CALCIUM

Sample solution: 1 g of Edetate Disodium in 20 mL of water

Analysis: To the *Sample solution* add 2 drops of methyl red TS, and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid dropwise until the solution is just acid, and then add 1 mL of ammonium oxalate TS. Acceptance criteria: No precipitate is formed.

#### • LIMIT OF NITRILOTRIACETIC ACID

Mobile phase: Add 10 mL of 1.0 M tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of  $7.5 \pm 0.1$ . Transfer the solution so obtained to a 1000-mL volumetric flask. Add 90 mL of methanol, and dilute with water to volume. Pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

Cupric nitrate solution: 10 mg/mL of cupric nitrate [ $Cu(NO_3)_2$ ]

Standard stock solution: Transfer 100 mg of nitrilotriacetic acid to a 10-mL volumetric flask. Add 0.5 mL of ammonium hydroxide, mix, and dilute with water to volume.

Standard solution: Transfer 1.0 g of Edetate Disodium to a 100-mL volumetric flask. Add 100  $\mu$ L of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

System suitability solution: Transfer 10 mg of Edetate Disodium to a 100-mL volumetric flask. Add 100  $\mu$ L of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

Sample solution: 10 mg/mL of Edetate Disodium in *Cupric nitrate solution*. If necessary, sonicate to dissolve.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm  $\times$  15-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 50  $\mu$ L

#### System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for nitrilotriacetic acid, copper, and edetate are about 0.35, 0.65, and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3 between nitrilotriacetic acid and copper, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: NMT 0.1%; the response of the nitrilotriacetic acid peak of the *Sample solution* does not exceed the difference between the nitrilotriacetic acid peak responses from the *Standard solution* and the *Sample solution*.

### SPECIFIC TESTS

#### • pH <791>

Sample solution: 50 mg/mL

Acceptance criteria: 4.0–6.0

#### • LOSS ON DRYING <731>

Analysis: Dry at 150° for 6 h.

Acceptance criteria: 8.7%–11.4%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS <11>

USP Edetate Disodium RS

## Edetate Disodium Injection

» Edetate Disodium Injection is a sterile solution of Edetate Disodium in Water for Injection, which, as a result of pH adjustment, contains varying amounts of the disodium and trisodium salts. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{10}H_{14}N_2Na_2O_8$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Edetate Disodium RS

USP Endotoxin RS

**Identification**—Transfer a volume of Injection, equivalent to about 1 g of edetate disodium, to an evaporating dish, adjust with 3 N hydrochloric acid to a pH of 5.0, and evaporate on a steam bath to dryness: the residue responds to Identification tests A and C under *Edetate Disodium*.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of edetate disodium.

**pH** (791): between 6.5 and 7.5.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

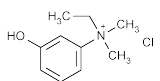
*Assay preparation*—Dilute an accurately measured volume of Injection, equivalent to about 2 g of edetate disodium, with water to volume in a 100-mL volumetric flask, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Edetate Disodium*. Calculate the weight, in mg, of  $C_{10}H_{14}N_2Na_2O_8$  in the volume of Injection taken by the formula:

$$(336.21/100.09)W(V_T/V)$$

in which 336.21 and 100.09 are the molecular weights of edetate disodium and calcium carbonate, respectively;  $W$  is the weight, in mg, of calcium carbonate;  $V_T$  is the volume, in mL, of the *Assay preparation*; and  $V$  is the volume, in mL, of the *Assay preparation* consumed in the titration.

## Edrophonium Chloride



$C_{10}H_{16}ClNO$  201.69

Benzenaminium, *N*-ethyl-3-hydroxy-*N,N*-dimethyl-, chloride. Ethyl(*m*-hydroxyphenyl)dimethylammonium chloride [116-38-1].

» Edrophonium Chloride contains not less than 98.0 percent and not more than 100.5 percent of  $C_{10}H_{16}ClNO$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Edrophonium Chloride RS

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 50 µg per mL.

*Medium:* 0.1 N hydrochloric acid.

Absorptivities at 273 nm, calculated on the dried basis, do not differ by more than 2.0%.

**C:** To 10 mL of a solution (1 in 10) add 1 drop of ferric chloride TS: a violet-blue color is produced.

**D:** It responds to the tests for *Chloride* (191).

**Melting range** (741): between 165° and 170°, with decomposition.

**pH** (791): between 4.0 and 5.0, in a solution (1 in 10).

**Loss on drying** (731)—Dry it in a suitable vacuum drying tube, using phosphorus pentoxide as the desiccant, for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals** (231)—Dissolve 1.0 g in 25 mL of water: the limit is 0.002%.

**Limit of dimethylaminophenol**—Dissolve about 500 mg, accurately weighed, in 5 mL of water in a separator. Add 5 mL of pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and shake. Extract with five 20-mL portions of chloroform, pooling the extracts in a 100-mL volumetric flask, and add chloroform to volume. The absorbance of this solution, determined in a 1-cm cell at 252 nm, with a suitable spectrophotometer, is not more than that of a 1 in 200,000 solution of dimethylaminophenol in chloroform, similarly measured (0.1%).

**Assay**—Transfer about 175 mg of Edrophonium Chloride, accurately weighed, to a suitable flask, and dissolve in about 20 mL of glacial acetic acid. Add 5 mL of mercuric acetate TS, then add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.17 mg of  $C_{10}H_{16}ClNO$ .

## Edrophonium Chloride Injection

» Edrophonium Chloride Injection is a sterile solution of Edrophonium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{10}H_{16}ClNO$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**Labeling**—Label the Injection in multiple-dose containers to indicate an expiration date of not later than 3 years after the date of manufacture, and label the Injection in single-dose containers to indicate an expiration date of not later than 4 years after the date of manufacture.

### USP Reference standards (11)—

USP Edrophonium Chloride RS

USP Endotoxin RS

### Identification—

**A:** *Ultraviolet Absorption* (197U)—Test solution versus standard solution prepared as directed in the Assay.

**B:** Place in a small separator a volume of Injection, equivalent to about 30 mg of edrophonium chloride, add 15 mL of pH 9.6 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and 5 mL of a 1 in 1000 solution of thymol blue in pH 9.6 alkaline borate buffer, and mix. Add 10 mL of chloroform, shake thoroughly, and allow to settle: a yellow color is produced in the chloroform layer.

**C:** It responds to the tests for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 8.33 USP Endotoxin Units per mg of edrophonium chloride.

**pH** (791): between 5.0 and 5.8.

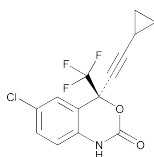
**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Pipet a volume of Injection, equivalent to about 50 mg of edrophonium chloride, into a glass-stoppered, 50-mL centrifuge tube. Add 5 mL of pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and 5 g of sodium chloride. Wash the solution with four 20-mL portions of a mixture of equal volumes of solvent hexane and ether. Transfer the aqueous phase to a 100-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Transfer a 5-mL aliquot of this solution to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix test solution. Dissolve an accurately weighed quantity of USP Edrophonium Chloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the acid to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of C<sub>10</sub>H<sub>16</sub>ClNO in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Edrophonium Chloride RS in the Standard solution; V is the volume, in mL, of Injection taken; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the test solution and the Standard solution, respectively.

## Efavirenz



C<sub>14</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>2</sub> 315.67  
 1*H*-Benzo[d][1,3]oxazin-2(4*H*)-one, 6-chloro-4-cyclopropylethynyl-4-trifluoromethyl;  
 (S)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one [154598-52-4].

### DEFINITION

Efavirenz contains NLT 98.0% and NMT 102.0% of C<sub>14</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>2</sub>, calculated on the anhydrous, solvent-free basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

**Sample:** Dry at 105° for 30 min, and cool in desiccator.

#### B. ULTRAVIOLET ABSORPTION (197U)

**Solvent:** Methanol

**Standard solution:** 10 µg/mL of USP Efavirenz RS in Solvent

**Sample solution:** 10 µg/mL of Efavirenz in Solvent

**Acceptance criteria:** Meets the requirements

### ASSAY

#### PROCEDURE

[NOTE—Protect solutions of efavirenz from light. Use of polypropylene HPLC vials is recommended to avoid possible degradation from certain types of glass vials.]

**Diluent:** Acetonitrile and water (1:1)

**Solution A:** Methanol, trifluoroacetic acid, and water (1:0.005:9). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

**Solution B:** Methanol, trifluoroacetic acid, and water (9:0.005:1). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
16	50	50
23	35	65
28	30	70
29	20	80
31	20	80
32	60	40
40	60	40

**Standard solution:** 250 µg/mL of USP Efavirenz RS and 1.0 µg/mL of USP Efavirenz Related Compound B RS in Diluent. [NOTE—Dissolve in about 65% of the flask volume in Diluent and shake for 30 min or until dissolved before diluting with Diluent to volume.]

**Sample solution:** 250 µg/mL of Efavirenz in Diluent.

[NOTE—Dissolve in about 65% of the flask volume of Diluent, and shake for 30 min or until dissolved before diluting with Diluent to volume.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L10

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 35 µL

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Resolution:** NLT 1.2 between efavirenz related compound B and efavirenz

**Relative standard deviation:** NMT 1.0% for efavirenz

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of C<sub>14</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>2</sub> in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r<sub>U</sub> = peak response for efavirenz from the Sample solution

r<sub>S</sub> = peak response for efavirenz from the Standard solution

C<sub>S</sub> = concentration of USP Efavirenz RS in the Standard solution (mg/mL)

C<sub>U</sub> = concentration of efavirenz in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

## IMPURITIES

### Inorganic Impurities

• **RESIDUE ON IGNITION** (281): NMT 0.2%, use a platinum crucible

• **HEAVY METALS**, Method II (231): NMT 20 ppm

### Organic Impurities

#### • PROCEDURE 1

**Diluent, Solution A, Solution B, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability solution:** Use the *Standard solution* prepared as directed in the Assay.

**Standard solution:** 1.25 µg/mL of USP Efavirenz RS in *Diluent*, prepared from the *System suitability solution*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.2 between efavirenz related compound B and efavirenz, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for efavirenz, *Standard solution*

## Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for any individual impurity from the *Sample solution*

$r_S$  = peak response for efavirenz from the *Standard solution*

$C_S$  = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Efavirenz in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any peak less than 0.05%.]

**Total impurities:** NMT 1.0%

#### • PROCEDURE 2

[NOTE— Perform *Procedure 2* in addition to *Procedure 1* if the result for the total of the three impurities at a relative retention time of 1.16 in *Procedure 1* exceeds 0.10%.]

**Diluent:** Acetonitrile, trifluoroacetic acid, and water (55:0.05:45)

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (4:0.005:6). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz aminoalcohol <sup>a</sup>	0.48	0.26	0.15
Efavirenz ethene analog <sup>b</sup> (efavirenz related compound B)	0.93	0.91	0.40
Efavirenz pent-3-ene-1-yne ( <i>cis</i> ) <sup>c</sup>	1.16	1.0	0.10 <sup>q</sup>
Efavirenz pent-3-ene-1-yne ( <i>trans</i> ) <sup>d</sup>	1.16	1.0	0.10 <sup>q</sup>
Efavirenz penteneyne <sup>e</sup>	1.16	1.0	0.10 <sup>q</sup>
Efavirenz pentyne analog <sup>f</sup>	1.2	1.0	0.15
Methyl efavirenz <sup>g</sup>	1.28	1.0	0.10
Efavirenz amino alcohol methyl carbamate <sup>h</sup>	1.33	0.83	0.10
N-Benzylefavirenz <sup>i</sup>	1.8	0.71	0.25
Efavirenz benzoylaminoalcohol <sup>j</sup>	1.9	0.56	0.15
Quinoline analog <sup>k</sup>	1.45	2.0	0.10
Efavirenz amino alcohol ethyl carbamate <sup>l</sup>	1.53	0.83	0.10
Unidentified impurity <sup>m</sup>	1.60	1.0	0.10
Efavirenz amino alcohol bis(ethoxycarbonyl) <sup>n</sup>	1.63	0.34	0.10
Unidentified impurity <sup>o</sup>	2.1	1.0	0.10
Cyclobutenylindole analog <sup>p</sup>	2.18	0.48	0.10
Any other unknown individual impurity	—	1.0	0.10

<sup>a</sup> (S)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.

<sup>b</sup> (S,E)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>c</sup> (S,E)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>d</sup> (S,Z)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>e</sup> (S)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>f</sup> (S)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>g</sup> (S)-6-Chloro-4-[(2R,2S)-2-methylcyclopropyl]ethynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>h</sup> (S)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.

<sup>i</sup> (S)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>j</sup> (S)-N-(4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenyl)-4-methoxybenzamide.

<sup>k</sup> 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.

<sup>l</sup> (S)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.

<sup>m</sup> Relative retention time of 1.60.

<sup>n</sup> (S)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.

<sup>o</sup> Relative retention time of 2.1.

<sup>p</sup> Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1H-indole-1-carboxylate.

<sup>q</sup> [NOTE—If results exceed 0.10%, perform *Procedure 2* to separate the three coeluting impurities and ensure that each impurity meets the limit.]

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (8:0.005:2). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
45	0	100
45.1	100	0
50	100	0

**Standard solution:** 1.25 µg/mL of USP Efavirenz RS in *Diluent*

**Sample solution:** 250 µg/mL of Efavirenz in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5 for efavirenz

**Relative standard deviation:** NMT 5.0% for efavirenz

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each of the three specified impurities in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each of the three specified impurities from the *Sample solution*

$r_S$  = peak response for efavirenz from the *Standard solution*

$C_S$  = concentration of USP Efavirenz RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Efavirenz in the *Sample solution* (µg/mL)

$F$  = relative response factor (see *Impurity Table 2*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 2*. [NOTE—Disregard any peak less than 0.05%.]

#### SPECIFIC TESTS

- **COMPLETENESS OF SOLUTION** <641>: Meets the requirements

**Solvent:** Methanol

**Sample solution:** 50 mg/mL of Efavirenz in *Solvent*

- **WATER DETERMINATION, Method 1c** <921>

**Sample:** 100 mg/mL of Efavirenz in methanol

**Acceptance criteria:** NMT 0.5%

- **ENANTIOMERIC PURITY**

**Mobile phase:** Hexane and ethanol (97:3)

**Retention time solution:** 1 mg/mL of USP Efavirenz RS in *Mobile phase*

**Standard solution:** 10 µg/mL of USP Efavirenz Racemic RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Efavirenz in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 25-cm column; 5-µm packing L10 connected in-line and before 4.6-mm × 25-cm column; 10-µm packing L40

**Column temperature:** 35°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *Retention time solution*

#### Suitability requirements

**Efavirenz identification:** Identify the (S)-efavirenz enantiomer peak, *Retention time solution*

**Resolution:** NLT 3.0 between (R)-efavirenz enantiomer and (S)-efavirenz enantiomer, *Standard solution*

**Relative standard deviation:** NMT 5.0% for (R)-efavirenz enantiomer

#### Analysis

**Samples:** *Retention time solution*, *Standard solution*, and *Sample solution*

[NOTE—Verify the identification of the efavirenz peak based on the chromatogram of the *Retention time solution*. The relative retention times for (R)-efavirenz enantiomer and (S)-efavirenz enantiomer are 0.88 and 1.00, respectively.]

Calculate the percentage of (R)-efavirenz enantiomer in the portion of Efavirenz taken:

$$\text{Result} = 100 \times [r_R/(r_R + r_S)]$$

$r_R$  = peak response of (R)-efavirenz enantiomer from the *Sample solution*

$r_S$  = peak response of (S)-efavirenz enantiomer from the *Sample solution*

**Acceptance criteria:** NMT 0.5% of (R)-efavirenz enantiomer

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store at controlled room temperature.

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz	1.0	1.0	—
Efavirenz pent-3-ene-1-yne ( <i>cis</i> ) <sup>a</sup>	1.10	1.1	0.10
Efavirenz pent-3-ene-1-yne ( <i>trans</i> ) <sup>b</sup>	1.13	1.1	0.10
Efavirenz penteneyne <sup>c</sup>	1.14	1.0	0.10

<sup>a</sup> (S,E)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>b</sup> (S,Z)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>c</sup> (S)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

- **USP REFERENCE STANDARDS** <11>  
USP Efavirenz RS  
USP Efavirenz Related Compound B RS  
(*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-  
2*H*-3,1-benzoxazin-2-one.  
 $C_{14}H_{11}ClF_3NO_2$  317.69  
USP Efavirenz Racemic RS

## Efavirenz Capsules

### DEFINITION

Efavirenz Capsules contain NLT 92.0% and NMT 108.0% of the labeled amount of efavirenz ( $C_{14}H_9ClF_3NO_2$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>  
**Sample solution:** Dissolve the contents of 1 Capsule in about 5 mL of acetonitrile by mixing on a vortex mixer. Allow to settle, remove about 3 mL of the solution, and centrifuge for about 5 min. Transfer 1–2 mL of supernatant to a clean suitable container, and evaporate to dryness under nitrogen. Mix 0.5–1 mg of the powder with 200 mg of potassium bromide.
- **B. ULTRAVIOLET ABSORPTION** <197U>  
**Solvent:** Acetonitrile  
**Standard solution:** 10 µg/mL in *Solvent*  
**Sample solution:** Dissolve the contents of 1 Capsule in about 40 mL of *Solvent* by shaking for about 30 min. Pass through a suitable nylon or PVDF membrane filter, discarding the first 2 mL of filtrate, and dilute a portion with acetonitrile to a concentration of 10 µg/mL of efavirenz.  
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as does the *Standard solution*.

### ASSAY

- **PROCEDURE**  
**Diluent:** Acetonitrile and water (1:1)  
**Solution A:** Methanol, trifluoroacetic acid, and water (1:0.005:9). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]  
**Solution B:** Methanol, trifluoroacetic acid, and water (9:0.005:1). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]  
**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
16	50	50
23	35	65
28	30	70
29	20	80
31	20	80
32	60	40
40	60	40

**Standard solution 1:** 0.2 mg/mL of USP Efavirenz Related Compound B RS in *Diluent*

**Standard solution 2:** 5 mg/mL of USP Efavirenz RS in acetonitrile. [NOTE—Sonicate to dissolve before diluting to final volume.]

**Standard solution:** 250 µg/mL of USP Efavirenz RS and 1 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* prepared from *Standard solution 2* and *Standard solution 1*, respectively. [NOTE—Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation has been noted with certain brands made of glass.]

**Sample stock solution:** Transfer the contents of NLT 10 Capsules to a suitable container, and extract the contents in acetonitrile by mixing for about 30 min to obtain a quantitative solution equivalent to about 5 mg/mL of efavirenz. [NOTE—Store protected from light.]

**Sample solution:** Filter a portion of the *Sample stock solution*, and dilute the filtrate with *Diluent* to obtain a solution of about 250 µg/mL of efavirenz. [NOTE—Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation has been noted with certain brands made of glass.]

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 15-cm; packing L10

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 35 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.2 between efavirenz related compound B and efavirenz

**Relative standard deviation:** NMT 2.0% for efavirenz

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of efavirenz ( $C_{14}H_9ClF_3NO_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of efavirenz from the *Sample solution*

$r_S$  = peak response of efavirenz from the *Standard solution*

$C_S$  = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of efavirenz in the *Sample solution* (mg/mL)

**Acceptance criteria:** 92.0%–108.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Medium:** 1.0% (w/v) sodium lauryl sulfate in water; 900 mL. [NOTE—Do not deaerate.]

**Apparatus 2:** 50 rpm, with helix sinker

**Time:** 45 min

**Standard solution:** (L/900) mg/mL of USP Efavirenz RS in *Medium*, where L is the Capsule label claim in mg. A small volume of methanol, NMT 10% of the final volume, could be used to solubilize efavirenz. Dilute this solution with *Medium* to obtain a final concentration of about 0.01 mg/mL for Capsules labeled to contain 50 mg, or about 0.02 mg/mL for Capsules labeled to contain 100 mg or 200 mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Medium* to obtain a theoretical concentration similar to the *Standard solution*, assuming complete dissolution of the Capsule label claim.

**Analytical wavelength:** UV 247 nm

**Cell:** 1 cm

**Blank:** *Medium*

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of efavirenz dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of efavirenz is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**Procedure for content uniformity**

**Standard solution:** 10 µg/mL of USP Efavirenz RS in acetonitrile

**Sample solution:** Transfer the contents of 1 Capsule into a suitable container, and dissolve in 40.0 mL of acetonitrile. Shake for about 30 min and pass through a suitable nylon or PVDF membrane filter. Dilute a portion of the filtrate to an efavirenz concentration of about 10 µg/mL.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV absorption spectroscopy

**Analytical wavelength:** UV 246 nm

**Cell:** 1 cm

**Blank:** Acetonitrile

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of efavirenz (C<sub>14</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>2</sub>) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A<sub>U</sub> = absorbance of efavirenz from the *Sample solution*

A<sub>S</sub> = absorbance of efavirenz from the *Standard solution*

C<sub>S</sub> = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of the *Sample solution*

D = dilution factor of the *Sample solution*

**IMPURITIES**

**Organic Impurities**

• **PROCEDURE**

**Diluent, Solution A, Solution B, Sample solution, and Chromatographic system:** Prepare as directed in the *Assay*.

**System suitability solution:** Use the *Standard solution* prepared as directed in the *Assay*.

**Standard solution:** 1.25 µg/mL of USP Efavirenz RS and 0.005 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* from the *System suitability solution*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.2 between efavirenz related compound B and efavirenz, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for efavirenz, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r<sub>U</sub> = peak response of any individual impurity (degradation product) from the *Sample solution*

r<sub>S</sub> = peak response of efavirenz from the *Standard solution*

C<sub>S</sub> = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

C<sub>U</sub> = nominal concentration of efavirenz in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*. [NOTE— Disregard any peak less than 0.05%.]

**Total impurities:** NMT 0.50%. [NOTE— Include only the degradation products in the calculation of the total impurities.]

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz aminoalcohol (degradation product) <sup>a</sup>	0.48	0.26	0.25
Efavirenz ethene analog <sup>b</sup>	0.93	—	*
Efavirenz pent-3-ene-1-yne ( <i>cis</i> ) <sup>c</sup>	1.16	—	*
Efavirenz pent-3-ene-1-yne ( <i>trans</i> ) <sup>d</sup>	1.16	—	*
Efavirenz penteneyne <sup>e</sup>	1.16	—	*
Efavirenz pentyne analog <sup>f</sup>	1.2	—	*
Methylefavirenz <sup>g</sup>	1.28	—	*
Efavirenz aminoalcohol methyl carbamate <sup>h</sup>	1.33	—	*
N-Benzylefavirenz <sup>i</sup>	1.8	—	*
Efavirenz benzoylaminoalcohol <sup>j</sup>	1.9	—	*
Quinoline analog (degradation product) <sup>k</sup>	1.45	2.0	0.20
Efavirenz aminoalcohol ethyl carbamate <sup>l</sup>	1.53	—	*
Unidentified impurity	1.60	—	*
Efavirenz aminoalcohol bis(ethoxycarbonyl) <sup>m</sup>	1.63	—	*
Unidentified impurity	2.1	—	*

<sup>a</sup> (S)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.  
<sup>b</sup> (S,E)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>c</sup> (S,E)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>d</sup> (S,Z)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>e</sup> (S)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>f</sup> (S)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>g</sup> (S)-6-Chloro-4-[[[(2R,2R)-2-methylcyclopropyl]ethynyl]-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>h</sup> (S)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.

<sup>i</sup> (S)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

<sup>j</sup> (S)-N-[4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenyl]-4-methoxybenzamide.

<sup>k</sup> 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.

<sup>l</sup> (S)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.

<sup>m</sup> (S)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.

<sup>n</sup> Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1H-indole-1-carboxylate.

\* For information purposes only. These are process impurities monitored in the drug substance and are not included in the total impurities.



**Impurity Table 1** (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclobutenylindole analog <sup>a</sup>	2.18	—	*
Any other individual degradation product	—	1.0	0.20

<sup>a</sup> (S)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.<sup>b</sup> (S,E)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>c</sup> (S,E)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>d</sup> (S,Z)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>e</sup> (S)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>f</sup> (S)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>g</sup> (S)-6-Chloro-4-[(2R,5,2R)-2-methylcyclopropyl]ethynyl-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>h</sup> (S)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.<sup>i</sup> (S)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.<sup>j</sup> (S)-N-[4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenyl]-4-methoxybenzamide.<sup>k</sup> 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.<sup>l</sup> (S)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.<sup>m</sup> (S)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.<sup>n</sup> Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1H-indole-1-carboxylate.

\* For information purposes only. These are process impurities monitored in the drug substance and are not included in the total impurities.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store in well-closed containers at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Efavirenz RS
  - USP Efavirenz Related Compound B RS
  - (S,E)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.
  - C<sub>14</sub>H<sub>11</sub>ClF<sub>3</sub>NO<sub>2</sub> 317.69

**Multiple Electrolytes Injection Type 1**

» Multiple Electrolytes Injection Type 1 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and gluconate, or acetate, gluconate, and phosphate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), chloride (Cl), acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>), gluconate (C<sub>6</sub>H<sub>11</sub>O<sub>7</sub>), and phosphate (PO<sub>4</sub>). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alterna-

tively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Potassium Gluconate RS

**Identification**—

**A:** It responds to the flame tests for *Sodium* (191) and *Potassium* (191), and to the tests for *Magnesium* (191) and *Chloride* (191).

**B:** The retention time of the acetate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay for acetate*.

**C:** Where gluconate is purported to be present, the retention time of the gluconate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay for gluconate*.

**D:** Where phosphate is purported to be present, add 5 mL of the Injection and 1 mL of ammonium molybdate TS to a test tube, and mix: a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 4.0 and 8.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for potassium and sodium**—

*Internal standard solution*, *Potassium stock solution*, *Sodium stock solution*, *Stock standard preparation*, and *Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution*—Transfer 17.69 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

*Dilute hydrochloric acid*—Prepare by mixing 678 mL of hydrochloric acid with sufficient water to make 3000 mL.

*Blank solution*—Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask, dilute with *Dilute hydrochloric acid* to volume, and mix.

*Magnesium stock solution*—Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Dilute hydrochloric acid* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Dilute hydrochloric acid* to volume, and mix. This solution contains 100 µg of magnesium (Mg) per mL.

**Standard preparations**—To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 10.0, 15.0, and 20 mL, respectively, of *Magnesium stock solution*. Dilute the contents of each flask with *Dilute hydrochloric acid* to volume, and mix. These three solutions contain 10.0, 15.0, and 20.0 µg, respectively, of magnesium (Mg) per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a magnesium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of magnesium in the *Assay preparation*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which *V* is the volume, in mL, of Injection taken to prepare the *Assay preparation*.

**Assay for chloride**—Transfer an accurately measured volume of Injection, equivalent to about 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to about 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg (0.1 mEq) of Cl.

#### Assay for acetate—

**Mobile phase**—Prepare a filtered and degassed solution of 0.05 N sulfuric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of sodium acetate trihydrate in water to obtain a *Standard preparation* having a known concentration of about 1.2 mg of sodium acetate trihydrate (about 0.0088 mEq of acetate) per mL.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.0088 mEq of acetate per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector, a 4.6-mm × 3-cm guard column containing packing L17, and a 7.8-mm × 30-cm analytical column containing packing L17. The column temperature is maintained at about 60°. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mEq per L, of acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>) in the Injection taken by the formula:

$$(C/136.08)(L/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of sodium acetate trihydrate in the *Standard preparation*; 136.08 is the molecular weight of sodium acetate trihydrate; *L* is the labeled quantity, in mEq per L, of acetate in the Injection; *D*

is the quantity, in mEq per mL, of acetate in the *Assay preparation*, based on the labeled quantity and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for gluconate (if present)—

**Mobile phase**—Prepare a filtered and degassed solution of 0.05 N sulfuric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Potassium Gluconate RS in water to obtain a *Standard preparation* having a known concentration of about 1 mg of USP Potassium Gluconate RS (about 0.0043 mEq of gluconate) per mL.

**Assay preparation**—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.004 mEq of gluconate per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector, a 4.6-mm × 3-cm guard column containing packing L17, and a 7.8-mm × 30-cm analytical column containing packing L17. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mEq per L, of gluconate (C<sub>6</sub>H<sub>11</sub>O<sub>7</sub>) in the Injection taken by the formula:

$$(C/234.25)(L/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Potassium Gluconate RS in the *Standard preparation*; 234.25 is the molecular weight of anhydrous potassium gluconate; *L* is the labeled quantity, in mEq per L, of gluconate in the Injection; *D* is the quantity, in mEq per mL, of gluconate in the *Assay preparation*, based on the labeled quantity and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the gluconate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for phosphate (if present)—

**Ammonium molybdate solution**—Transfer 25 g of ammonium molybdate to a 500-mL volumetric flask, add about 300 mL of water, and swirl to dissolve solution. Add 75 mL of sulfuric acid, and swirl. Allow to cool, dilute with water to volume, and mix.

**Hydroquinone solution**—Dissolve 0.5 g of hydroquinone in 100 mL of water, add 1 drop of sulfuric acid, and mix. [NOTE—Prepare this solution fresh daily.]

**Sodium sulfite solution**—Dissolve 1 g of sodium sulfite in water to make 5 mL of solution. [NOTE—Prepare this solution fresh daily.]

**Standard preparation**—Dissolve an accurately weighed quantity of monobasic potassium phosphate in water to obtain a solution having a known concentration of about 0.11 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 4 mg (0.126 mEq) of phosphate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Blank**—Use water.

**Procedure**—Transfer 2.0 mL each of the *Standard preparation*, the *Assay preparation*, and the *Blank* to separate test tubes. To each test tube add 1.0 mL of *Ammonium molybdate solution*, mix, and allow to stand for 3 minutes. Add 1.0 mL of *Hydroquinone solution*, and mix. Add 1.0 mL of *Sodium sulfite solution*, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the *Assay*

preparation and the *Standard preparation* at 640 nm, using water to zero the instrument. Calculate the quantity, in mg, of phosphate ( $\text{PO}_4$ ) in each mL of the Injection taken by the formula:

$$50(94.97/136.09)(C/V)(A_U / A_S)$$

in which 94.97 is the formula weight of phosphate ( $\text{PO}_4$ ); 136.09 is the molecular weight of monobasic potassium phosphate; C is the concentration, in mg per mL, of monobasic potassium phosphate in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, corrected for any absorbance of the solution from the *Blank*.

## Multiple Electrolytes Injection Type 2

» Multiple Electrolytes Injection Type 2 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, calcium, magnesium, and chloride ions. In addition, the salts provide ions of either acetate and citrate, or acetate and lactate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chloride (Cl), acetate ( $\text{C}_2\text{H}_3\text{O}_2$ ), citrate ( $\text{C}_6\text{H}_5\text{O}_7$ ), and lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

### USP Reference standards (11)—

USP Citric Acid RS  
USP Endotoxin RS  
USP Sodium Lactate RS

### Identification—

**A:** It responds to the flame tests for *Sodium* (191) and *Potassium* (191), to the oxalate test for *Calcium* (191), and to the tests for *Magnesium* (191) and *Chloride* (191).

**B:** The retention time of the acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetate*.

**C:** Where citrate is purported to be present, the retention time of the citrate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for citrate*.

**D:** Where lactate is purported to be present, the retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lactate*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 4.0 and 8.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay for potassium and sodium—

*Internal standard solution*, *Potassium stock solution*, *Sodium stock solution*, *Stock standard preparation*, and *Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution*, *Dilute hydrochloric acid*, *Blank solution*, *Magnesium stock solution*, and *Standard preparations*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in  $\mu\text{g}$ , of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which V is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and the other terms are as defined therein.

**Assay for calcium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution*, *Dilute hydrochloric acid*, and *Blank solution*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Calcium stock solution*—Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Dilute hydrochloric acid*, and swirl to dissolve the calcium carbonate. Dilute with water to volume, and mix. This solution contains 1000  $\mu\text{g}$  of calcium (Ca) per mL.

*Standard preparations*—To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL, respectively, of *Calcium stock solution*. Dilute the contents of each flask with *Dilute hydrochloric acid* to volume, and mix. These three solutions contain 10.0, 15.0, and 20.0  $\mu\text{g}$ , respectively, of calcium (Ca) per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of

calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the calcium emission line at 422.7 nm, with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a calcium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in  $\mu\text{g}$  per mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu\text{g}$  per mL, of calcium in the *Assay preparation*. Calculate the quantity, in  $\mu\text{g}$ , of calcium in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which  $V$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*.

**Assay for chloride**—Using the Injection, proceed as directed for the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

#### **Assay for acetate**—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.0088 mEq of acetate per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mEq per L, of acetate ( $\text{C}_2\text{H}_3\text{O}_2$ ) in the Injection taken by the formula:

$$(C/136.08)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

#### **Assay for citrate (if present)**—

*Mobile Phase and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* (345).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide to obtain a solution having a known concentration of about 0.3 mEq of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ ) per L.

**Assay preparation**—Dilute an accurately measured volume of Injection quantitatively with freshly prepared sodium hydroxide to obtain a solution having a concentration of about 0.3 mEq of citrate per L in 1 mM sodium hydroxide.

**Procedure**—Proceed as directed for *Procedure* under general chapter (345), calculate the quantity, in mEq per L, of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ ) in the Injection taken by the formula:

$$C_S D(r_U / r_S)$$

in which  $C_S$  is the concentration, in mEq per L, of citrate in the *Standard preparation*;  $D$  is the dilution factor; and  $r_U$  and  $r_S$  are the citrate peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### **Assay for lactate (if present)**—

*Mobile phase*—Prepare a solution in water containing about 1 mL of formic acid and 1 mL of dicyclohexylamine per L, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Prepare a solution in water containing about 3 mg of anhydrous sodium acetate and 3 mg of USP Sodium Lactate RS per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Sodium Lactate RS in water to obtain a

solution having a known concentration of about 2 mg per mL.

**Assay preparation**—Where the labeled quantity of lactate is greater than 20 mEq per L, quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per L, use the undiluted Injection as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  10-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between acetate and lactate is not less than 2. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mEq per L, of lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ) in the Injection taken by the formula:

$$(C/112.06)(L/D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Sodium Lactate RS in the *Standard preparation*; 112.06 is the molecular weight of sodium lactate;  $L$  is the labeled quantity, in mEq per L, of lactate in Injection;  $D$  is the quantity, in mEq per mL, of lactate in the *Assay preparation*, based on the labeled quantity and the extent of dilution; and  $r_U$  and  $r_S$  are the lactate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## **Multiple Electrolytes and Dextrose Injection Type 1**

» Multiple Electrolytes and Dextrose Injection Type 1 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and gluconate, or acetate and phosphate, or phosphate and lactate, or phosphate and sulfate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), acetate ( $\text{C}_2\text{H}_3\text{O}_2$ ), gluconate ( $\text{C}_6\text{H}_{11}\text{O}_7$ ), phosphate ( $\text{PO}_4$ ), lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ), and sulfate ( $\text{SO}_4$ ), not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl), and not less than 90.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L.

When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** <11>—

USP Endotoxin RS  
USP Potassium Gluconate RS  
USP Sodium Lactate RS

**Identification**—

**A:** It responds to the *Identification* test under *Dextrose*.

**B:** It responds to the flame tests for *Sodium* <191> and *Potassium* <191>, and to the tests for *Magnesium* <191> and *Chloride* <191>.

**C:** Where acetate is purported to be present, the retention time of the acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetate*.

**D:** Where gluconate is purported to be present, the retention time of the gluconate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for gluconate*.

**E:** Where phosphate is purported to be present, add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube, and mix: a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

**F:** Where lactate is purported to be present, the retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lactate*.

**G:** Where sulfate is purported to be present, it responds to the barium chloride test for *Sulfate* <191>.

**Bacterial endotoxins** <85>—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** <791>: between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay for potassium and sodium**—

*Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride* in *Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride* in *Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which V is the volume, in mL, of Injection taken to prepare the *Assay preparation*.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

**Assay for acetate** (if present)—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.0088 mEq of acetate per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mEq per L, of acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>) in the Injection taken by the formula:

$$(C/136.08)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for gluconate** (if present)—

*Mobile phase*—Dissolve 7.0 g of calcium acetate in 1900 mL of water, and adjust with glacial acetic acid to a pH of 4.5 ± 0.1. Dilute with water to 2000 mL, mix, pass through a filter having a 0.5-µm porosity or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>). Maintain the *Mobile phase* at 70 ± 2° at all times.

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Potassium Gluconate RS in water to obtain a solution having a known concentration of about 4.6 mg per mL.

*Resolution solution*—Prepare an aqueous solution containing about 5 mg each of USP Sodium Lactate RS and USP Potassium Gluconate RS per mL.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with water, if necessary, to obtain a solution containing about 0.023 mEq of gluconate per mL.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a refractive index detector and a 7.8-mm × 30-cm column that contains packing L19 in the calcium form and is maintained at 70 ± 2°. The flow rate is about 1 mL per minute. Condition the column for about 60 minutes by pumping *Mobile phase* through it. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution R<sub>s</sub> between lactate and gluconate is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mEq per L, of gluconate ( $\text{C}_6\text{H}_{11}\text{O}_7$ ) in the Injection taken by the formula:

$$(C/234.25)(L/D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Potassium Gluconate RS in the *Standard preparation*; 234.25 is the molecular weight of potassium gluconate;  $L$  is the labeled quantity, in mEq per L, of gluconate in the Injection;  $D$  is the quantity, in mEq per L, of gluconate in the *Assay preparation*, based on the labeled quantity and the extent of dilution, if any; and  $r_U$  and  $r_S$  are the gluconate peak responses for the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for phosphate (if present)—

*Ammonium molybdate solution*, *Hydroquinone solution*, *Sodium sulfite solution*, *Standard preparation*, and *Blank*—Prepare as directed in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 4 mg (0.126 mEq) of phosphate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mg, of phosphate ( $\text{PO}_4$ ) in each mL of the Injection taken by the formula:

$$50(94.97/136.08)(C/V)(A_U / A_S)$$

in which the terms are as defined therein.

#### Assay for lactate (if present)—

*Mobile phase*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*.

*Assay preparation*—Where the labeled quantity of lactate is greater than 20 mEq per L, quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per L, use the undiluted Injection as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in mEq per L, of lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ) in the Injection taken by the formula:

$$(C/112.06)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for sulfate (if present)**—Transfer an accurately measured volume of Injection, equivalent to about 120 mg (1.22 mEq) of sulfate, to a suitable vessel. Dilute if necessary to 200 mL, and add 1 mL of hydrochloric acid. Heat to boiling, and gradually add, in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 hour, collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.4116, represents its equivalent of sulfate ( $\text{SO}_4$ ). Each mg of barium sulfate represents 0.004285 mEq of sulfate.

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a polarimeter tube (see *Optical Rotation* <781>). Calculate the percentage (g per 100 mL) of dex-

trose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

## Multiple Electrolytes and Dextrose Injection Type 2

» Multiple Electrolytes and Dextrose Injection Type 2 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, calcium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and citrate, or acetate and lactate, or gluconate and sulfate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), acetate ( $\text{C}_2\text{H}_3\text{O}_2$ ), citrate ( $\text{C}_6\text{H}_5\text{O}_7$ ), lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ), gluconate ( $\text{C}_6\text{H}_{11}\text{O}_7$ ), and sulfate ( $\text{SO}_4$ ), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl), and not less than 90.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

#### USP Reference standards <11>—

USP Citric Acid RS  
USP Endotoxin RS  
USP Potassium Gluconate RS  
USP Sodium Lactate RS

#### Identification—

**A:** It responds to the *Identification* test under *Dextrose*.

**B:** It responds to the flame tests for *Sodium* <191> and *Potassium* <191>, to the tests for *Magnesium* <191> and *Chloride* <191>, and to the oxalate test for *Calcium* <191>.

**C:** Where acetate is purported to be present, the retention time of the acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetate*.

**D:** Where citrate is purported to be present, the retention time of the citrate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for citrate*.

**E:** Where lactate is purported to be present, the retention time of the lactate peak in the chromatogram of the *Assay*

preparation corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lactate*.

**F:** Where gluconate is purported to be present, the retention time of the gluconate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for gluconate*.

**G:** Where sulfate is purported to be present, it responds to the barium chloride test for *Sulfate* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

#### **Assay for potassium and sodium—**

*Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which V is the volume, in mL, of Injection taken to prepare the *Assay preparation*.

**Assay for calcium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, and Blank solution*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Calcium stock solution and Standard preparations*—Prepare as directed in the *Assay for calcium* under *Multiple Electrolytes Injection Type 2*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

*Procedure*—Proceed as directed in the *Assay for calcium* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in µg, of calcium in each mL of the Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

**Assay for acetate (if present)**—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.0088 mEq of acetate per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mEq per liter, of acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>) in the Injection taken by the formula:

$$(C/136.08)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for citrate (if present)**—

*Mobile Phase and Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* (345).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide to obtain a solution having a known concentration of about 0.3 mEq of citrate (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) per L.

*Assay preparation*—Dilute an accurately measured volume of Injection quantitatively with freshly prepared sodium hydroxide to obtain a solution having a concentration of about 0.3 mEq of citrate per L in 1 mM sodium hydroxide.

*Procedure*—Proceed as directed for *Procedure* under general chapter (345). Calculate the quantity, in mEq per L, of citrate (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) in the portion of Injection taken by the formula:

$$C_S D(r_U / r_S)$$

in which C<sub>S</sub> is the concentration, in mEq per L, of citrate in the *Standard preparation*; D is the dilution factor; and r<sub>U</sub> and r<sub>S</sub> are the citrate peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for lactate (if present)**—

*Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*.

*Assay preparation*—Where the labeled quantity of lactate is greater than 20 mEq per L, quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per L, use the undiluted Injection as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in mEq per L, of lactate (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>) in the Injection taken by the formula:

$$(C/112.06)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for gluconate (if present)**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay for gluconate* under *Multiple Electrolytes and Dextrose Injection Type 1*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.023 mEq of gluconate per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for gluconate* under *Multiple Electrolytes and Dextrose Injection Type 1*. Calculate the quantity, in mEq per L, of gluconate ( $C_6H_{11}O_7$ ) in the Injection taken by the formula:

$$(C/234.25)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for sulfate (if present)**—Using the Injection, proceed as directed in the *Assay for sulfate* under *Multiple Electrolytes and Dextrose Injection Type 1*.

**Assay for dextrose**—Using the Injection, proceed as directed in the *Assay for dextrose* under *Multiple Electrolytes and Dextrose Injection Type 1*.

## Multiple Electrolytes and Dextrose Injection Type 3

» Multiple Electrolytes and Dextrose Injection Type 3 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, and chloride ions. In addition, the salts provide ions of ammonium, or acetate and phosphate, or phosphate and lactate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), ammonium ( $NH_4$ ), acetate ( $C_2H_3O_2$ ), phosphate ( $PO_4$ ), and lactate ( $C_3H_5O_3$ ), not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl), and not less than 90.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards (11)**—

USP Endotoxin RS

USP Sodium Lactate RS

**Identification**—

**A:** It responds to the *Identification* test under *Dextrose*.

**B:** It responds to the flame tests for *Sodium* (191) and *Potassium* (191), and to the tests for *Chloride* (191) and *Ammonium* (191).

**C:** Where acetate is purported to be present, the retention time of the acetate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay for acetate*.

**D:** Where phosphate is purported to be present, add 5 mL of the Injection and 1 mL of ammonium molybdate TS to a test tube, and mix: a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

**E:** Where lactate is purported to be present, the retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lactate*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for potassium and sodium**—

*Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

**Assay for ammonium (if present)**—Transfer an accurately measured volume of Injection, equivalent to about 63 mg (3.5 mEq) of ammonium, to a 500-mL Kjeldahl flask, dilute with water to 200 mL, mix, and add 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 1.804 mg (0.1 mEq) of ammonium ( $NH_4$ ).

**Assay for acetate (if present)**—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.0088 mEq of acetate per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for acetate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mEq per L, of acetate ( $C_2H_3O_2$ ) in the Injection taken by the formula:

$$(C/136.08)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for phosphate (if present)**—

*Ammonium molybdate solution, Hydroquinone solution, Sodium sulfite solution, Standard preparation, and Blank*—Pre-



pare as directed in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 4 mg (0.126 mEq) of phosphate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for phosphate* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mg, of phosphate ( $\text{PO}_4$ ) in each mL of the Injection taken by the formula:

$$50(94.97/136.09)(C/V)(A_U / A_S)$$

in which the terms are as defined therein.

#### **Assay for lactate (if present)**—

**Mobile phase, Resolution solution, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*.

**Assay preparation**—Where the labeled quantity of lactate is greater than 20 mEq per L, quantitatively dilute an accurately measured volume of Injection with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per L, use the undiluted Injection as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for lactate* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in mEq per L, of lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ) in the Injection taken by the formula:

$$(C/112.06)(L/D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for dextrose**—Using the Injection, proceed as directed in the *Assay for dextrose* under *Multiple Electrolytes and Dextrose Injection Type 1*.

## **Multiple Electrolytes and Dextrose Injection Type 4**

» Multiple Electrolytes and Dextrose Injection Type 4 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, magnesium, calcium, chloride, gluconate, and sulfate ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), magnesium (Mg), calcium (Ca), gluconate ( $\text{C}_6\text{H}_{11}\text{O}_7$ ), and sulfate ( $\text{SO}_4$ ), not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl), and not less than 90.0 percent and not more than 105.0 percent of the labeled amount of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

#### **USP Reference standards** (11)—

USP Endotoxin RS

USP Potassium Gluconate RS

#### **Identification**—

**A:** It responds to the *Identification* test under *Dextrose*.

**B:** It responds to the flame test for *Sodium* (191), to the tests for *Magnesium* (191) and *Chloride* (191), to the oxalate test for *Calcium* (191), and to the barium chloride test for *Sulfate* (191).

**C:** The retention time of the gluconate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay for gluconate*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 4.2 and 5.2.

**Other requirements**—It meets the requirements under *Injections* (1).

#### **Assay for sodium**—

**Internal standard solution**—Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, dilute with water to volume, and mix.

**Standard preparation**—Transfer 7.31 g of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix. Each mL of this solution contains 0.0292 mg of sodium chloride, equivalent to 0.0115 mg (0.0005 mEq) of sodium (Na).

**Assay preparation**—Dilute an accurately measured volume of Injection quantitatively with *Internal standard solution* to obtain a solution containing about 0.0115 mg (0.0005 mEq) of sodium per mL.

**Procedure**—Using a flame photometer, adjusted to read zero with *Internal standard solution*, concomitantly determine the flame emission readings for the *Standard preparation* and the *Assay preparation* at the wavelengths of maximum emission for sodium and lithium (589 nm and 671 nm, respectively). Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$(22.99 / 58.44)(C)(L / D)(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which 22.99 is the atomic weight of sodium, 58.44 is the molecular weight of sodium chloride, C is the concentration, in mg per mL, of sodium chloride in the *Standard preparation*, L is the labeled quantity, in mg, of sodium in each mL of the Injection, D is the concentration, in mg per mL, of sodium in the *Assay preparation*, based on the volume of Injection taken and the extent of dilution, and  $R_{U,589}$  and  $R_{U,671}$  are the emission readings at the wavelengths identified by the subscript numbers obtained for the *Assay preparation*, and  $R_{S,589}$  and  $R_{S,671}$  are the emission readings at the wavelengths identified by the subscript numbers obtained from the *Standard preparation*. Each mg of sodium is equivalent to 0.0435 of mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

**Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations**—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*. Calculate the quantity, in  $\mu\text{g}$ , of magnesium (Mg) in each mL of Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

**Assay for calcium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution*, *Dilute hydrochloric acid*, and *Blank solution*—Prepare as directed in the *Assay for magnesium* under *Multiple Electrolytes Injection Type 1*.

*Calcium stock solution* and *Standard preparations*—Prepare as directed in the *Assay for calcium* under *Multiple Electrolytes Injection Type 2*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for calcium* under *Multiple Electrolytes Injection Type 2*. Calculate the quantity, in  $\mu\text{g}$ , of calcium in each mL of Injection taken by the formula:

$$1000(C/V)$$

in which the terms are as defined therein.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

**Assay for gluconate**—

*Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Prepare as directed in the *Assay for gluconate* under *Multiple Electrolytes and Dextrose Injection Type 1*.

*Assay preparation*—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.023 mEq of gluconate per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for gluconate* under *Multiple Electrolytes and Dextrose Injection Type 1*. Calculate the quantity, in mEq per liter, of gluconate ( $\text{C}_6\text{H}_{11}\text{O}_7$ ) in the Injection taken by the formula:

$$(C/234.25)(L/D)(r_U/r_S)$$

in which the terms are as defined therein.

**Assay for sulfate**—Using the Injection, proceed as directed in the *Assay for sulfate* under *Multiple Electrolytes and Dextrose Injection Type 1*.

**Assay for dextrose**—Using the Injection, proceed as directed in the *Assay for dextrose* under *Multiple Electrolytes and Dextrose Injection Type 1*.

## Multiple Electrolytes and Invert Sugar Injection Type 1

» Multiple Electrolytes and Invert Sugar Injection Type 1 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, magnesium, chloride, phosphate, and lactate ions. It contains not

less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), phosphate ( $\text{PO}_4$ ), lactate ( $\text{C}_3\text{H}_5\text{O}_3$ ), and invert sugar ( $\text{C}_6\text{H}_{12}\text{O}_6$ ), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—  
USP Sodium Lactate RS

**Identification**—

**A:** It responds to the *Identification* test under *Invert Sugar Injection*.

**B:** It responds to the flame tests for *Sodium* (191) and *Potassium* (191), and to the tests for *Magnesium* (191) and *Chloride* (191).

**C:** Add 5 mL of the Injection and 1 mL of ammonium molybdate TS to a test tube, and mix: a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed (presence of phosphate).

**D:** The retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay for lactate*.

**Pyrogen**—It meets the requirements of the *Pyrogen Test* (151). [NOTE—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of 10% invert sugar.]

**pH** (791): between 3.0 and 6.0.

**Completeness of inversion**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the test for *Completeness of inversion* under *Invert Sugar Injection*.

*Test preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the test for *Completeness of inversion* under *Invert Sugar Injection*. Calculate the quantity, in mg, of sucrose in the volume of the Injection taken by the formula:

$$100C(r_U/r_S)$$

in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of the Injection taken, based on the labeled amount, is found.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for potassium and sodium**—

*Internal standard solution*, *Potassium stock solution*, *Sodium stock solution*, *Stock standard preparation*, and *Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the *Standard preparations* and the *Assay preparation* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations*—Prepare as directed in the *Assay for magnesium under Multiple Electrolytes Injection Type 1*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of *Lanthanum chloride solution*. Dilute the contents of the flask with *Dilute hydrochloric acid* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for magnesium under Multiple Electrolytes Injection Type 1*. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C / V)$$

in which the terms are as defined therein.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride under Multiple Electrolytes Injection Type 1*.

**Assay for phosphate**—

*Ammonium molybdate solution, Hydroquinone solution, Sodium sulfite solution, Standard preparation, and Blank*—Prepare as directed in the *Assay for phosphate under Multiple Electrolytes Injection Type 1*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 4 mg (0.126 mEq) of phosphate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for phosphate under Multiple Electrolytes Injection Type 1*. Calculate the quantity, in mg, of phosphate (PO<sub>4</sub>) in each mL of the Injection taken by the formula:

$$50(94.97 / 136.09)(C / V)(A_U / A_S)$$

in which the terms are as defined therein.

**Assay for lactate**—

*Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay for lactate under Multiple Electrolytes Injection Type 2*.

**Assay preparation**—Where the labeled quantity of lactate is greater than 20 mEq per liter, dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per liter, use the undiluted Injection as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for lactate under Multiple Electrolytes Injection Type 2*. Calculate the quantity, in mEq per liter, of lactate (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>) in the Injection taken by the formula:

late the quantity, in mEq per liter, of lactate (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>) in the Injection taken by the formula:

$$(C / 112.06)(L / D)(r_U / r_S)$$

in which the terms are as defined therein.

**Assay for invert sugar**—Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Transfer 50.0 mL of freshly mixed alkaline cupric tartrate TS to a 400-mL beaker. To the beaker add the boiling chips or glass beads from the tared crucible, 48 mL of water, and 2.0 mL of Injection that has been diluted quantitatively with water, if necessary, to a 5.0% concentration of invert sugar. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5 to 4 minutes. Boil the solution for 2.0 minutes, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight, and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 193.6 mg and not more than 234.5 mg, corresponding to between 90.0 and 110.0 mg of invert sugar (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>).

## Multiple Electrolytes and Invert Sugar Injection Type 2

» Multiple Electrolytes and Invert Sugar Injection Type 2 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, magnesium, calcium, chloride, and lactate ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), lactate (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>), and invert sugar (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** <11>—

USP Sodium Lactate RS

**Identification**—

**A:** It responds to the *Identification* test under *Invert Sugar Injection*.

**B:** It responds to the flame tests for *Sodium* <191> and *Potassium* <191>, to the tests for *Magnesium* <191> and *Chloride* <191>, and to the oxalate test for *Calcium* <191>.

**C:** The retention time of the lactate peak in the chromatogram of the *Assay preparation* corresponds to that of the

Standard preparation obtained as directed in the Assay for lactate.

**Pyrogen**—It meets the requirements of the Pyrogen Test (151). [NOTE—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of 10% invert sugar.]

**pH** (791): between 4.5 and 6.0.

**Completeness of inversion—**

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the test for Completeness of inversion under Invert Sugar Injection.

*Test preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for Procedure in the test for Completeness of inversion under Invert Sugar Injection. Calculate the quantity, in mg, of sucrose in the volume of Injection taken by the formula:

$$100C(r_u / r_s)$$

in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of Injection taken, based on the labeled amount, is found.

**Other requirements**—It meets the requirements under Injections (1).

**Assay for potassium and sodium—**

*Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation*—Prepare as directed in the Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with Internal standard solution to volume, and mix.

*Procedure*—Proceed as directed for Procedure in the Assay for potassium and sodium under Potassium Chloride in Sodium Chloride Injection. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for magnesium**—[NOTE—Concentrations of the Standard preparations and the Assay preparation may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, Blank solution, Magnesium stock solution, and Standard preparations*—Prepare as directed in the Assay for magnesium under Multiple Electrolytes Injection Type 1.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1.65 mEq) of magnesium, to a 1000-mL volumetric flask containing 50.0 mL of Lanthanum chloride solution. Dilute the contents of the flask with Dilute hydrochloric acid to volume, and mix.

*Procedure*—Proceed as directed for Procedure in the Assay for magnesium under Multiple Electrolytes Injection Type 1. Calculate the quantity, in µg, of magnesium (Mg) in each mL of the Injection taken by the formula:

$$1000(C / V)$$

in which the terms are as defined therein.

**Assay for calcium**—[NOTE—Concentrations of the Standard preparations and the Assay preparation may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

*Lanthanum chloride solution, Dilute hydrochloric acid, and Blank solution*—Prepare as directed in the Assay for magnesium under Multiple Electrolytes Injection Type 1.

*Calcium stock solution and Standard preparations*—Prepare as directed in the Assay for calcium under Multiple Electrolytes Injection Type 2.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 20 mg (1 mEq) of calcium, to a 1000-mL volumetric flask containing 50.0 mL of Lanthanum chloride solution. Dilute the contents of the flask with Dilute hydrochloric acid to volume, and mix.

*Procedure*—Proceed as directed for Procedure in the Assay for calcium under Multiple Electrolytes Injection Type 2. Calculate the quantity, in µg, of calcium in each mL of the Injection taken by the formula:

$$1000(C / V)$$

in which the terms are as defined therein.

**Assay for chloride**—Using the Injection, proceed as directed in the Assay for chloride under Multiple Electrolytes Injection Type 1.

**Assay for lactate—**

*Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay for lactate under Multiple Electrolytes Injection Type 2.

*Assay preparation*—Where the labeled quantity of lactate is greater than 20 mEq per liter, dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 0.02 mEq of lactate per mL. Where the labeled quantity of lactate is 20 mEq or less per liter, use the undiluted Injection as the Assay preparation.

*Procedure*—Proceed as directed for Procedure in the Assay for lactate under Multiple Electrolytes Injection Type 2. Calculate the quantity, in mEq per liter, of lactate ( $C_3H_5O_3$ ) in the Injection taken by the formula:

$$(C / 112.06)(L / D)(r_u / r_s)$$

in which the terms are as defined therein.

**Assay for invert sugar**—Using the Injection, proceed as directed in the Assay for invert sugar under Multiple Electrolytes and Invert Sugar Injection Type 1.

## Multiple Electrolytes and Invert Sugar Injection Type 3

» Multiple Electrolytes and Invert Sugar Injection Type 3 is a sterile solution of a mixture of equal amounts of Dextrose and Fructose, or an equivalent solution produced by the hydrolysis of Sucrose, and suitable salts in Water for Injection to provide sodium, potassium, chloride, and ammonium ions. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of sodium (Na), potassium (K), ammonium ( $NH_4$ ), and invert sugar ( $C_6H_{12}O_6$ ), and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloride (Cl). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**Labeling**—The label states the content of each electrolyte in terms of milliequivalents in a given volume. The label states the total osmolar concentration in mOsmol per L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**Identification**—

**A:** It responds to the *Identification* test under *Invert Sugar Injection*.

**B:** It responds to the flame tests for *Sodium* <191> and *Potassium* <191>, and to the tests for *Chloride* <191> and *Ammonium* <191>.

**Pyrogen**—It meets the requirements of the *Pyrogen Test* <151>. [NOTE—Dilute, with Water for Injection, Injection containing more than 10% of invert sugar to give a concentration of 10% invert sugar.]

**pH** <791>: between 3.0 and 5.5.

**Completeness of inversion**—

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the test for *Completeness of inversion* under *Invert Sugar Injection*.

*Test preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of invert sugar, to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the test for *Completeness of inversion* under *Invert Sugar Injection*. Calculate the quantity, in mg, of sucrose in the volume of Injection taken by the formula:

$$100C(r_u / r_s)$$

in which the terms are as defined therein. Not more than 1.5% of the quantity of invert sugar in the volume of Injection taken, based on the labeled amount, is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay for potassium and sodium**—

*Internal standard solution, Potassium stock solution, Sodium stock solution, Stock standard preparation, and Standard preparation*—Prepare as directed in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*.

*Assay preparation*—Transfer 5.0 mL of Injection to a 500-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for potassium and sodium* under *Potassium Chloride in Sodium Chloride Injection*. Calculate the quantity, in mg, of potassium (K) in each mL of the Injection taken by the formula:

$$C(R_{U,766} / R_{U,671})(R_{S,671} / R_{S,766})$$

in which the terms are as defined therein. Each mg of potassium is equivalent to 0.02558 mEq of potassium. Calculate the quantity, in mg, of sodium (Na) in each mL of the Injection taken by the formula:

$$C(R_{U,589} / R_{U,671})(R_{S,671} / R_{S,589})$$

in which the terms are as defined therein. Each mg of sodium is equivalent to 0.04350 mEq of sodium.

**Assay for chloride**—Using the Injection, proceed as directed in the *Assay for chloride* under *Multiple Electrolytes Injection Type 1*.

**Assay for ammonium**—Using the Injection, proceed as directed in the *Assay for ammonium* under *Multiple Electrolytes and Dextrose Injection Type 3*.

**Assay for invert sugar**—Using the Injection, proceed as directed in the *Assay for invert sugar* under *Multiple Electrolytes and Invert Sugar Injection Type 1*.

## Trace Elements Injection

» Trace Elements Injection is a sterile solution in Water for Injection of two or more of the following: Zinc Chloride or Zinc Sulfate, Cupric Chloride or Cupric Sulfate, Chromic Chloride, Manganese Chloride or Manganese Sulfate, Selenious Acid, Sodium Iodide, and Ammonium Molybdate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), selenium (Se), iodine (I), and molybdenum (Mo).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

**Labeling**—Label the Injection to specify that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration. The label shows by an appropriate number juxtaposed to the official name, the number of trace elements contained in the Injection according to the following: zinc and copper (2), and then cumulatively, chromium (3), manganese (4), selenium (5), iodine (6), and molybdenum (7). Other combinations are indicated separately by citing the number of trace elements contained in each followed by an asterisk that is repeated with the list of labeled ingredients. Label the Injection for its contents of zinc chloride (ZnCl<sub>2</sub>), zinc sulfate (ZnSO<sub>4</sub> · 7H<sub>2</sub>O), cupric chloride (CuCl<sub>2</sub>), cupric sulfate (CuSO<sub>4</sub>), chromic chloride (CrCl<sub>3</sub>), manganese chloride (MnCl<sub>2</sub>), manganese sulfate (MnSO<sub>4</sub>), selenious acid (H<sub>2</sub>SeO<sub>3</sub>), sodium iodide (NaI), and ammonium molybdate [(NH<sub>4</sub>)Mo<sub>7</sub> · 4H<sub>2</sub>O], and for elemental zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), selenium (Se), iodine (I), and molybdenum (Mo), as appropriate in relation to the ingredients claimed to be present.

**Identification**—

**A:** The *Assay preparation* prepared as directed in the *Assay for zinc*, the *Assay for copper*, the *Assay for chromium*, the *Assay for manganese*, the *Assay for selenium*, and the *Assay for molybdenum*, as appropriate, based on the elements claimed in the labeling to be present, exhibit maximum absorption at the relevant wavelengths specified when tested as directed for the *Procedures* in the respective *Assays*.

**B:** If iodine is claimed in the labeling to be present, the retention time of the iodine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for iodine*.

**Pyrogen**—When diluted with Sodium Chloride Injection so that each mL contains not more than 20 µg of zinc, 20 µg of copper, 0.1 µg of chromium, 4 µg of manganese, 4 µg of selenium, 5 µg of iodine, or 5 µg of molybdenum, it meets the requirements of the *Pyrogen Test* <151>.

**pH** <791>: between 1.5 and 3.5.

**Particulate matter** <788>: meets the requirements.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay for zinc**—

*Standard preparations*—Prepare as directed in the *Assay* under *Zinc Chloride Injection*.

**Assay preparation**—Using the Injection, proceed as directed for *Assay preparation* in the Assay under *Zinc Chloride Injection*.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Zinc Chloride Injection*. Calculate the quantity, in  $\mu\text{g}$ , of zinc (Zn) in each mL of the Injection taken by the formula:

$$5C/V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of zinc in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

#### **Assay for copper—**

**Sodium chloride solution, Copper stock solution, and Standard preparations**—Prepare as directed in the Assay preparation under *Cupric Chloride Injection*.

**Assay preparation**—Using the Injection, proceed as directed for the *Assay preparation* in the Assay under *Cupric Chloride Injection*.

**Procedure**—Proceed as directed for *Procedure* in the Assay preparation under *Cupric Chloride Injection*. Calculate the quantity, in mg, of copper (Cu) in each mL of the Injection taken by the formula:

$$2C/3V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of copper in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

#### **Assay for chromium (if present)—**

INJECTIONS CONTAINING 3  $\mu\text{g}$  OR MORE OF Cr PER mL—

**Sodium chloride solution, Chromium stock solution, Standard preparations, Assay preparation, and Procedure**—Proceed as directed in the Assay under *Chromic Chloride Injection*. Calculate the quantity, in  $\mu\text{g}$ , of chromium (Cr) in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

INJECTIONS CONTAINING LESS THAN 3  $\mu\text{g}$  OF Cr PER mL—

**Sodium chloride solution and Chromium stock solution**—Proceed as directed in the Assay under *Chromium Chloride Injection*.

**Standard preparations**—Prepare as directed in the Assay under *Chromic Chloride Injection*. If the Injection is not labeled as containing sodium chloride, omit the addition of the *Sodium chloride solution*.

**Assay preparation**—Use the undiluted Injection.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Chromic Chloride Injection*. Calculate the quantity, in  $\mu\text{g}$ , of chromium (Cr) in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

#### **Assay for manganese (if present)—**

**Sodium chloride solution, Manganese stock solution, and Standard preparations**—Prepare as directed in the Assay under *Manganese Chloride Injection*.

**Assay preparation**—Using the Injection, proceed as directed for the *Assay preparation* in the Assay under *Manganese Chloride Injection*.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Manganese Chloride Injection*. Calculate the quantity,

in  $\mu\text{g}$ , of manganese (Mn) in each mL of the Injection taken by the formula:

$$0.5C/V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of manganese in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

#### **Assay for selenium (if present)—**

**Selenium stock solution and Standard preparations**—Prepare as directed in the Assay under *Selenious Acid Injection*.

**Assay preparation**—Using the Injection, proceed as directed for *Assay preparation* in the Assay under *Selenious Acid Injection*.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Selenious Acid Injection*. Calculate the quantity, in mg, of selenium (Se) in each mL of the Injection taken by the formula:

$$LC/D$$

in which L is the labeled quantity, in mg per mL, of selenium in the Injection taken, C is the concentration, in  $\mu\text{g}$  per mL, in the *Assay preparation*, and D is the concentration, in  $\mu\text{g}$  of selenium per mL, of the *Assay preparation* on the basis of the labeled quantity in the Injection and the extent of dilution.

#### **Assay for iodine (if present)—**

**Mobile phase**—Prepare a suitable mixture of 0.05 M dibasic sodium phosphate, 0.0025 M cetyltrimethylammonium chloride (prepared by diluting 3.20 g of a 25% solution of cetyltrimethylammonium chloride with water to 1000 mL), and acetonitrile (45:25:25). Filter this mixture through a suitable filter of 0.5  $\mu\text{m}$  or finer porosity, adjust with phosphoric acid to a pH of  $6.8 \pm 0.1$ , and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparations**—Transfer about 52 mg of potassium iodide, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 5.2  $\mu\text{g}$  of potassium iodide per mL (equivalent to about 4  $\mu\text{g}$  of iodine per mL).

**Assay preparation**—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 4  $\mu\text{g}$  of iodine per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 226-nm detector, a precolumn containing packing L3, and a  $4.6 \times 25\text{-cm}$  column that contains packing L1. The flow rate is about 2.5 mL per minute. [NOTE—Equilibrate the column with *Mobile phase* recirculating at a rate of about 1 mL per minute for at least 12 hours before use.] Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the iodine peak is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the iodine peaks. Calculate the quantity, in  $\mu\text{g}$ , of iodine in each mL of the Injection taken by the formula:

$$(126.9 / 166.0)(CL / D)$$

in which 126.9 is the atomic weight of iodine, 166.0 is the molecular weight of potassium iodide, C is the concentration, in  $\mu\text{g}$  per mL, of potassium iodide, calculated on the dried basis, in the *Standard preparation*, L is the labeled concentration, in  $\mu\text{g}$  per mL, of iodine in the Injection, and D is the concentration, in  $\mu\text{g}$  per mL, of iodine in the *Assay prep-*

aration based on the labeled concentration and the extent of dilution.

#### Assay for molybdenum (if present)—

Ammonium hydroxide diluent, Sodium sulfate solution, Molybdenum stock solution, and Standard preparations—Prepare as directed in the Assay under Ammonium Molybdate Injection.

Assay preparation—Using the Injection, proceed as directed for the Assay preparation in the Assay under Ammonium Molybdate Injection.

Procedure—Proceed as directed for Procedure in the Assay under Ammonium Molybdate Injection. Calculate the quantity, in  $\mu\text{g}$ , of molybdenum (Mo) in each mL of the Injection taken by the formula:

$$25C / V$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of molybdenum in the Assay preparation, and V is the volume, in mL, of Injection taken.

## Elm

### DEFINITION

Elm is the dried inner bark of *Ulmus rubra* Muhl. (*Ulmus fulva* Michx.) (Fam. Ulmaceae).

### IDENTIFICATION

#### • A. MUCILAGINOUS SUBSTANCE

Sample: 1 g of finely powdered Elm

Analysis: Macerate the Sample with 40 mL of cold water for 1 h.

Acceptance criteria: The resulting mixture is of a thick mucilaginous consistency and yellowish brown in color.

#### • B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution: 0.025% rutin in methanol

Sample solution: Extract 1 g of powdered Elm with 10 mL of 60% methanol on a water bath for 15 min. Cool, filter, and concentrate the filtrate to 2.5 mL.

##### Chromatographic system

(See Chromatography <621>, Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20 cm long (TLC plates)

Application volume: 20  $\mu\text{L}$

Developing solvent system: Ethyl acetate, anhydrous formic acid, glacial acetic acid, and water (100:11:11:27)

Spray reagent: 1% solution of 2-aminoethyl diphenylborinate ester in methanol, followed by a 5% solution of polyethylene glycol 4000 in alcohol

##### Analysis

Samples: Standard solution and Sample solution

Develop the chromatograms in the Developing solvent system until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow to air-dry. Spray the plate with Spray reagent, and examine the plate under UV light at 366 nm.

Acceptance criteria: The  $R_f$  values of the principal spots relative to rutin are 1.05 (blue) and 0.8 (orange).

### SPECIFIC TESTS

#### • BOTANIC CHARACTERISTICS

##### Macroscopic

Unground Elm: Unground Elm occurs as broad, flat, oblong pieces 1–4 mm in thickness. The outer surface is yellow-orange with some brown outer bark or cork layers attached; the inner surface, which is pale yellow,

is marked faintly with striated phloem lines. The fracture is fibrous with projections of five bast bundles.

Powdered Elm: Weak yellowish orange with a distinctive fenugreek-like odor

##### Microscopic

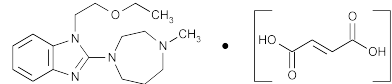
Powdered Elm: Bast fibers are numerous, very long, usually broken, up to 25  $\mu\text{m}$  in diameter, thick-walled, unligified, or with only a thin outer sheath of the wall lignified; have calcium oxalate prisms 10–35  $\mu\text{m}$  in length; have starch grains that are spheroidal, or polygonal, usually 3–15  $\mu\text{m}$  in diameter, occasionally up to 25  $\mu\text{m}$  in length; and have numerous mucilage fragments, frequently lamellated. Cork cells are few or absent.

- **OUTER BARK:** Contains NMT 2% of adhering outer bark
- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter <561>:** NMT 2%
- **LOSS ON DRYING <731>:** Dry 2 g at 105° to constant weight: it loses NMT 12% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN, Total Ash <561>:** NMT 10% on the dried basis
- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash <561>:** NMT 0.65% on the dried basis

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store in a cool, dry place.

## Emedastine Difumarate



$\text{C}_{17}\text{H}_{26}\text{N}_4\text{O} \cdot 2\text{C}_4\text{H}_4\text{O}_4$  534.56

1*H*-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)-, (*E*)-2-butenedioate (1:2). 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)benzimidazole fumarate (1:2) [87233-62-3].

» Emedastine Difumarate contains not less than 98.5 percent and not more than 101.0 percent of  $\text{C}_{17}\text{H}_{26}\text{N}_4\text{O} \cdot 2\text{C}_4\text{H}_4\text{O}_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, at controlled room temperature.

#### USP Reference standards <11>—

USP Emedastine Difumarate RS

#### Identification—

**A:** Infrared Absorption <197K>—

Test specimen—Dry for 3 hours at 105°.

**B:** The retention time of the major peak in the chromatogram of the Test solution corresponds to that of emedastine in the chromatogram of the System suitability solution, as obtained from the Chromatographic purity test.

**C:** Dissolve about 23 mg of Emedastine Difumarate in 25 mL of water. Add 1 mL of a solution prepared by mixing 20 mL of cupric sulfate solution (1 in 5) and 8 mL of pyridine: a precipitate is formed in the blue solution within one minute.

**pH <791>:** between 3.0 and 4.5, in a solution (2 in 1000).

**Loss on drying <731>—**Dry at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 0.002%.

**Chromatographic purity—**

**Buffer solution**—Dissolve 3.9 g of monobasic sodium phosphate and 2.5 g of sodium dodecyl sulfate in 1.0 L of water. Adjust with phosphoric acid to a pH of 2.4.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Mobile phase* containing 0.10 mg of USP Emedastine Difumarate RS and 0.04 mg of 4-methylbenzophenone per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Emedastine Difumarate RS in *Mobile phase*, and dilute, stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.003 mg per mL.

**Test solution**—Prepare a solution of Emedastine Difumarate in *Mobile phase* containing about 1.0 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.2 for fumaric acid, 1.0 for emedastine, and 2.0 for 4-methylbenzophenone; the resolution, *R*, is not less than 2.0; the column efficiency determined from the emedastine peak is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Mobile phase*, *Standard solution*, and *Test solution* into the chromatograph, and record the chromatograms, allowing the elution to continue for a period of not less than twice the retention time of emedastine. Measure the areas for all of the peaks, disregarding the fumaric acid peak and peaks corresponding to those obtained from the *Mobile phase*. Calculate the percentage of each impurity in the portion of Emedastine Difumarate taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response for emedastine in the *Standard solution*: not more than 0.3% of any individual impurity is found; and not more than 1.0% of total impurities is found.

**Assay**—Dissolve about 200 mg of Emedastine Difumarate, accurately weighed, in 50.0 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using suitable electrodes (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 26.73 mg of C<sub>17</sub>H<sub>26</sub>N<sub>4</sub>O · 2C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>.

## Emedastine Ophthalmic Solution

» Emedastine Ophthalmic Solution is a sterile, aqueous solution containing an amount of Emedastine Difumarate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of emedastine (C<sub>17</sub>H<sub>26</sub>N<sub>4</sub>O).

**Packaging and storage**—Preserve in tight, light-resistant containers, in a refrigerator or at controlled room temperature.

**USP Reference standards** (11)—

USP Emedastine Difumarate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 8.0.

**Assay—**

**Buffer solution**—Dissolve 13.8 g of monobasic sodium phosphate and 10 mL of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of 5.7, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (83:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Emedastine Difumarate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.057 mg of emedastine per mL.

**System suitability solution**—Add 50 μL of 30 percent hydrogen peroxide to 2 mL of *Standard preparation*, and heat at 100° for 30 minutes. Add another 2 mL of *Standard preparation*, mix, and use immediately.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution into a suitable volumetric flask to obtain a solution having a known concentration of about 0.057 mg of emedastine per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for emedastine and 1.2 for emedastine *N*-oxide; the resolution, *R*, between emedastine and emedastine *N*-oxide is not less than 1.5; the column efficiency determined from the emedastine peak is not less than 1000 theoretical plates; and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

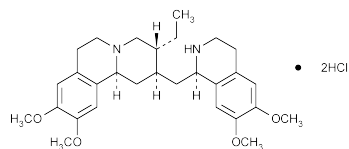
**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for emedastine. Calculate the quantity, in mg, of emedastine (C<sub>17</sub>H<sub>26</sub>N<sub>4</sub>O) in each mL of the Ophthalmic Solution taken by the formula:

$$(302.42/534.57)C(V_1/V_2)(r_U/r_S)$$

in which 302.42 and 534.57 are the molecular weights of emedastine and emedastine difumarate, respectively; *C* is the concentration, in mg per mL, of USP Emedastine Difumarate RS in the *Standard preparation*; *V<sub>1</sub>* is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation*; *V<sub>2</sub>* is the volume, in mL, of Ophthalmic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Emetine Hydrochloride



$C_{29}H_{40}N_2O_4 \cdot 2HCl$  553.56

Emetan, 6',7',10,11-tetramethoxy-, dihydrochloride.

Emetine dihydrochloride [316-42-7].

» Emetine Hydrochloride is the hydrochloride of an alkaloid obtained from *Ipecac*, or prepared by methylation of cephaeline, or prepared synthetically. It contains not less than 98.0 percent and not more than 101.5 percent of  $C_{29}H_{40}N_2O_4 \cdot 2HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Cephaeline Hydrobromide RS

USP Emetine Hydrochloride RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 50 µg per mL.

*Medium:* 0.5 N sulfuric acid.

**C:** A solution (1 in 20) responds to the tests for *Chloride* (191).

**Water**, *Method I* (921): between 15.0% and 19.0%.

**Residue on ignition** (281): not more than 0.2%.

**Acidity**—Dissolve 100 mg in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.5 mL is required to produce a yellow color.

**Limit of cephaeline**—[NOTE—Conduct this test in subdued light until after the chromatogram has been completely developed.]

*Standard preparation*—Dissolve 23 mg of USP Cephaeline Hydrobromide RS in 100.0 mL of methanol.

*Test preparation*—Dissolve 100 mg of Emetine Hydrochloride in 10.0 mL of methanol.

*Spray reagent*—Dissolve 300 mg of *p*-nitroaniline in 25 mL of 2 N hydrochloric acid, and cool to about 4°. Slowly add 5 mL of sodium nitrite solution (1 in 25), maintaining the temperature at about 4°. Freshly prepare the solution for each test.

*Procedure*—Apply 10-µL portions of the *Standard preparation* and the *Test preparation*, respectively, to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in a chromatographic tank containing a mixture of 9 volumes of chloroform and 1 volume of diethylamine, and develop the chromatogram until the solvent front has moved about 12 cm. Remove the plate from the tank, and allow the plate to air-dry for 20 minutes. Spray the dried plate with 2.5 N sodium hydroxide solution, and dry at 50° for 5 minutes. Then spray the plate with *Spray reagent*: any cephaeline spot from the *Test preparation* is not larger or more intense than that produced by the *Standard preparation* (2%).

**Assay**—Dissolve about 150 mg of Emetine Hydrochloride, accurately weighed, in 5 mL of glacial acetic acid, warming, if necessary. Allow the solution to cool, add 10 mL of diox-

ane, 5 mL of mercuric acetate TS, and 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.68 mg of  $C_{29}H_{40}N_2O_4 \cdot 2HCl$ .

## Emetine Hydrochloride Injection

» Emetine Hydrochloride Injection is a sterile solution of Emetine Hydrochloride in Water for Injection. It contains an amount of anhydrous emetine hydrochloride ( $C_{29}H_{40}N_2O_4 \cdot 2HCl$ ) equivalent to not less than 84.0 percent and not more than 94.0 percent of the labeled amount of emetine hydrochloride.

**Packaging and storage**—Preserve in single-dose, light-resistant containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Cephaeline Hydrobromide RS

USP Emetine Hydrochloride RS

USP Endotoxin RS

**Identification**—

**A:** Evaporate 1 mL of Injection on a steam bath to dryness: the residue responds to *Identification test A* under *Emetine Hydrochloride*.

**B:** The Injection responds to *Identification test C* under *Emetine Hydrochloride*.

**Bacterial endotoxins** (85)—It contains not more than 5.4 USP Endotoxin Units per mg of emetine hydrochloride.

**pH** (791): between 3.0 and 5.0.

**Limit of cephaeline**—[NOTE—Conduct this test in subdued light until after the chromatogram has been completely developed.]

*Standard preparation* and *Spray reagent*—Prepare as directed in the test for *Limit of cephaeline* under *Emetine Hydrochloride*.

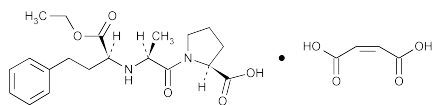
*Test preparation*—Evaporate 1.0 mL of Injection on a steam bath with the aid of a stream of nitrogen just to dryness. Dissolve the residue in a sufficient volume of methanol to obtain a *Test preparation* having a concentration of 10 mg of emetine hydrochloride per mL.

*Procedure*—Proceed as directed for *Procedure* in the test for *Limit of cephaeline* under *Emetine Hydrochloride*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 120 mg of emetine hydrochloride, to a suitable extraction apparatus containing about 20 mL of water. Add 6 N ammonium hydroxide until the solution is strongly alkaline, and extract with ether until 0.5 mL of the water layer, slightly acidified with hydrochloric acid, remains unaffected by the addition of a few drops of mercuric-potassium iodide TS. Evaporate the combined ether extracts on a steam bath, allowing the last few mL of the ether to evaporate spontaneously. Add to the residue 2 mL of neutralized alcohol and 30.0 mL of 0.02 N sulfuric acid VS, and warm gently until the alkaloid is dissolved. Cool, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS. Each mL of 0.02 N sulfuric acid is equivalent to 5.536 mg of  $C_{29}H_{40}N_2O_4 \cdot 2HCl$ .

## Enalapril Maleate



$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  492.52

L-Proline, 1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-  
(S), (Z)-2-butenedioate (1:1).

1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1'-  
ethyl ester, maleate (1:1) [76095-16-4].

» Enalapril Maleate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Enalapril Maleate RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between  $-41.0^\circ$  and  $-43.5^\circ$ .

*Test solution*: 10 mg per mL, in methanol.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals**, *Method II* (231): 0.001%.

**Related compounds**—

*pH 6.8 Phosphate buffer*, *pH 2.5 Phosphate buffer*, *Solution A*, *Solution B*, *Mobile phase*, *Diluent*, *Enalapril diketopiperazine solution*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Enalapril Maleate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 3  $\mu$ g per mL.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the percentage of each impurity in the portion of Enalapril Maleate taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Enalapril Maleate in the *Test solution*;  $r_i$  is the peak area of each impurity obtained from the *Test solution*; and  $r_S$  is the peak area of enalapril obtained from the *Standard solution*: not more than 1.0% of any impurity having a relative retention time of about 1.10 is found; not more than 0.3% of any other individual impurity is found; and not more than 2% of total impurities is found.

**Assay**—

*pH 6.8 Phosphate buffer*—Dissolve 2.8 g of monobasic sodium phosphate in about 900 mL of water in a 1000-mL

volumetric flask. Adjust with a 9 M sodium hydroxide solution to a pH of about 6.8, dilute with water to volume, and mix.

*pH 2.5 Phosphate buffer*—Dissolve 2.8 g of monobasic sodium phosphate in about 900 mL of water in a 1000-mL volumetric flask. Adjust with phosphoric acid to a pH of about 2.5, dilute with water to volume, and mix.

*Solution A*—Prepare a filtered and degassed mixture of *pH 6.8 Phosphate buffer* and acetonitrile (19:1).

*Solution B*—Prepare a filtered and degassed mixture of acetonitrile and *pH 6.8 Phosphate buffer* (33:17).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of *pH 2.5 Phosphate buffer* and acetonitrile (95:5).

*Enalapril diketopiperazine solution*—Carefully place about 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting. Heat for about 5 to 10 minutes until the solid is melted. Immediately remove the beaker from the hot plate, and allow to cool. [NOTE—Avoid overheating to prevent heat-induced degradation, which would give rise to a brown color.] To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few minutes to dissolve. The solution typically contains, in each mL, between 0.2 mg and 0.4 mg of enalapril diketopiperazine.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Enalapril Maleate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL.

*System suitability solution*—Add 1 mL of *Enalapril diketopiperazine solution* to a 50-mL portion of the *Standard preparation*, and mix.

*Assay preparation*—Transfer about 30 mg of Enalapril Maleate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.1-mm  $\times$  15-cm column that contains packing L21. The flow rate is about 1.5 mL per minute. The column temperature is maintained at  $70^\circ$ . The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0-20	95 $\rightarrow$ 40	5 $\rightarrow$ 60	linear gradient
20-25	40	60	isocratic
25-26	40 $\rightarrow$ 95	60 $\rightarrow$ 5	linear gradient
26-30	95	5	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for enalapril and 2.1 for enalapril diketopiperazine; and the resolution,  $R$ , between enalapril and enalapril diketopiperazine is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  in the portion of Enalapril Maleate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Add the following:

### ▲Enalapril Maleate Oral Suspension

#### DEFINITION

Enalapril Maleate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of enalapril maleate ( $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ ).

Prepare Enalapril Maleate Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Enalapril Maleate tablets <sup>a</sup> equivalent to	100 mg
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> (regular or sugar-free) and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Vasotec 20-mg tablets, Merck Sharp & Dohme, West Point, PA.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an enalapril maleate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

#### ASSAY

##### • PROCEDURE

**Buffer solution:** 1 M monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 4.0

**Mobile phase:** Acetonitrile, *Buffer solution*, and water (30:2:68). Filter and degas.

**Standard solution:** 0.1 mg/mL of USP Enalapril Maleate RS in *Mobile phase*

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of the Oral Suspension into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.1 mg/mL of enalapril maleate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for enalapril maleate is about 8.8 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 2.1% for the replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ( $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of enalapril maleate in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

• **pH (791):** 4.2–5.3

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or at controlled cold temperature.

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded, when stored at controlled cold temperature or controlled room temperature

• **USP REFERENCE STANDARDS (11)**

USP Enalapril Maleate RS<sup>▲</sup><sub>USP36</sub>

### Enalapril Maleate Tablets

» Enalapril Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards (11)**—

USP Enalapril Maleate RS

USP Enalaprilat RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**Dissolution (711)**—

**Medium:** pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  dissolved as directed for *Procedure for content uniformity* under *Uniformity of dosage units* except to use pH 6.8 phosphate buffer instead of the *Buffer solution* to prepare the *Standard preparation*, to use a filtered portion of the solution under test as the *Test preparation*, and to make any necessary modifications for appropriate sample and standard concentrations.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Buffer solution and Mobile phase**—Prepare as directed in the *Assay*.

**Standard preparation**—Transfer about 10 mg of USP Enalapril Maleate RS to a 100-mL volumetric flask. Add about 50 mL of *Buffer solution*, shake, and use sonication if

necessary to dissolve. Dilute with *Buffer solution* to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Enalapril Maleate RS per mL.

**Test preparation**—Transfer one Tablet to a volumetric flask of capacity such that, when filled to volume, will produce a solution having a concentration of about 0.1 mg of enalapril maleate per mL. Proceed as directed for *Assay preparation* in the *Assay*, beginning with “Add a volume of *Buffer solution* that is about one-half the nominal volume of the flask.”

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The column temperature is maintained at 50°, and the flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure*: the column efficiency is not less than 300 theoretical plates; the tailing factor is not more than 2.0; the capacity factor,  $K'$ , is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—The enalapril peak tailing factor may be minimized by controlling the column temperature between 45° and 50° and by raising the pH of the aqueous component of the *Mobile phase* from 2.2 to 2.6; the capacity factor may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.]

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  in the Tablet taken by the formula:

$$(TC/D)(r_U / r_S)$$

in which  $T$  is the labeled quantity, in mg, of enalapril maleate in the Tablet;  $C$  is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation*;  $D$  is the concentration, in mg per mL, of enalapril maleate in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and  $r_U$  and  $r_S$  are the enalapril peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

#### Related compounds—

*Buffer solution*, *Mobile phase*, *Enalaprilat standard solution*, *Enalapril diketopiperazine solution*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Test preparation**—Use the *Assay preparation*.

**Related compounds standard solution**—Transfer 1.0 mL of the *Standard preparation* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard preparation*, the *Test preparation*, the *Related compounds standard solution*, and the *Buffer solution*, into the chromatograph, record the chromatograms, and measure the responses for all of the peaks in the *Test preparation* greater than 0.1% of the response of the enalapril peak that are not observed in the *Buffer solution*. Calculate the percentage of anhydrous enalaprilat (as enalapril maleate) present in the portion of Tablets taken by the formula:

$$(492.52/348.39)(CV/N)(r_U / r_S)(100/L)$$

in which 492.52 and 348.39 are the molecular weights of enalapril maleate and anhydrous enalaprilat, respectively;  $C$  is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation*;  $V$  is the nominal capacity, in mL, of the volumetric flask containing the *Test preparation*;  $N$  is the number of Tablets taken for the *Test preparation*;  $r_U$  and  $r_S$  are the enalaprilat peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively; and  $L$  is the labeled amount of enalapril maleate in the Tablet.

Calculate the percentage of enalapril diketopiperazine (as enalapril maleate) present in the portion of Tablets taken by the formula:

$$(492.52/358.44)(C'V/N)(r_U / 1.25 r_S)(100/L)$$

in which 492.52 and 358.44 are the molecular weights of enalapril maleate and enalapril diketopiperazine, respectively;  $C'$  is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Related compounds standard solution*;  $V$  is the nominal capacity, in mL, of the volumetric flask containing the *Test preparation*;  $N$  is the number of Tablets taken for the *Test preparation*;  $r_U$  is the enalapril diketopiperazine peak response obtained from the *Test preparation*; 1.25 is the response for enalapril diketopiperazine relative to that for enalapril maleate;  $r_S$  is the enalapril peak response obtained from the *Related compounds standard solution*; and  $L$  is the labeled amount, in mg, of enalapril maleate in the Tablet.

Calculate the percentage of any other related compound by the formula:

$$(C'V/N)(r_R / r_S)(100/L)$$

in which  $r_R$  is the sum of the responses of any related compound, other than those from maleic acid, enalapril, enalaprilat, and enalapril diketopiperazine obtained from the *Test preparation*;  $r_S$  is the enalapril peak response obtained from the *Related compounds standard solution*; and  $C'$ ,  $V$ ,  $N$ , and  $L$  are as defined above: the sum of all related compounds including those from enalaprilat and enalapril diketopiperazine is not greater than 5.0%.

#### Assay—

**Buffer solution**—Dissolve 1.38 g of monobasic sodium phosphate in about 800 mL of water. Adjust with phosphoric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Enalaprilat standard solution**—Dissolve an accurately weighed quantity of USP Enalaprilat RS in water to obtain a solution having a known concentration of about 0.4 mg per mL.

**Enalapril diketopiperazine solution**—Carefully place about 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting to melt the solid. When melting is observed, (after 5 to 10 minutes of heating), immediately remove the beaker from the hot plate, and allow it to cool. [NOTE—Avoid over heating beyond the melting initially observed to prevent heat-induced degradation, which would give rise to a brown color.] To the cooled residue in the beaker add 50 mL of acetonitrile and sonicate for a few minutes to dissolve the residue. The solution typically contains, in each mL, between 0.2 mg and 0.4 mg of enalapril diketopiperazine.

**Standard preparation**—Transfer about 20 mg of USP Enalapril Maleate RS, accurately weighed, to a 100-mL volumetric flask. Transfer 0.5 mL of *Enalaprilat standard solution* to the flask, and add about 50 mL of *Buffer solution* to dissolve, using sonication if necessary. Dilute with *Buffer solution* to volume, and mix to obtain a solution having known concentrations of about 0.2 mg of USP Enalapril Maleate RS per mL and 0.002 mg of USP Enalaprilat RS per mL.

**System suitability solution**—Transfer 0.5 mL of *Enalapril diketopiperazine solution* to a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.

**Assay preparation**—Transfer not fewer than 10 Tablets to a volumetric flask of capacity such that, when filled to volume, will produce a solution having a concentration of

about 0.2 mg of enalapril maleate per mL. Add a volume of *Buffer solution* that is about one-half the nominal volume of the flask, sonicate for 15 minutes, and shake by mechanical means for 30 minutes. Dilute with *Buffer solution* to volume, shake well, and sonicate for 15 minutes. Pass through a filter of 0.45- $\mu$ m or finer porosity, discarding the first portion of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L7. The column temperature is maintained at 50°, and the flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for maleic acid, 0.5 for enalaprilat, 1.0 for enalapril, and 1.5 for enalapril diketopiperazine. [NOTE—A peak response for heat-induced degradation product of enalapril diketopiperazine (if present with relative retention time about 1.2) is not greater than 15% of the response for enalapril diketopiperazine.] The column efficiency is not less than 1000 theoretical plates for enalaprilat, not less than 300 theoretical plates for enalapril, and not less than 2500 theoretical plates for enalapril diketopiperazine; the tailing factor for enalapril is not more than 2.0; the resolution, *R*, between maleic acid and enalaprilat is not less than 2.0, between enalaprilat and enalapril is not less than 2.0, and between enalapril and enalapril diketopiperazine is not less than 2.0. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0% for the enalapril peak, and responses for the enalaprilat peak agree within 5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of enalapril maleate ( $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ ) in each Tablet taken by the formula:

$$(CV/N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation*; *V* is the nominal capacity, in mL, of the volumetric flask containing the *Assay preparation*; *N* is the number of Tablets taken for the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the enalapril peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Enalapril Maleate and Hydrochlorothiazide Tablets

» Enalapril Maleate and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Enalapril Maleate ( $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ ) and Hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Enalaprilat RS

USP Enalapril Maleate RS

USP Hydrochlorothiazide RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for enalapril maleate*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrochlorothiazide*.

**Dissolution** <711>—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

Determine the amount of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  dissolved, using filtered portions of the solution under test and following the *Procedure for content uniformity of enalapril maleate* in the test for *Uniformity of dosage units*, making any necessary volumetric adjustments, in comparison with a Standard solution of USP Enalapril Maleate RS having similar concentrations in the same *Medium*.

Determine the amount of  $C_7H_8ClN_3O_4S_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 320 nm and at 360 nm in 1-cm cells, on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Hydrochlorothiazide RS dissolved in 20 mL of methanol and diluted with *Medium*. Calculate the quantity, in mg, of hydrochlorothiazide dissolved by the formula:

$$(TC/D)(A_{320} - A_{360})_U / (A_{320} - A_{360})_S$$

in which *T* is the Tablet label claim, in mg, for hydrochlorothiazide; *C* is the concentration, in mg per mL, of hydrochlorothiazide in the Standard solution; *D* is the concentration, in mg per mL, of hydrochlorothiazide in the solution under test; and  $(A_{320} - A_{360})_U$  and  $(A_{320} - A_{360})_S$  are the differences in the absorbances at 320 and 360 nm of the solution under test and the Standard solution, respectively.

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of enalapril maleate ( $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ ) and not less than 60% (*Q*) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) are dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY OF ENALAPRIL MALEATE—

**Buffer solution A**—Dissolve 136 g of monobasic potassium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 4.0, dilute with water to 1000 mL, and mix.

**Buffer solution B**—Transfer 20.0 mL of *Buffer solution A* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and *Buffer solution A* (34:15:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Dissolve an accurately weighed quantity of USP Enalapril Maleate RS in *Buffer solution B* to obtain a solution having a known concentration of about 100  $\mu$ g per mL.

**Test solution**—Transfer one finely powdered Tablet to a 50-mL volumetric flask, add about 30 mL of *Buffer solution B*, and sonicate for 15 minutes. Shake by mechanical means for 30 minutes, dilute with *Buffer solution B* to volume, sonicate for 30 minutes, mix, and filter, discarding the first portion of the filtrate. Dilute a portion of the filtrate with *Buffer solution B* quantitatively to obtain a solution containing about 100  $\mu$ g per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  20-cm column containing 10- $\mu$ m packing L7 and maintained at a temperature of 80°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, is not less than 2.5; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the

analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  in the Tablet taken by the formula:

$$(TC/D)(r_U / r_S)$$

in which  $T$  is the labeled quantity, in mg, of enalapril maleate in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Enalapril Maleate RS in the *Standard solution*;  $D$  is the concentration, in  $\mu$ g per mL, of enalapril maleate in the *Test solution*; based upon the labeled quantity per Tablet and the extent of dilution; and  $r_U$  and  $r_S$  are the enalapril peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

#### PROCEDURE FOR CONTENT UNIFORMITY OF HYDROCHLOROTHIAZIDE—

**Buffer solution A** and **Buffer solution B**—Prepare as directed under *Procedure for content uniformity of enalapril maleate*.

**Standard solution**—Transfer about 50 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 200-mL volumetric flask. Add 20 mL of methanol to dissolve the material, dilute with *Buffer solution B* to volume, and mix to obtain a stock solution. Transfer 5.0 mL of the stock solution to a 25-mL volumetric flask, dilute with *Buffer solution B* to volume, and mix to obtain a solution having a known concentration of about 50  $\mu$ g per mL.

**Test solution**—Transfer 1 Tablet to a volumetric flask of a suitable size such that, when the hydrochlorothiazide is dissolved from the Tablet, a solution having a concentration of about 250  $\mu$ g per mL is obtained. Add a volume of *Buffer solution B* equal to about half the capacity of the flask, and sonicate with occasional shaking for 15 minutes. Shake by mechanical means for 30 minutes, dilute with *Buffer solution B* to volume, sonicate for 30 minutes, mix, and filter, discarding the first portion of the filtrate. Transfer 5.0 mL of the clear filtrate to a 25-mL volumetric flask, dilute with *Buffer solution B* to volume, and mix.

**Procedure**—Determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells at the wavelength of maximum absorbance at about 320 nm and at 360 nm, with a suitable spectrophotometer, relative to *Buffer solution B* as the blank. Calculate the quantity, in mg, of  $C_7H_8ClN_3O_4S_2$  in the Tablet taken by the formula:

$$(TC / D)(A_{320} - A_{360})_U / (A_{320} - A_{360})_S$$

in which  $T$  is the labeled quantity, in mg, of hydrochlorothiazide in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Hydrochlorothiazide RS in the *Standard solution*;  $D$  is the concentration, in  $\mu$ g per mL, of hydrochlorothiazide in the *Test solution*, based upon the labeled quantity per Tablet and the extent of dilution; and the parenthetical expressions are the differences in absorbances of the two solutions at the wavelengths indicated by the subscripts for the *Test solution* ( $U$ ) and the *Standard solution* ( $S$ ), respectively.

#### Related compounds—

**Buffer solution** and **Mobile phase**—Proceed as directed in the *Assay for enalapril maleate*.

**Test solution**—Use the *Assay preparation* prepared as directed in the *Assay for enalapril maleate*.

**Enalaprilat solution**—Transfer about 10 mg of USP Enalaprilat RS, accurately weighed, to a 25-mL volumetric flask, dilute with water to volume, and mix.

**Enalapril diketopiperazine solution**—Carefully place about 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting. Heat for about 5 to 10 minutes

until the solid is melted. Immediately remove the beaker from the hot plate, and allow to cool. [NOTE—Avoid overheating to prevent heat-induced degradation, which would give rise to a brown color.] To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few minutes to dissolve. The solution typically contains, in each mL, between 0.2 mg and 0.4 mg of enalapril diketopiperazine.

**Standard solution**—Transfer about 40 mg of USP Enalapril Maleate RS, accurately weighed, to a 200-mL volumetric flask, and dissolve with about 50 mL of methanol. Pipet 1 mL each of *Enalaprilat solution* and *Enalapril diketopiperazine solution* into the volumetric flask, dilute with *Buffer solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L7. The column temperature is maintained at 65°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3, 0.4, and 1.0 for enalaprilat, enalapril diketopiperazine, and enalapril, respectively; the resolution,  $R$ , between any of the peaks is not less than 1.3; the column efficiency is not less than 700 theoretical plates for enalapril, 1500 for enalaprilat, and 1500 for enalapril diketopiperazine; the tailing factor is not more than 3.5; and the relative standard deviation for replicate injections is not more than 5.0% for enalaprilat and enalapril diketopiperazine and not more than 2.0% for enalapril.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the peaks. Calculate the quantity, in mg, of enalaprilat (enalapril related compound A) in the portion of Tablets taken by the formula:

$$0.2C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of enalaprilat in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of enalaprilat obtained from the *Test solution* and the *Standard solution*, respectively.

Calculate the quantity, in mg, of enalapril diketopiperazine in the portion of Tablets taken by the formula:

$$0.2C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of enalapril diketopiperazine in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of enalapril diketopiperazine obtained from the *Test solution* and the *Standard solution*, respectively: not more than 5.0% of total related compounds is found, calculated on the basis of the portion of Tablets taken as directed under *Assay for enalapril maleate*.

#### Assay for enalapril maleate—

**Buffer solution**—Transfer 136 mg of monobasic potassium phosphate to a 1000-mL volumetric flask, add 800 mL of water, adjust with phosphoric acid to a pH of 2.0, dilute with water to volume, and mix.

**Mobile phase**—Prepare a degassed and filtered solution of *Buffer solution* and acetonitrile (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 40 mg of USP Enalapril Maleate RS, accurately weighed, to a 200-mL volumetric flask, add about 50 mL of methanol to dissolve, dilute with *Buffer solution* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of enalapril maleate, to a 200-mL volumetric flask, add about 50 mL of *Buffer solution*, and sonicate for 15 minutes. Add about 50 mL of

methanol to the flask, sonicate for 15 minutes, dilute with *Buffer solution* to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The column temperature is maintained at 65°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of enalapril maleate (C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Tablets taken by the formula:

$$200C(r_u / r_s)$$

in which C is the concentration, in μg per mL, of USP Enalapril Maleate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for hydrochlorothiazide—

**Buffer solution**—Prepare as directed under *Assay for enalapril maleate*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (9:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 20 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 200-mL volumetric flask, add about 50 mL of methanol to dissolve, dilute with *Buffer solution* to volume, and mix.

**Assay preparation**—Prepare as directed for the *Assay preparation* under *Assay for enalapril maleate*, except to weigh a portion of the powdered Tablets equivalent to about 20 mg of hydrochlorothiazide.

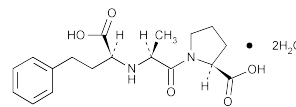
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 310-nm detector and a 4.6-mm × 20-cm column that contains 10-μm packing L7. The column temperature is maintained at 30°, and the flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 2.0; the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Tablets taken by the formula:

$$200C(r_u / r_s)$$

in which C is the concentration, in μg per mL, of USP Hydrochlorothiazide RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Enalaprilat



C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub> · 2H<sub>2</sub>O 384.42

L-Proline, 1-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]-, dihydrate, (S)-.

1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline dihydrate [84680-54-6].

» Enalaprilat contains not less than 98.0 percent and not more than 101.0 percent of C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Enalaprilat RS

#### Identification—

**A: Infrared Absorption** <197M>—[NOTE—If the spectrum is not comparable to that of the Reference Standard, expose the specimen and Reference Standard to an environment of 98% relative humidity (use a chamber conditioned with a saturated solution of calcium sulfate) for 1 to 3 days to equilibrate them. Prepare dispersions from the equilibrated specimen and Reference Standard, and record the spectra.]

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**Specific rotation** <781S>: between −53.0° and −56.0°.

**Test solution:** 10 mg per mL, in methanol.

**Water, Method I** <921>: between 7.0% and 11.0%.

**Residue on ignition** <281>: not more than 0.2%.

**Heavy metals, Method II** <231>: 0.002%.

#### Assay—

**pH 3 buffer**—Dissolve 1.36 g of monobasic potassium phosphate in 950 mL of water, adjust with phosphoric acid to a pH of 3.0 ± 0.1, dilute with water to 1000 mL, and mix.

**Solvent mixture**—Prepare a mixture of acetonitrile, methanol, and pH 3 buffer (2:2:1). Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and mix.

**Diluent**—Prepare a mixture of pH 3 buffer and *Solvent mixture* (92:8), and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of pH 3 buffer and *Solvent mixture* (85:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Enalaprilat RS in *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL. [NOTE—Use this solution within 24 hours.]

**Assay preparation**—Transfer about 30 mg of Enalaprilat, accurately weighed, to a 100-mL volumetric flask, dissolve in *Diluent*, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 15-cm column that contains 4-μm packing L1 and is maintained at 70°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 500 theoretical plates; the tailing factor for the analyte peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{24}N_2O_5$  in the portion of Enalaprilat taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the enalaprilat peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Enalaprilat Injection

» Enalaprilat Injection is a sterile solution of enalaprilat in a suitable vehicle for injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of enalaprilat ( $C_{18}H_{24}N_2O_5$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Benzyl Alcohol RS  
USP Enalaprilat RS  
USP Enalapril Maleate RS  
USP Endotoxin RS

### Identification—

**A:** The retention time of the enalaprilat peak in the chromatogram of the *Assay preparation* corresponds to that of the corresponding peak in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**Bacterial endotoxins** (85)—It contains no more than 280 USP Endotoxin Units per mg of enalaprilat.

**Sterility** (71): meets the requirements.

**pH** (791): between 6.5 and 7.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Osmolality** (785): between 276 to 305 mOsm per kg.

### Related compounds—

**Diluent**—Prepare a mixture of water and acetonitrile (84:16).

**Buffer solution**—Dilute 3.4 mL of phosphoric acid with water to 1 L. Adjust with potassium hydroxide to a pH of 2.5.

**Solution A**—Use a mixture of *Buffer solution* and acetonitrile (84:16).

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Stock solution A**—Dissolve 20 mg of USP Enalaprilat RS in a 100-mL volumetric flask with approximately 80 mL of *Diluent*. Heat at 80° for 24 hours to generate enalaprilat related compound A. Cool the solution to room temperature, and dilute with *Diluent* to volume.

**Stock solution B**—Dissolve 10 mg of USP Enalapril Maleate RS in a 200-mL volumetric flask, and dilute with *Diluent* to volume.

**Stock solution C**—Dissolve accurately weighed quantities of benzyl alcohol, benzaldehyde, and benzoic acid in *Diluent*. Dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of 0.1 mg per mL of each of the three substances.

**System suitability solution**—Pipet 4.0 mL of *Stock solution A*, 5 mL of *Stock solution B*, and 5 mL of *Stock solution C* into a 25-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution containing about 0.0032 mg per mL of enalaprilat related impurity A, 0.01 mg per mL of enalapril, and 0.02 mg per mL of each of benzyl alcohol, benzaldehyde, and benzoic acid, respectively.

**Test solution**—Transfer an accurately measured volume of Injection, equivalent to about 12.5 mg of enalaprilat, to a 25-mL volumetric flask. Dilute with *Diluent* to volume, and mix.

**Diluted test solution**—Dilute the *Test solution* (1 in 100) with *Diluent* to obtain a solution having a known concentration of 0.005 mg per mL (corresponds to 1% of the *Test solution*).

**Chromatographic system** (see *Chromatography* (621))—The chromatograph is equipped with 215-nm detector and a 4.6-mm  $\times$  15-cm, 5- $\mu$ m column that contains packing L1. The column temperature is maintained at 60°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows:

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	97.0	3.0	isocratic
5–20	97→77.5	3→22.5	linear gradient
20–25	77.5→10	22.5→90	linear gradient
25–25.01	10→97	90→3.0	step gradient
25.01–30	97	3.0	re-equilibrium

Chromatograph the *System suitability solution* and the *Diluted test solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between enalapril maleate and enalaprilat related impurity A is not less than 1.2; the capacity factor for enalaprilat is not less than 1.5; and the relative standard deviation of the enalaprilat peak in replicate injections of the *Diluted test solution* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the peak response for enalaprilat in the *Diluted test solution* and all of the peak responses from the *Test solution* that do not correspond to enalaprilat, benzyl alcohol, benzoic acid, benzaldehyde, and benzyl alcohol related compounds (see *Table 1* for RRT). Calculate the percentage of specified and unspecified impurities using the formula:

$$(r_i / r_S)$$

in which  $r_i$  is the peak response for each specified impurity in the *Test solution*; and  $r_S$  is the peak response for enalaprilat in the *Diluted test solution*: the impurities meet the specified limits in *Table 1*.



Table 1

Component	Relative Retention Time (minutes)	Limit (w/w, %)
Enalaprilat heat degradation product	0.6 vs enalaprilat	0.5
Enalaprilat	1 vs enalaprilat	—
Benzyl alcohol	1 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 1	1.2 vs benzyl alcohol	—
Benzoic acid	1.4 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 2	1.7 vs benzyl alcohol	—
Benzaldehyde	2.1 vs benzyl alcohol	—
Enalapril maleate	4.7 vs enalaprilat	0.25
Enalaprilat related impurity A	5.1 vs enalaprilat	1.0
Any other unspecified individual impurity	—	0.10
Total impurities	—	2.0

**Change to read:****Benzyl alcohol content** (if present)—

*Buffer solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Benzyl Alcohol RS in **Mobile phase**. (ERR 1-Jul-2012) to obtain a solution having a known concentration of 0.72 mg per mL.

*Test solution*—Use the Assay preparation, prepared as directed in the Assay.

*Procedure*—Proceed as directed in the Assay. Calculate the percentage of benzyl alcohol, based on the label claim, in the volume of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Benzyl Alcohol RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of benzyl alcohol in the *Test solution*; and  $r_U$  and  $r_S$  are the benzyl alcohol peak responses obtained from the *Test solution* and the *Standard solution*, respectively: between 75.0% and 120.0% of the labeled amount is found.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Buffer solution*—Prepare a solution of 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (84 : 16). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability preparation*—Use the *Standard preparation*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Enalaprilat RS and USP Benzyl Alcohol RS in *Buffer solution*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL of enalaprilat and 0.72 mg per mL of benzyl alcohol.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of enalaprilat, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

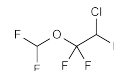
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm and 258-nm detector (use 215 nm as the initial wavelength, and switch to 258 nm after the elution of enalaprilat and before the elution of benzyl alcohol) and a 4.6-mm×15-cm, 5-μm

column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 60°. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between benzyl alcohol and enalaprilat is not less than 3.0; the tailing factor for benzyl alcohol and the enalaprilat peaks is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the quantity, in percentage of the label claim, of enalaprilat ( $C_{18}H_{24}N_2O_5$ ) in the portion of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of enalaprilat in the *Assay preparation*, based on the label claim; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Enflurane**

$C_3H_2ClF_5O$  184.49  
Ethane, 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoro-, (±)-; (±)-2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether [13838-16-9].

**DEFINITION**

Enflurane contains NLT 99.9% and NMT 100.0% of enflurane ( $C_3H_2ClF_5O$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197F)

**ASSAY**

- **PROCEDURE**

**Sample solution:** Enflurane

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 4-mm × 3-m stainless steel; packed with 20% liquid phase G4 on 60- to 80-mesh S1A

**Temperatures**

Injection port: 200°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	6	125	0

Carrier gas: Dry helium

Flow rate: 60 mL/min

Injection volume: NMT 30 µL

**Analysis**Sample: *Sample solution*

Calculate the percentage of purity:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak area of Enflurane $r_T$  = sum of all the peak areas

Acceptance criteria: 99.9%–100.0%

**IMPURITIES**• **CHLORIDE AND SULFATE**, Chloride <221>

Sample: Shake 25 mL with 25 mL of water for 5 min, and allow the liquids to separate completely. Draw off the water layer, and add to it 1 drop of nitric acid and 5 drops of silver nitrate TS.

Acceptance criteria: Any turbidity produced is not greater than that produced in a solution containing 0.35 mL of 0.020 N hydrochloric acid.

• **LIMIT OF FLUORIDE**

[NOTE—Use plasticware throughout this test.]

Buffer: Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, and dilute with water to volume; the pH of this solution is 5.0–5.5.

Standard stock solution: 1 mg/mL of fluoride ion, prepared as follows. Transfer 221 mg of sodium fluoride, previously dried at 150° for 4 h, to a 100-mL volumetric flask. Add 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), and dilute with water to volume. [NOTE—Store in a tightly closed plastic container.]

Standard solution A: 100 mL of a solution containing 1 µg/mL of fluoride ion in Buffer, from Standard stock solution

Standard solution B: 100 mL of a solution containing 3 µg/mL of fluoride ion in Buffer, from Standard stock solution

Standard solution C: 100 mL of a solution containing 5 µg/mL of fluoride ion in Buffer, from Standard stock solution

Standard solution D: 100 mL of a solution containing 10 µg/mL of fluoride ion in Buffer, from Standard stock solution

Sample stock solution: Enflurane and water (1:1). Shake the Sample stock solution for 5 min, allow the liquids to separate completely, and use the water layer.

Sample solution: Sample stock solution and Buffer (1:1). Use volumetric glassware.

Electrode system: Use a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved, calomel-fluoride, specific-ion electrode system (see pH <791>).

**Analysis**Samples: *Standard solutions* and *Sample solution*

Transfer the solution to a 150-mL beaker, add a polytetrafluoroethylene-coated stirring bar, and immerse the electrodes in the solution. Stir with a magnetic stirrer having an insulated top until equilibrium is obtained (1–2 min), and record the potential, in mV. [NOTE—Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]

Plot the logarithm of the fluoride-ion concentrations, in µg/mL, of the *Standard solutions* versus the potential, in mV. From the measured potential of the *Sample solution* and the standard curve, determine the concentration, in µg/mL, of fluoride ions in the *Sample solution*.

Acceptance criteria: NMT 10 µg/mL

• **LIMIT OF NONVOLATILE RESIDUE**

Analysis: Allow 10.0 mL to evaporate at room temperature in a tared evaporating dish, dry the residue at 50° for 2 h, and weigh.

Acceptance criteria: NMT 2 mg of residue remains.

**SPECIFIC TESTS**• **SPECIFIC GRAVITY** <841>: 1.516–1.519• **REFRACTIVE INDEX** <831>: 1.3020–1.3038 at 20°• **WATER DETERMINATION**, Method I <921>: NMT 0.14%• **ACIDITY OR ALKALINITY**

Sample: Shake 20 mL of Enflurane with 20 mL of carbon dioxide-free water for 3 min, and allow the layers to separate. Draw off the water layer, add bromocresol purple TS as the indicator, and titrate with 0.010 N sodium hydroxide or 0.010 N hydrochloric acid.

Acceptance criteria: NMT 0.10 mL of 0.010 N sodium hydroxide or NMT 0.60 mL of 0.010 N hydrochloric acid is required for neutralization.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

• **USP REFERENCE STANDARDS** <11>  
USP Enflurane RS

## Endotoxin Indicator for Depyrogenation

**DEFINITION**

An Endotoxin Indicator is an article (challenge vial of endotoxin or a carrier spiked with endotoxin) designed for use in depyrogenation studies. The endotoxin (a purified lipopolysaccharide) is validated for use in or on an Endotoxin Indicator. The carrier is made from a material appropriate for the intended depyrogenation processes to which it will be subjected. The endotoxin on a carrier is added at a concentration sufficient to allow recovery of a minimum of 1000 USP Endotoxin Units/carrier. The Endotoxin Indicator would allow for accurate indication of at least a 3-log reduction in USP Endotoxin Units during depyrogenation process challenges.

**IDENTIFICATION**• **CHARACTERISTICS**

The endotoxin (lipopolysaccharide) has equivalent characteristics to those of the USP Endotoxin RS.

**PERFORMANCE TESTS**

- **CARRIER:** The carrier should be the same as or chemically similar to the surface or material used for measuring depyrogenation, e.g., glass or stainless steel. If not similar, then the carrier and endotoxin combination must be at least as resistant to the depyrogenation process as the surface or material being measured. The carrier must be depyrogenated, or the inherent endotoxin level of the carrier should be determined before the addition of the endotoxin to the carrier.

• **ENDOTOXIN RECOVERY TESTS**

**Analysis:** Proceed as directed for the relevant technique under *Bacterial Endotoxins Test* (85).

**Acceptance criteria:** The determined endotoxin concentration is within a factor of 2 of the labeled endotoxin concentration.

**SPECIFIC TESTS**• **PURITY****Absence of assay enhancing substances**

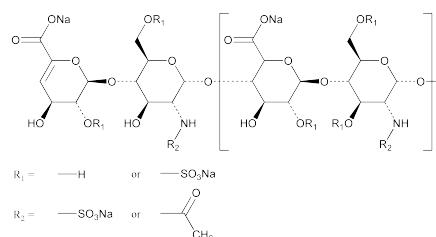
**Analysis:** Proceed as directed in the test for interfering factors in *Bacterial Endotoxins Test* (85).

**Acceptance criteria:** The Endotoxin Indicator should contain no substances (e.g., glucans) that can result in enhancement of endotoxin spike recovery.

**Absence of depyrogenation enhancing/inhibiting substances:** No substance (e.g., lactose, albumin, polyethylene glycol) should be present as filler for the endotoxin, as this can result in enhanced or inhibited depyrogenation effects.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store in the original package under conditions recommended on the label, and protect from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article, when used as directed on the labeling.
- **EXPIRATION DATE:** The expiration date is determined on the basis of stability studies from the date of manufacture, which is the date on which the first determination of Endotoxin Units was made.
- **LABELING:** The label states that the article is or may be used as an Endotoxin Indicator. It indicates the concentration in USP Endotoxin Units/indicator and the recommended storage conditions. The labeling states the source of the endotoxin (e.g. species and strain number) and includes instructions for preparation and safe disposal of the indicator. If the carrier is labeled, the label must be either easily removable or heat resistant, with no substances that could interfere with the assay. For Endotoxin Indicator vials, the label must include instructions for removal of the stopper from vials before use. If the stopper is heat resistant and designed to be left in the vial, the maximum processing temperature is stated.
- **DISPOSAL:** For destruction or discard, follow instructions recommended by the manufacturer, or depyrogenate at 250° for NLT 120 min.
- **USP REFERENCE STANDARDS**  
USP Endotoxin RS

**Enoxaparin Sodium**

R =	$X = 15 - 25\%$		$n = 0 \text{ to } 20$
	100-X	H	$n = 1 \text{ to } 21$

[9041-08-1].

**DEFINITION**

Enoxaparin Sodium is the sodium salt of a depolymerized heparin. It is obtained by alkaline depolymerization of heparin benzyl ester. The starting material, heparin, is obtained exclusively from porcine intestinal mucosa. Heparin source material used in the manufacture of Enoxaparin Sodium complies with the compendial requirements stated in the Heparin Sodium monograph. Enoxaparin Sodium consists of a complex set of oligosaccharides that have not yet been completely characterized. The majority of the components have a 4-enopyranose uronate structure at the nonreducing end of their chain. About 20% of the materials contain a 1,6-anhydro derivative on the reducing end of the chain, the range being between 15% and 25%. The weight-average molecular weight of Enoxaparin Sodium is 4500 Da, the range being between 3800 and 5000 Da; about 16% have a molecular weight of less than 2000 Da, the range being between 12.0% and 20.0%; about 74% have a molecular weight between 2000 and 8000 Da, the range being between 68.0% and 82.0%. NMT 18.0% have a molecular weight higher than 8000 Da. When prepared as a solution, the solution is analyzed for clarity and degree of color using a validated method. The degree of sulfation is NLT 1.8 per disaccharide unit. It has a potency of NLT 90 and NMT 125 Anti-Factor Xa International Units (IU)/mg, and NLT 20.0 and NMT 35.0 Anti-Factor IIa IU/mg, calculated on the dried basis. The ratio of Anti-Factor Xa activity to Anti-Factor IIa activity is between 3.3 and 5.3.

**IDENTIFICATION**• **A. ULTRAVIOLET ABSORPTION (197U)**

**Medium:** 0.01 N hydrochloric acid

**Sample solution:** 500 µg/mL

**Acceptance criteria:** The spectra exhibit maxima at  $231 \pm 2$  nm.

• **B. <sup>13</sup>C NMR SPECTRUM**

(See *Nuclear Magnetic Resonance* (761).)

**Standard solution:** Dissolve 200 mg of USP Enoxaparin Sodium RS in a mixture of 0.2 mL of deuterium oxide and 0.8 mL of water. Add 0.05 mL of deuterated methanol to serve as an internal reference.

**Sample solution:** Dissolve 200 mg of Enoxaparin Sodium in a mixture of 0.2 mL of deuterium oxide and 0.8 mL of water. Add 0.05 mL of deuterated methanol.

**Analysis:** Transfer the *Standard solution* and the *Sample solution* to NMR tubes of 5-mm diameter. Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 75 MHz for  $^{13}\text{C}$ , record the  $^{13}\text{C}$  NMR spectra of the *Standard solution* and the *Sample solution* at 40°.

**Acceptance criteria:** The spectra are similar.

- C.** The ratio of the numerical value of the Anti-Factor Xa activity, in Anti-Factor Xa IU/mg, to the numerical value of the Anti-Factor IIa activity, in Anti-Factor IIa IU/mg, as determined by the *Assay (Anti-Factor Xa Activity)* and *Impurities (Anti-Factor IIa Activity)*, respectively, is NLT 3.3 and NMT 5.3.

**D. MOLECULAR WEIGHT DISTRIBUTION AND WEIGHT-AVERAGE MOLECULAR WEIGHT**

**Mobile phase:** Prepare a 0.5 M lithium nitrate solution. Pass through a membrane filter of 0.45- $\mu\text{m}$  or smaller pore size, and degas with helium.

**Standard solution:** 10 mg/mL of USP Enoxaparin Sodium RS in *Mobile phase*

**Sample solution:** 10 mg/mL of Enoxaparin Sodium in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** Size exclusion LC

**Detector:** Differential refractive index

**Column**

**Guard:** 6-mm  $\times$  40-mm; packing L59

**Analytical:** Two 7.8-mm  $\times$  300-mm columns in series; packing L59

**Temperature:** Room temperature

**Flow rate:** 0.6 mL/min maintained constant to  $\pm 1.0\%$

**Analysis:** Reconstitute 1 vial each of USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS in 1 mL of *Mobile phase*. Separately inject 20  $\mu\text{L}$  of USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS, record the chromatograms, and measure the retention times. Inject in duplicate 20  $\mu\text{L}$  each of the *Standard solution* and the *Sample solution*, and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks. Calculate the total peak areas under each of the *Standard solution* and *Sample solution* chromatograms, excluding salt and solvent peaks at the end.

**Calibration curve:** Plot the retention times on the *x*-axis against the peak molecular weights on the *y*-axis for the peaks from USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS, and fit the data to a third-order polynomial, using suitable gel permeation chromatography (GPC) software.

**Calculations:** Compute the data, using the same GPC software; determine the weight-average molecular weight,  $M_w$ , for each of the duplicate chromatograms of the *Standard solution* and the *Sample solution*; and take the average for each solution. Correct the mean value of  $M_w$  to the nearest 50. The *Chromatographic system* is suitable if  $M_w$  for USP Enoxaparin Sodium RS is within 150 Da of the labeled  $M_w$  value. The  $M_w$  for the *Sample solution* is between 3800 and 5000 Da. Using the same software, determine for each of the duplicate *Sample solution* chromatograms the percentage of Enoxaparin Sodium chains with molecular weights lower than 2000 Da,  $M_{2000}$ , the percentage of Enoxaparin Sodium chains with molecular weights in the range 2000–8000 Da,  $M_{2000-8000}$ , and the percentage of Enoxaparin Sodium chains with molecular weights greater than 8000 Da,  $M_{8000}$ . Average the duplicate values, and express to the nearest 0.5%.

**Acceptance criteria:**  $M_{2000}$  is between 12.0% and 20.0%,  $M_{2000-8000}$  is between 68.0% and 82.0%, and  $M_{8000}$  is NMT 18.0%.

- E. IDENTIFICATION TESTS—GENERAL, Sodium <191>:** Meets the requirements

**ASSAY**

**• ANTI-FACTOR Xa ACTIVITY**

**Acetic acid solution:** Glacial acetic acid and water (42:58)

**pH 7.4 polyethylene glycol 6000 buffer:** Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000, adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

**pH 7.4 buffer:** Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

**pH 8.4 buffer:** Dissolve 3.03 g of tris(hydroxymethyl)aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.

**Human antithrombin III solution:** Reconstitute a vial of antithrombin III (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin III Units/mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Anti-thrombin III Unit/mL.

**Factor Xa solution:** Reconstitute a weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min when assayed as described below but using as an appropriate volume, *V*, the volume in  $\mu\text{L}$  of *pH 7.4 buffer* instead of *V*  $\mu\text{L}$  of the enoxaparin solution.

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for factor Xa in water to obtain a concentration of about 3 mM. Dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM.

**Standard solutions:** Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions in the concentration range between 0.025 and 0.2 Anti-Factor Xa IU/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Enoxaparin Sodium similar to those obtained for the *Standard solutions*.

**Analysis**

**Samples:** *Standard solutions*, *Sample solutions*, *Human antithrombin III solution*, *pH 7.4 buffer*, *Factor Xa solution*, *Chromogenic substrate solution*, and *Acetic acid solution*

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions*. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V* (20–50  $\mu\text{L}$ ), of *Human antithrombin III solution* and an equal volume, *V*, of either the blank (*pH 7.4 buffer*) or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 min. Add to each tube 2*V* (40–100  $\mu\text{L}$ ) of *Factor Xa solution*, and incubate for 1.0 min. Add a 5*V* (100–250  $\mu\text{L}$ ) volume of *Chromogenic substrate solution*. Stop the reaction after 4.0 min with a 5*V* (100–250  $\mu\text{L}$ ) volume of *Acetic acid solution*. Measure the absorbance of each solution at 405 nm, using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>) against blank B1. The reading of blank B2 relative to blank B1 is NMT  $\pm 0.05$  absorbance unit.

**Calculations:** For each series, calculate the regression of the absorbance against log concentrations of the *Sam-*

ple solutions and of the Standard solutions, and calculate the potency of the Enoxaparin Sodium in IU of Anti-Factor Xa activity/mL, using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the Anti-Factor Xa activity of Enoxaparin Sodium/mg.

**Acceptance criteria:** The potency is NLT 90 and NMT 125 Anti-Factor Xa IU/mg on the dried basis.

## OTHER COMPONENTS

### • BENZYL ALCOHOL CONTENT

**Mobile phase:** Acetonitrile, methanol, and water (3:1:16)

**Standard solution:** 0.1 mg/mL of USP Benzyl Alcohol RS in water

**Sample solution:** Weigh 0.5 g of Enoxaparin Sodium into a 10-mL volumetric flask, and dissolve in 5.0 mL of 1 N sodium hydroxide. Allow to stand at room temperature for about 1 h. Add 1.0 mL of glacial acetic acid, dilute with water to volume, and mix.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 256 nm

**Column:** 4.6-mm × 15-cm stainless steel; packing L7

**Flow rate:** 1.0 mL/min, maintained constant to ±10%

**Injection volume:** 20 µL

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of benzyl alcohol in the portion of Enoxaparin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of benzyl alcohol from the *Sample solution*

$r_S$  = peak area of benzyl alcohol from the *Standard solution*

$C_S$  = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

$C_U$  = concentration of Enoxaparin Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.1%

### • NITROGEN DETERMINATION, Method II <461>: 1.8%–2.5% on the dried basis

### • SODIUM CONTENT

(See *Spectrophotometry and Light-Scattering* <851>.)

**Cesium chloride solution:** 1.27 mg/mL of cesium chloride in 0.1 N hydrochloric acid

**Standard solution A:** 0.0025% of sodium chloride in *Cesium chloride solution*

**Standard solution B:** 0.0050% of sodium chloride in *Cesium chloride solution*

**Standard solution C:** 0.0075% of sodium chloride in *Cesium chloride solution*

**Sample solution:** Transfer 50.0 mg of Enoxaparin Sodium to a 100-mL volumetric flask, and dissolve in and dilute with *Cesium chloride solution* to volume.

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Cesium chloride solution*, and *Sample solution*

Concomitantly determine the absorbances of the *Cesium chloride solution* (blank), *Sample solution*, and *Standard solutions* at 330.3 nm, using a sodium hollow-cathode lamp and an air-acetylene flame. Using the absorbances of *Standard solutions A–C*, determine the sodium content in the *Sample solution* after an appropriate blank correction.

**Acceptance criteria:** 11.3%–13.5% on the dried basis

## IMPURITIES

- **HEAVY METALS, Method I <231>:** NMT 30 µg/g, using a 2.7% solution in water

## SPECIFIC TESTS

- **pH <791>:** 6.2–7.7 for a 10.0% solution in water
- **LOSS ON DRYING <731>:** Dry 1 g in a vacuum at 70° for 6 h: it loses NMT 10.0% of its weight.

### • SPECIFIC ABSORBANCE

(See *Spectrophotometry and Light-Scattering* <851>.)

**Sample solution:** 0.5 mg/mL of Enoxaparin Sodium in 0.01 N hydrochloric acid

**Analysis:** Obtain the UV spectra of the *Standard solution* and the *Sample solution* between 200 nm and 300 nm against a 0.01 N hydrochloric acid blank.

Calculate the specific absorbance at the wavelength of maximum absorbance at  $231 \pm 2$  nm, with reference to the dried substance:

$$\text{Result} = A \times 100 \times 1000 / [M \times l \times (100 - E)]$$

$A$  = absorbance at the wavelength of maximum absorbance

$M$  = weight of Enoxaparin Sodium in the *Sample solution* (mg)

$l$  = path length (typically 1 cm)

$E$  = loss on drying (%)

**Acceptance criteria:** 14.0–20.0 on the dried basis

- **BACTERIAL ENDOTOXINS TEST <85>:** It contains NMT 0.01 USP Endotoxin Unit/IU of Anti-Factor Xa activity.

### • ANTI-FACTOR IIa ACTIVITY

**Acetic acid solution, pH 7.4 polyethylene glycol 6000**

**buffer, pH 7.4 buffer, pH 8.4 buffer, and Human antithrombin III solution:** Proceed as directed in the *Assay for Anti-Factor Xa Activity*, except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit/mL.

**Thrombin human solution:** Reconstitute thrombin human (see *Reagents, Indicators and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL.

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Immediately before use, dilute with *pH 8.4 buffer* to 0.5 mM.

**Standard solutions:** Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.

**Sample solutions:** Proceed as directed under *Standard solutions* to obtain concentrations of Enoxaparin Sodium similar to those obtained for the *Standard solutions*.

**Analysis:** Proceed as directed in the *Assay for Anti-Factor Xa Activity*, except to use *Thrombin human solution* instead of *Factor Xa solution* and to use *Human antithrombin III solution* as described above.

**Calculations:** For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the Enoxaparin Sodium in IU of Anti-Factor IIa activity/mg, using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the Anti-Factor IIa activity of Enoxaparin Sodium/mg.

**Acceptance criteria:** It has a potency of NLT 20.0 and NMT 35.0 Anti-Factor IIa IU/mg on the dried basis.

• **MOLAR RATIO OF SULFATE TO CARBOXYLATE**

**Mobile phase:** Carbon dioxide-free water

**Sample solution:** 5 mg/mL of Enoxaparin Sodium in carbon dioxide-free water

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Ion

**Column:** Two columns; one 1.5-cm × 2.5-cm column, packed with an anion-exchange resin L64 packing; and one 1.5-cm × 7.5-cm column, packed with a cation-exchange resin L65 packing. The outlet of the anion-exchange column is connected to the inlet of the cation-exchange column.

**Flow rate:** 1 mL/min

**Analysis**

**Sample:** *Sample solution*

[NOTE—Regenerate the anion-exchange column and the cation-exchange column with 1 N sodium hydroxide and 1 N hydrochloric acid, respectively, between two injections.]

Inject the *Sample solution* into the anion-exchange column, and collect the eluate from the cation-exchange column in a beaker at the outlet until the ion detector reading returns to the baseline value. Quantitatively transfer the eluate to a titration vessel containing a magnetic stirring bar, and dilute with carbon dioxide-free water to about 60 mL. Position the titration vessel on a magnetic stirrer, and immerse the electrodes. Note the initial conductivity reading, and titrate with approximately 0.1 N sodium hydroxide added in 100-μL portions. [NOTE—Prepare the sodium hydroxide solution in carbon dioxide-free water.] Record the buret reading and the conductivity meter reading after each addition of the sodium hydroxide solution.

**Calculations:** Plot the conductivity measurements on the y-axis against the volumes of sodium hydroxide added on the x-axis. The graph will have three linear sections—an initial downward slope, a middle slight rise, and a final rise. For each of these sections draw the best-fit straight lines, using linear regression analysis. At the points where the first and second straight lines intersect and where the second and third lines intersect, draw perpendiculars to the x-axis to determine the volumes of sodium hydroxide taken up by the sample at those points. The point where the first and second lines intersect corresponds to the volume of sodium hydroxide taken up by the sulfate groups ( $V_S$ ). The point where the second and third lines intersect corresponds to the volume of sodium hydroxide consumed by the sulfate and the carboxylate groups together ( $V_T$ ). Calculate the molar ratio of sulfate to carboxylate:

$$\text{Result} = V_S / (V_T - V_S)$$

**Acceptance criteria:** The molar ratio of sulfate to carboxylate is NLT 1.8.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 40°, preferably at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Benzyl Alcohol RS
  - USP Endotoxin RS
  - USP Enoxaparin Sodium RS
  - USP Enoxaparin Sodium Molecular Weight Calibrant A RS
  - USP Enoxaparin Sodium Molecular Weight Calibrant B RS
  - USP Enoxaparin Sodium for Bioassays RS

## Enoxaparin Sodium Injection

**DEFINITION**

Enoxaparin Sodium Injection is a sterile solution of Enoxaparin Sodium in Water for Injection. Its appearance is analyzed for clarity and degree of color, using a validated method. Its potency value is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU). It may contain, in multiple-dose containers, a suitable antimicrobial preservative, such as benzyl alcohol.

**IDENTIFICATION**

• **A.**

**Analysis:** Transfer the total contents of a single-dose container or 0.4 mL from a multiple-dose container to a glass test tube, add 2 mL of water and 1 mL of 2% (w/v) protamine sulfate solution, and mix.

**Acceptance criteria:** A creamy white precipitate is formed.

• **B. ULTRAVIOLET ABSORPTION** (197U)

**Medium:** 0.01 N hydrochloric acid

**Standard solution:** 500 μg/mL

**Sample solution:** Transfer the total content of a single-dose container or 0.4 mL from a multiple-dose container to a 100-mL volumetric flask. Dilute with *Medium* to volume.

**Acceptance criteria:** The spectra exhibit maxima at  $231 \pm 2$  nm.

• **C. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements

**ASSAY**

• **ANTI-FACTOR Xa ACTIVITY**

**Acetic acid solution:** Glacial acetic acid and water (42:58)

**pH 7.4 polyethylene glycol 6000 buffer:** Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000, adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

**pH 7.4 buffer:** Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

**pH 8.4 buffer:** Dissolve 3.03 g of tris(hydroxymethyl)aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.

**Human antithrombin III solution:** Reconstitute a vial of antithrombin III (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin III Units/mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Antithrombin III Unit/mL.

**Factor Xa solution:** Reconstitute a weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min when assayed as described below but using as an appropriate volume,  $V$ , the volume in μL of *pH 7.4 buffer* instead of  $V$  μL of the enoxaparin solution.

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for Factor Xa in water to obtain a concentration of about 3 mM. Dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM.

**Standard solutions:** Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS

with water, and dilute with *pH 7.4 buffer* to obtain four dilutions in the concentration range between 0.025 and 0.2 Anti-Factor Xa IU/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.

#### Analysis

**Samples:** *Standard solutions*, *Sample solutions*, *Human antithrombin III solution*, *pH 7.4 buffer*, *Factor Xa solution*, *Chromogenic substrate solution*, and *Acetic acid solution*

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions*. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V* (20–50 µL), of *Human antithrombin III solution* and an equal volume, *V*, of either the blank (*pH 7.4 buffer*) or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 min. Add to each tube 2*V* (40–100 µL) of *Factor Xa solution*, and incubate for 1.0 min. Add a 5*V* (100–250 µL) volume of *Chromogenic substrate solution*. Stop the reaction after 4.0 min with a 5*V* (100–250 µL) volume of *Acetic acid solution*. Measure the absorbance of each solution at 405 nm, using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>) against blank B1. The reading of blank B2 relative to blank B1 is NMT ±0.05 absorbance unit.

**Calculations:** For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor Xa activity/mL, using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated.

**Acceptance criteria:** The potency is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU).

- **ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO:** The ratio of the numerical value of the Anti-Factor Xa activity in Anti-Factor Xa IU/mL to the numerical value of the Anti-Factor IIa activity in Anti-Factor IIa IU/mL, as determined by *Anti-Factor Xa Activity* and *Anti-Factor IIa Activity*, respectively, is NLT 3.3 and NMT 5.3.

#### OTHER COMPONENTS

##### • BENZYL ALCOHOL CONTENT (IF PRESENT)

**Mobile phase:** Acetonitrile, methanol, and water (3:1:16)

**Standard solution:** 1.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

**Sample solution:** Transfer exactly 5.0 mL of the Injection to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 256 nm

**Column:** 4.6-mm × 15-cm stainless steel; packing L7<sup>1</sup>

**Flow rate:** 1.0 mL/min, maintained constant to ±10%

**Injection volume:** 20 µL

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C$$

*r<sub>U</sub>* = peak area of benzyl alcohol from the *Sample solution*

*r<sub>S</sub>* = peak area of benzyl alcohol from the *Standard solution*

*C* = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

**Acceptance criteria:** 1.35%–1.65%

#### SPECIFIC TESTS

- **pH <791>:** 5.5–7.5

- **BACTERIAL ENDOTOXINS TEST <85>:** It contains less than 0.01 USP Endotoxin Unit/unit of Anti-Factor Xa activity in Anti-factor Xa IU.

##### • ANTI-FACTOR IIa ACTIVITY

**Acetic acid solution, pH 7.4 polyethylene glycol 6000 buffer, pH 7.4 buffer, pH 8.4 buffer, and Human antithrombin III solution:** Proceed as directed in the

*Assay for Anti-Factor Xa Activity*, except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit/mL.

**Thrombin human solution:** Reconstitute thrombin human (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL.

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Immediately before use, dilute with *pH 8.4 buffer* to 0.5 mM.

**Standard solutions:** Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.

**Standard solutions:** Dilute USP Enoxaparin Sodium Solution for Bioassays RS with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.

**Sample solutions:** Proceed as directed under *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.

**Analysis:** Proceed as directed in the *Assay for Anti-Factor Xa Activity*, except to use *Thrombin human solution* instead of *Factor Xa solution* and to use *Human antithrombin III solution* as described above.

**Calculations:** For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor IIa activity/mL, using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits.

**Acceptance criteria:** The Anti-Factor IIa activity IU (or IU/mL) is NLT 20.0% and NMT 35.0% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU or IU/mL).

##### • FREE SULFATE CONTENT

**Mobile phase:** 3.0 mM sodium carbonate solution

**System suitability solution:** 3 µg/mL of sulfate anion and 5 µg/mL of oxalate anion

**Standard sulfate stock solution:** Prepare a solution of sodium sulfate in *Mobile phase* in a suitable sulfate-free container such that the concentration of sulfate is accurately known at about 1 mg/mL. Transfer 5 g of the solution to a similar container, and add *Mobile phase* to obtain 25 g of solution.

**Standard solution A:** 0.1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

**Standard solution B:** 0.5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

**Standard solution C:** 1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

<sup>1</sup> Available as Lichrospher 100 RP 18, pore size 100 Å, particle size 5 µm, or equivalent.

**Standard solution D:** 2 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

**Standard solution E:** 4 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

**Standard solution F:** 5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

**Sample solution:** Transfer a known quantity, *m*, of Enoxaparin Sodium Injection, accurately weighed, to a suitable previously tared sulfate-free vial. Add *Mobile phase* to obtain a solution having a known concentration of about 10 mg/g.

#### Chromatographic system

(See *Chromatography* <621>, *System suitability*.)

**Mode:** Ion chromatography

**Detector:** Conductivity

#### Column

**Guard:** 4-mm × 5-cm; packing L61

**Analytical:** 4-mm × 25-cm; packing L61

[NOTE—Use a micromembrane anion autosuppressor<sup>2</sup> or a suitable chemical suppression system.]

**Flow rate:** 2.0 mL/min

**Injection size:** 25 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1 between the sulfate and oxalate peaks

#### Analysis

**Samples:** *Standard solutions A–F* and *Sample solution*  
Plot the standard curve of sulfate peak height as a function of sulfate concentration (in µg/g) in *Standard solutions A–F*. From the sulfate peak height determine the concentration of sulfate, *C*, in µg/g, in the *Sample solution*, using the standard curve.

Calculate the percentage of free sulfate content (w/w) in the portion of Injection taken:

$$\text{Result} = [(C \times M_s)/10m]$$

*M<sub>s</sub>* = total mass of the *Sample solution* (g)

*m* = mass of Injection taken to prepare the *Sample solution* (mg)

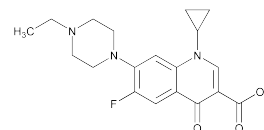
**Acceptance criteria:** The percentage of free sulfate is NMT 0.12% (w/w).

- **STERILITY TESTS** <71>: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* <1>.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers in Type I glass. Store between 20° and 25°, excursions permitted between 15° and 30°.
- **LABELING:** Label it to indicate the amount (mg) of Enoxaparin Sodium in the total volume of contents. The label states also that the Enoxaparin Sodium starting material is porcine derived.
- **USP REFERENCE STANDARDS** <11>
  - USP Benzyl Alcohol RS
  - USP Endotoxin RS
  - USP Enoxaparin Sodium RS
  - USP Enoxaparin Sodium for Bioassays RS

## Enrofloxacin



C<sub>19</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>3</sub> 359.39

3-Quinolonecarboxylic acid, 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-;

1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid [93106-60-6].

» Enrofloxacin contains not less than 98.5 percent and not more than 101.5 percent of C<sub>19</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>3</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, tight, light-resistant containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards <11>—

USP Enrofloxacin RS

USP Fluoroquinolonic Acid RS

USP N-Ethylpiperazine RS

USP Enrofloxacin Related Compound Mixture RS

Contains a mixture of USP Enrofloxacin RS, desfluoro-enrofloxacin, and USP Ciprofloxacin RS.

*Desfluoro-enrofloxacin*: 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid.

#### Clarity of solution—

**Hydrazine sulfate solution**—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours.

**Hexamethylenetetramine solution**—Transfer 2.5 g of hexamethylenetetramine to a 100-mL volumetric flask, add 25.0 mL of water, insert a glass stopper, and mix to dissolve.

**Primary opalescent suspension**—[NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well-mixed before use.] Transfer 25.0 mL of the *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 hours.

**Opalescence standard**—[NOTE—This suspension should not be used beyond 24 hours after preparation.] Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Reference suspension**—Transfer 10.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

**Procedure**—Transfer a sufficient portion of the *Test solution*, the *Reference suspension*, and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm to obtain a depth of 40 mm. Compare the *Test solution*, the *Reference suspension*, and water in diffused daylight 5 minutes after preparation of the *Reference suspension*, viewing vertically against a black background (see *Visual Comparison* under *Spectrophotometry and Light-Scattering* <851>). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* shows the same clarity as that of water or its opalescence is not more pronounced than that of the *Reference suspension*.

<sup>2</sup>Available as Anion Self-Regenerating Suppressor (ASRS) from Dionex Inc, or equivalent.



**Color of solution—**

**Standard stock solution**—Combine 9.6 mL of ferric chloride CS, 0.2 mL of cobaltous chloride CS, and 0.2 mL of cupric sulfate CS, and mix.

**Standard solution**—[NOTE—Prepare the *Standard solution* immediately before use.] Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g per 1000 mL).

**Test solution**—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

**Procedure**—Transfer a sufficient portion of the *Test solution*, the *Standard solution*, and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm to obtain a depth of 40 mm. Compare the *Test solution*, the *Standard solution*, and water in diffused daylight, viewing vertically against a white background (see *Visual Comparison under Spectrophotometry and Light-Scattering* (851)). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

**Identification—**

**A: Infrared Absorption** (197K).

**B: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution:** Prepare a solution containing 10 mg of Enrofloxacin per mL of 0.05 M hydrochloric acid.

**Application volume:** 5  $\mu$ L.

**Developing solvent solution:** methylene chloride, methanol, 25% ammonia solution, and acetonitrile (2:2:1:0.5).

**Procedure**—Proceed as directed in the chapter except apply 5- $\mu$ L portions of the *Test solution* and the *Standard solution* to the starting line of the chromatographic plate. Place the plate in an atmosphere of ammonia for approximately 15 minutes. Develop the chromatogram in an unsaturated chamber with the *Developing solvent solution*.

**Loss on drying** (731)—Dry a 2-g sample, accurately weighed, 5 to 7 hours under vacuum at 120° to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%, based on a test specimen of about 2 g.

**Chloride** (221)—Add 58 mL of water to 0.5 g of Enrofloxacin, shake for 5 minutes, and pass through a chloride-free filter paper, add 2 mL of 2 M acetic acid, and mix. Transfer 15.0 mL of the filtrate to a 50-mL color-comparison tube (*Test solution*). To a second matched 50-mL color-comparison tube transfer 10.0 mL of a *Standard solution* of sodium chloride having a concentration of 8.2  $\mu$ g per mL, corresponding to 5  $\mu$ g of chloride per mL, add 5.0 mL of water, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix. Allow the solutions to stand for 5 minutes, protected from light. Examine the tubes vertically against a black background (see *Visual Comparison under Spectrophotometry and Light-Scattering* (851)). Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

**Sulfate** (221)—Dissolve 0.5 g of Enrofloxacin in 5.0 mL of 2 N acetic acid and 15.0 mL of water (*Test solution*). To each of two 50-mL matched color-comparison tubes transfer 1.50 mL of a *Standard solution* of potassium sulfate in 30% alcohol having a concentration of 18.1  $\mu$ g per mL, equivalent to 10  $\mu$ g of sulfate per mL. To each tube add, successively and with continuous shaking, 1.0 mL of barium chloride solution (1 in 4), and allow to stand for 1 minute. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Test solution* and 0.5 mL of 30% acetic acid, and mix. Allow solutions to stand for 5 minutes. Examine the tubes vertically against a black background (see *Visual Comparison under Spectrophotometry and Light-Scattering* (851)).

Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

**Heavy metals, Method II** (231): 0.002%.

**Limit of N-ethylpiperazine—**

**Internal standard solution**—Dissolve an accurately weighed quantity of *n*-decane in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 0.1 mg per mL.

**Standard stock solution**—Dissolve an accurately weighed quantity of USP N-Ethylpiperazine RS in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 9.0 mg per mL.

**Standard solution**—To 2.0 mL of the *Internal standard solution* add 20  $\mu$ L of the *Standard stock solution*, and mix.

**Test solution**—To 200 mg of Enrofloxacin, accurately weighed, add 2.0 mL of the *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a split injector system and contains a 0.32-mm  $\times$  50-m column with 100% liquid phase G1 with a film thickness of about 5.0  $\mu$ m. The carrier gas is hydrogen (helium may be used), flowing at a rate of about 2.9 mL per minute. The auxiliary gas is nitrogen flowing at a rate of about 30 mL per minute. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then the temperature is increased at a rate of 10° per minute to 240°, and maintained at 240° for 15 minutes. The split injector (25:1 split ratio) temperature is maintained at 200°, and the detector is maintained at 250°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.90 for N-ethylpiperazine and 1.0 for *n*-decane.

**Procedure**—Inject a volume (about 1.0  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of the impurity in the portion of Enrofloxacin taken by the formula:

$$100(C_S / C_U)(R_U / R_S)$$

in which  $C_S$  is the concentration of the impurity, in mg per mL, in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of Enrofloxacin in the *Test solution*;  $R_U$  is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Test solution*; and  $R_S$  is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Standard solution*. Not more than 0.1% of the impurity is found.

**Related compounds—**

TEST 1 (FOR FLUOROQUINOLONIC ACID)—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution**—Prepare as directed for the *Test solution* in Identification test B.

**Diluent**—Transfer 0.1 mL of 6 M ammonium to a 100-mL volumetric flask, mix, and dilute with water to volume.

**Standard stock solution**—Dissolve and mix an accurately weighed quantity of USP Fluroquinolonic Acid RS with *Diluent* to prepare a solution containing about 0.10 mg per mL, and mix.

**Standard solution 1 (0.1%)**—Transfer 1.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.

**Standard solution 2 (0.2%)**—Transfer 2.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.

**Application volume:** 5  $\mu$ L.

**Developing solvent system**—[NOTE—Carefully follow the mixing order stated below.] Shake butyl acetate, *n*-butanol, water, and glacial acetic acid (50:9:15:25), and allow to settle. Use the upper layer as the mobile phase and discard the lower layer.

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Apply separately the *Test solution*, *Standard solution 1*, and *Standard solution 2* to the thin-layer plate, and chromatograph using the *Developing solvent system*. Dry the developed chromatogram in the air under a fume hood for 30 to 60 minutes, then view under short-wavelength UV light. Determine the quantity of fluoroquinolonic acid by comparing the size and intensity of the spots from the *Test solution* to the *Standard solutions*. The intensity of any spot from the *Test solution* at about the same retardation factor,  $R_f$ , as that of the *Standard solutions* is not greater than the intensity of the *Standard solution 2* (0.2%) spot.

**TEST 2 (FOR CIPROFLOXACIN, DES-FLUORO COMPOUND AND OTHER UNSPECIFIED IMPURITIES)**—

**Phosphoric acid buffer**—Prepare 25 mM phosphoric acid, and adjust with triethylamine to a pH of 3.0.

**Mobile phase**—Prepare a solution containing *Phosphoric acid buffer* and acetonitrile (87:13).

**Control solution**—Dissolve about 5 mg of USP Enrofloxacin Related Compound Mixture RS, accurately weighed, in *Mobile phase* in a 5-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution 1**—Dissolve about 50 mg of Enrofloxacin, accurately weighed, in *Mobile phase* in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution 2**—Transfer 1.0 mL of *Test solution 1* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm stainless steel column that contains 5-μm packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Control solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.58 for the des-fluoro compound, 0.74 for ciprofloxacin, and 1.0 for enrofloxacin. The resolution,  $R$ , between the des-fluoro compound and ciprofloxacin is not less than 1.5.

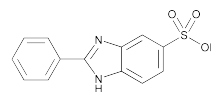
**Procedure**—Inject a volume (about 25 μL) of *Test solution 1*, *Test solution 2*, and the *Control solution* into the chromatograph, record the chromatogram, and measure the peak responses. Identify the ciprofloxacin and the des-fluoro compound peaks in *Test solution 2* by comparing their retention times with those from the *Control solution*. Calculate the percentage of each related compound in the portion of Enrofloxacin taken by the formula:

$$100C(r_1 / r_2)$$

in which  $C$  is the concentration of Enrofloxacin in *Test solution 2* as a percentage of *Test solution 1* (0.2%);  $r_1$  is the individual peak response of each related compound obtained from *Test solution 1*; and  $r_2$  is the individual peak area of enrofloxacin obtained from *Test solution 2*: not more than 0.1% of des-fluoro compound, not more than 0.3% of ciprofloxacin, not more than 0.1% of any unspecified impurity, and not more than 0.5% of total impurities are found.

**Assay**—Transfer about 250 mg of Enrofloxacin, accurately weighed, to a 125-mL flask, dissolve in 100 mL of anhydrous acetic acid, and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M perchloric acid is equivalent to 35.94 mg of  $C_{19}H_{22}FN_3O_3$ .

## Ensulizole



$C_{13}H_{10}N_2O_3S$  274.30  
1H-Benzimidazole-5-sulfonic acid-2-phenyl-;  
2-Phenylbenzimidazole-5-sulfonic acid [27503-81-7].

### DEFINITION

Ensulizole contains NLT 98.0% and NMT 102.0% of  $C_{13}H_{10}N_2O_3S$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)

**Sample solution:** 5 μg/mL

[NOTE—Transfer 100 mg of Ensulizole to a 100-mL volumetric flask, dissolve in 4 mL of 1 N sodium hydroxide, and dilute with water to volume. Transfer 0.5 mL of the solution to a 100-mL volumetric flask, and dilute with water to volume.]

**Acceptance criteria:** The absorptivities, calculated on the dried basis, at the wavelength of maximum absorbance at about 302 nm, do not differ by more than 3.0%.

### ASSAY

#### PROCEDURE

**Sample solution:** 48 mg/mL of Ensulizole in 0.5 N sodium hydroxide

**Titratant:** 0.5 N hydrochloric acid VS

**Analysis:** To the *Sample solution*, add phenolphthalein TS, and titrate the excess with *Titratant*. Perform a blank determination, and make any necessary corrections (see *Titrimetry* (541)). Each mL of 0.5 N sodium hydroxide is equivalent to 137.15 mg of  $C_{13}H_{10}N_2O_3S$ .

Calculate the percentage of  $C_{13}H_{10}N_2O_3S$  in the portion of Ensulizole taken:

$$\text{Result} = [(N \times (V_B - V_A)) / (W \times (1 - \text{LOD}))] \times M_{r1} \times 100$$

$N$  = actual normality of the *Titratant*

$V_B$  = volume of *Titratant* used for the blank (mL)

$V_A$  = volume of *Titratant* used for the *Sample solution* (mL)

$W$  = weight of Ensulizole taken for the *Sample solution* (mg)

$\text{LOD}$  = percentage of loss on drying expressed as a decimal fraction, as determined in the test for *Loss on Drying*

$M_{r1}$  = molecular weight of ensulizole (274.30)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

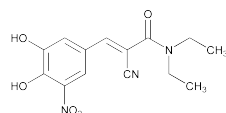
### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 2.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place.
- **USP REFERENCE STANDARDS** (11)  
USP Ensulizole RS

## Entacapone



$C_{14}H_{15}N_3O_5$  305.29  
(*E*)- $\alpha$ -Cyano-*N,N*-diethyl-3,4-dihydroxy-5-nitrocinnamamide;  
2-Propenamides, 2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N,N*-diethyl-, (*E*)- [130929-57-6].

### DEFINITION

Entacapone contains NLT 98.0% and NMT 102.0% of  $C_{14}H_{15}N_3O_5$ , calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 2.34 g/L of monobasic sodium phosphate dihydrate. Adjust with phosphoric acid to a pH of 2.1.

**Diluent:** Methanol and tetrahydrofuran (7:3)

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer* (22:1:27)

**System suitability solution:** 1  $\mu$ g/mL each of USP Entacapone Related Compound A RS and USP Entacapone RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Entacapone RS in *Diluent*

**Sample solution:** 0.1 mg/mL of Entacapone in *Diluent*

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L11

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for entacapone related compound A and entacapone are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between entacapone related compound A and entacapone, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{15}N_3O_5$  in the portion of Entacapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of entacapone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS**, *Method II* (231): NMT 10 ppm

#### Organic Impurities

##### PROCEDURE

**Buffer, Diluent, and Mobile phase:** Proceed as directed in the *Assay*.

**Standard solution:** Use the *System suitability solution* prepared as directed in the *Assay*.

**Sample solution:** 1.0 mg/mL of Entacapone in *Diluent*

**Chromatographic system:** Prepare as directed in the *Assay*.

**Run time:** 2 times the retention time of the entacapone peak

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between entacapone related compound A and entacapone

**Relative standard deviation:** NMT 10.0% based on the entacapone peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of any individual impurity in the portion of Entacapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any impurity from the *Sample solution*

$r_S$  = peak response of entacapone from the *Standard solution*

$C_S$  = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Entacapone in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.2%

[NOTE— Do not include entacapone related compound A in the calculation of total impurities.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Entacapone related compound A <sup>a</sup>	0.8	0.1
Entacapone	1.0	—
Any other unknown individual impurity	—	0.10
Total impurities	—	0.2

<sup>a</sup> (Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N,N*-diethylacrylamide.

### SPECIFIC TESTS

#### Loss on Drying (731)

**Analysis:** Dry 1.0 g of the sample in vacuum at a pressure not exceeding 49 mm of mercury at 65° to constant weight.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

#### USP REFERENCE STANDARDS (11)

USP Entacapone RS

USP Entacapone Related Compound A RS

(Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N,N*-diethylacrylamide.

$C_{14}H_{15}N_3O_5$  305.29

## Entacapone Tablets

### DEFINITION

Entacapone Tablets contain an amount of entacapone equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of entacapone ( $C_{14}H_{15}N_3O_5$ ).

### IDENTIFICATION

#### Change to read:

- **A. INFRARED ABSORPTION** (197K): The sample shows a medium band at about  $2216\text{ cm}^{-1}$  and strong bands at about  $1628, 1604, 1544, 1512, 1440, 1376, 1348, 1296, 1280$ , and  $1208\text{ cm}^{-1}$  (ERR 1-Jul-2012) similar to the reference preparation.
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Protect solutions from light.]

**Buffer:** 2.1 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.1.

**Diluent:** Methanol and tetrahydrofuran (7:3)

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer* (22:1:27)

**Standard solution:** 0.5 mg/mL of USP Entacapone RS in *Diluent*

**Sample solution:** 0.5 mg/mL of entacapone from finely powdered Tablets (NLT 20). Add tetrahydrofuran up to 30% of the final volume, and sonicate for 3 min. Add methanol up to 30% of the final volume, and shake for 5 min. Dilute with methanol to volume. Centrifuge a portion of this solution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L11

**Flow rate:** 1 mL/min

**Run time:** 1.5 times the retention time of the entacapone peak

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{15}N_3O_5$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of entacapone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** pH 5.5 Phosphate buffer (6.8 g/L of monobasic potassium phosphate in water. Adjust with 1 M sodium hydroxide to a pH of 5.5); 900 mL.

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard stock solution:** 0.22 mg/mL of USP Entacapone RS, prepared by dissolving in 2% of the flask volume in tetrahydrofuran and diluting with *Medium* to volume. Protect this solution from the light.

**Standard solution:** 22  $\mu$ g/mL of USP Entacapone RS in *Medium* from the *Standard stock solution*. Protect this solution from the light.

**Sample solution:** Pass a portion of the solution through a suitable filter of 20- $\mu$ m pore size. Dilute with *Medium*, if necessary. Protect this solution from the light.

#### Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** 313 nm

**Path length:** 1 cm

**Blank:** Tetrahydrofuran and *Medium* (0.2:99.8)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{14}H_{15}N_3O_5$  dissolved:

$$\text{Result} = A_U/A_S \times C_S/L \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{14}H_{15}N_3O_5$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

### IMPURITIES

#### Organic Impurities

#### PROCEDURE

[NOTE—Protect solutions from light.]

**Buffer, Diluent, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 0.03 mg/mL each of USP Entacapone RS and USP Entacapone Related Compound A RS in *Diluent*

**Standard solution:** 3  $\mu$ g/mL of USP Entacapone RS in *Diluent*

**Sample solution:** 3 mg/mL of entacapone from finely powdered Tablets (NLT 20). Add tetrahydrofuran up to 30% of the final volume, and sonicate for 3 min. Add methanol up to 30% of the final volume, and sonicate for 5 min. Dilute with methanol to volume. Centrifuge a portion of this solution. [NOTE—Prepare the *Sample solution* fresh and use within 7 h of preparation.]

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between entacapone related compound A and entacapone, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of entacapone from the *Standard solution*

$C_S$  = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of entacapone in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.2%

[NOTE— Do not include entacapone related compound A in the calculation of total impurities.]

**Impurity Table 1**

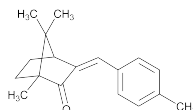
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Entacapone related compound A <sup>a</sup>	0.8	0.2
Entacapone	1.0	—
Any individual unspecified degradation product	—	0.1

<sup>a</sup> (Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylacrylamide.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Entacapone RS
  - USP Entacapone Related Compound A RS
  - (Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylacrylamide.
  - $C_{14}H_{15}N_3O_5$

## Enzacamene



$C_{18}H_{22}O$  254.37

3-(4-Methylbenzylidene)-camphor [36861-47-9].

» Enzacamene contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{22}O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Methyl Benzylidene Camphor RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* methanol.

Absorptivities at the wavelength of maximum absorbance, about 299 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range, Class Ia (741):** between 66° and 68°.

**Loss on drying (731)**—Dry it in vacuum at 50° to constant weight; it loses not more than 0.2% of its weight.

#### Related compounds—

*Standard solution*—Use the *Standard preparation* prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Chromatographic system*—Proceed as directed in the *Assay*.

*Procedure*—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of each impurity in the portion of Enzacamene taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response of enzacamene obtained from the *Standard solution*: not more than 0.02% of camphor is found; not more than 0.1% of any other individual impurity is found; and not more than 0.5% of total impurities is found.

#### Assay—

*Standard preparation*—Dissolve accurately weighed quantities of camphor and USP Methyl Benzylidene Camphor RS in dichloromethane, and dilute quantitatively, and stepwise if necessary, with dichloromethane to obtain a solution having a known concentration of about 250 µg of each per mL.

*Assay preparation*—Transfer about 25 mg of Enzacamene, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with dichloromethane to volume.

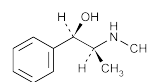
*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m fused-silica capillary column coated with a 0.25-µm layer of phase G1. The carrier gas is helium flowing at a rate of about 1.2 mL per minute. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 100°, then 5 minutes after injection the temperature is increased at a rate of 10° per minute to 230°, and maintained at 230° for 10 minutes. The temperature is then increased at a rate of 10° per minute to 300°, and maintained at 300° until the end of the procedure. The injection port temperature is maintained at 295°, and the detector is maintained at 305°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.2 for camphor and 1.0 for enzacamene; the tailing factor is not more than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{22}O$  in the portion of Enzacamene taken by the formula:

$$100C(r_U / r_s)$$

in which C is the concentration, in mg per mL, of USP Methyl Benzylidene Camphor RS in the *Standard preparation*; and  $r_U$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ephedrine



$C_{10}H_{15}NO$  165.23

Benzenemethanol, α-[1-(methylamino)ethyl]-, [R-(R\*,S\*)]-.

(-)-Ephedrine [299-42-3].

Hemihydrate 174.24 [50906-05-3].

» Ephedrine is anhydrous or contains not more than one-half molecule of water of hydration. It

contains not less than 98.5 percent and not more than 100.5 percent of  $C_{10}H_{15}NO$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, in a cold place.

**Labeling**—Label it to indicate whether it is hydrous or anhydrous. Where the quantity of Ephedrine is indicated in the labeling of any preparation containing Ephedrine, this shall be understood to be in terms of anhydrous Ephedrine.

**USP Reference standards** (11)—

USP Ephedrine Sulfate RS

**Identification**—Accurately weigh about 100 mg, and add by buret the exact volume of 0.1 N sulfuric acid, determined in the Assay, to neutralize it. Dilute with water in a volumetric flask to 25 mL. Mix 2 mL with 10 mL of alcohol, and evaporate on a steam bath with the aid of a current of air to dryness: the residue so obtained responds to *Identification* test A under *Ephedrine Sulfate*.

**Specific rotation** (781S): between  $-40.3^\circ$  and  $-43.3^\circ$ .

*Test solution*: 25 mg per mL, in 0.6 N hydrochloric acid.

**Water**, *Method 1b* (921): between 4.5% and 5.5%, for hydrated Ephedrine; not more than 0.5% for anhydrous Ephedrine.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—A solution of 500 mg shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.030%).

**Sulfate**—Dissolve 100 mg in 40 mL of water, and add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity develops within 10 minutes.

**Ordinary impurities** (466)—

*Test solution*: methanol.

*Standard solution*: methanol.

*Eluant*: a mixture of isopropyl alcohol, ammonium hydroxide, and chloroform (80:15:5).

*Visualization*: 1, followed by 4.

**Assay**—Dissolve about 500 mg of Ephedrine, accurately weighed, in 10 mL of neutralized alcohol, and add 5 drops of methyl red TS and 40.0 mL of 0.1 N hydrochloric acid VS. Titrate the excess acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N hydrochloric acid is equivalent to 16.52 mg of  $C_{10}H_{15}NO$ .

## Ephedrine Hydrochloride

$C_{10}H_{15}NO \cdot HCl$  201.69

Benzenemethanol,  $\alpha$ -[1-(methyamino)ethyl]-, hydrochloride,  $[R-(R^*, S^*)]$ -.

(-)-Ephedrine hydrochloride [50-98-6].

» Ephedrine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of  $C_{10}H_{15}NO \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Ephedrine Sulfate RS

**Identification**—

A: Dissolve 100 mg in 5 mL of water, add 1 mL of potassium carbonate solution (1 in 5), and extract with 2 mL of chloroform: the IR absorption spectrum of the chloroform extract so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ephedrine Sulfate RS.

B: A solution of it responds to the tests for *Chloride* (191).

**Melting range**, *Class I* (741): between  $217^\circ$  and  $220^\circ$ .

**Specific rotation** (781S): between  $-33.0^\circ$  and  $-35.5^\circ$ .

*Test solution*: 50 mg per mL, in water.

**Acidity or alkalinity**—Dissolve 1.0 g in 20 mL of water, and add 1 drop of methyl red TS. If the solution is yellow, it is changed to red by not more than 0.10 mL of 0.020 N sulfuric acid. If the solution is pink, it is changed to yellow by not more than 0.20 mL of 0.020 N sodium hydroxide.

**Loss on drying** (731)—Dry it at  $105^\circ$  for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Sulfate**—Dissolve 50 mg in 40 mL of water, and add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity develops within 10 minutes.

**Ordinary impurities** (466)—

*Test solution*: alcohol.

*Standard solution*: alcohol.

*Eluant*: a mixture of isopropyl alcohol, ammonium hydroxide, and chloroform (80:15:5).

*Visualization*: 1, followed by 4.

**Assay**—Dissolve about 500 mg of Ephedrine Hydrochloride, accurately weighed, in 25 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.17 mg of  $C_{10}H_{15}NO \cdot HCl$ .

## Ephedrine Sulfate

$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$  428.54

Benzenemethanol,  $\alpha$ -[1-(methyamino)ethyl]-,  $[R-(R^*, S^*)]$ -, sulfate (2:1) (salt).

(-)-Ephedrine sulfate (2:1) (salt) [134-72-5].

» Ephedrine Sulfate contains not less than 98.0 percent and not more than 101.0 percent of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Ephedrine Sulfate RS

**Identification**—

A: *Infrared Absorption* (197K).

B: A solution of it responds to the tests for *Sulfate* (191).

**Specific rotation** (781S): between  $-30.5^\circ$  and  $-32.5^\circ$ .

*Test solution*: 50 mg per mL, in water.

**Acidity or alkalinity**—Dissolve 1.0 g in 20 mL of water, and add 1 drop of methyl red TS. If the solution is yellow, it is changed to red by not more than 0.10 mL of 0.020 N sulfuric acid. If the solution is pink, it is changed to yellow by not more than 0.20 mL of 0.020 N sodium hydroxide.

**Loss on drying** (731)—Dry about 500 mg, accurately weighed, at  $105^\circ$  for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—A 200-mg portion shows no more chloride than corresponds to 0.40 mL of 0.020 N hydrochloric acid (0.14%).

**Ordinary impurities** <466>—

*Test solution:* alcohol.

*Standard solution:* alcohol.

*Eluant:* a mixture of isopropyl alcohol, ammonium hydroxide, and chloroform (80:15:5).

*Visualization:* 1, followed by 4.

**Assay**—Transfer about 300 mg of Ephedrine Sulfate, accurately weighed, to a separator, and dissolve in about 10 mL of water. Saturate the solution with sodium chloride (about 3 g), add 5 mL of 1 N sodium hydroxide, and extract with four 25-mL portions of chloroform. Wash the combined chloroform extracts by shaking with 10 mL of a saturated solution of sodium chloride, and filter through chloroform-saturated purified cotton into a beaker. Extract the wash solution with 10 mL of chloroform, and add to the main chloroform extract. Add methyl red TS, and titrate with 0.1 N perchloric acid in dioxane VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.43 mg of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ .

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**Ephedrine Sulfate Capsules**


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» Ephedrine Sulfate Capsules contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Ephedrine Sulfate RS

**Identification**—Macerate the contents of a sufficient number of Capsules, equivalent to about 200 mg of ephedrine sulfate, with 15 mL of warm alcohol for 20 minutes, filter, and evaporate the filtrate on a steam bath to dryness: the residue so obtained responds to the *Identification* tests under *Ephedrine Sulfate*.

**Dissolution** <711>—

*Medium:* water; 500 mL.

*Apparatus 1:* 100 rpm.

*Time:* 30 minutes.

**Procedure**—Dilute filtered portions of the solutions under test with water to a concentration of about 25 µg per mL. Transfer 5.0-mL portions to suitable tubes. Add 1 mL of a saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (2 in 100) to each, mix, and allow to stand for 10 minutes. Add 20.0 mL of hexanes, shake for 30 seconds, and allow the phases to separate. Measure the absorbances of the hexanes extract in 1-cm cells at the wavelength of maximum absorbance, at about 242 nm, with a suitable spectrophotometer, using hexanes as the blank. Determine the amount of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$  dissolved by comparison with a similarly treated Standard solution having a known concentration of USP Ephedrine Sulfate RS in water. Remove the contents of 1 Capsule as completely as possible, with the aid of a current of air, dissolve the empty capsule shell in the *Medium*, determine the absorbance at the same dilution and in the same manner as for the Capsules, and make any necessary corrections.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

**Standard preparation**—Weigh accurately about 25 mg of USP Ephedrine Sulfate RS, transfer to a 50-mL volumetric

flask with the aid of 10 mL of water, add methanol to volume, and mix. Dilute 5.0 mL of this solution with water to 100.0 mL.

**Assay preparation**—Weigh accurately the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the mixture, equivalent to about 25 mg of ephedrine sulfate, to a glass-stoppered conical flask, and add by pipet 50 mL of a 1 in 5 mixture of water in methanol. Shake by mechanical means for 10 minutes, and filter. Dilute 5.0 mL of the filtrate with water to 100.0 mL.

**Procedure**—Transfer 5-mL portions of the *Assay preparation* and the *Standard preparation* to separate glass-stoppered, 50-mL centrifuge tubes. Add 1 mL of saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (1 in 50) to each tube, mix, and allow to stand for 10 minutes. Pipet 20 mL of *n*-hexane into each tube, shake for 30 seconds, and allow the phases to separate. Concomitantly determine the absorbances of the *n*-hexane extracts in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$  in the portion of Capsule contents taken by the formula:

$$C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*, and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the hexane extracts of the *Assay preparation* and the *Standard preparation*, respectively.

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**Ephedrine Sulfate Injection**


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» Ephedrine Sulfate Injection is a sterile solution of Ephedrine Sulfate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose, light-resistant containers, preferably of Type I glass.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Ephedrine Sulfate RS

**Identification**—

**A:** Mix 1 mL of Injection with 5 mL of alcohol, and evaporate on a steam bath with the aid of a current of air to dryness: the residue so obtained responds to *Identification* tests under *Ephedrine Sulfate*.

**B:** It responds to the tests for *Sulfate* <191>.

**Bacterial endotoxins** <85>—It contains not more than 1.7 USP Endotoxin Units per mg of ephedrine sulfate.

**pH** <791>: between 4.5 and 7.0.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 250 mg of ephedrine sulfate, to a separator, add water, if necessary, to make about 10 mL, and proceed as directed in the *Assay* under *Ephedrine Sulfate*, beginning with "Saturate the solution."

## Ephedrine Sulfate Nasal Solution

» Ephedrine Sulfate Nasal Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Ephedrine Sulfate RS

**Identification**—It responds to the *Identification* tests under *Ephedrine Sulfate Injection*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Assay**—

**Standard preparation**—Weigh accurately about 26 mg of USP Ephedrine Sulfate RS, transfer to a 50-mL volumetric flask with the aid of 10 mL of water, add methanol to volume, and mix. Pipet 5 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Nasal Solution, equivalent to about 26 mg of ephedrine sulfate, to a 50-mL volumetric flask, dilute with a 1 in 5 mixture of water in methanol to volume, and mix. Pipet 5 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Transfer 5-mL portions of the *Assay preparation* and the *Standard preparation* to separate glass-stoppered, 50-mL centrifuge tubes. Add 1 mL of saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (1 in 50) to each tube, mix, and allow to stand for 10 minutes. Pipet 20 mL of *n*-hexane into each tube, shake for 30 seconds, and allow the phases to separate. Concomitantly determine the absorbances of the *n*-hexane extracts in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of  $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$  in each mL of the Nasal Solution taken by the formula:

$$(C / V)(A_U / A_S)$$

in which *V* is the volume, in mL, of Nasal Solution taken, *C* is the concentration, in  $\mu\text{g}$  per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*, and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the hexane extracts of the *Assay preparation* and the *Standard preparation*, respectively.

## Ephedrine Sulfate Oral Solution

» Ephedrine Sulfate Oral Solution contains, in each 100 mL, not less than 360 mg and not more than 440 mg of ephedrine sulfate  $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Ephedrine Sulfate RS

**Identification**, *Angular rotation* (781A)—Use the 0.1 N sulfuric acid extract of the chloroform solution obtained as directed for *Assay preparation*: the angular rotation is levorotatory.

**Alcohol content** (611): between 2.0% and 4.0% of  $C_2H_5OH$ .

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ephedrine Sulfate RS in 0.1 N sulfuric acid to obtain a solution having a known concentration of about 20  $\mu\text{g}$  per mL.

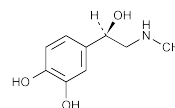
**Assay preparation**—Transfer 5 mL of Oral Solution to a separator, add 1 mL of 1 N sulfuric acid, and extract with 10 mL of chloroform. Discard the extract, and add 5 mL of potassium carbonate solution (1 in 5). After gas evolution has ceased, extract the solution with three 10-mL portions of chloroform, and combine the extracts in a second separator. Extract the chloroform solution with 50.0 mL of 0.1 N sulfuric acid. Filter the acid layer through paper, and dilute 5.0 mL of it with 0.1 N sulfuric acid to 100.0 mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Ephedrine Sulfate Capsules*. Calculate the quantity, in mg, of ephedrine sulfate  $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$  in the portion of Oral Solution taken by the formula:

$$C(A_U / A_S)$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Epinephrine



$C_9H_{13}NO_3$  183.20  
1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (*R*)-; (-)-3,4-Dihydroxy- $\alpha$ -[(methylamino)methyl]benzyl alcohol [51-43-4].

### DEFINITION

Epinephrine contains NLT 97.0% and NMT 100.5% of  $C_9H_{13}NO_3$ , calculated on the dried basis.

### IDENTIFICATION

- A.** To 5 mL of pH 4.0 acid phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) add 0.5 mL of a slightly acid solution of Epinephrine (1 in 1000) and 1.0 mL of 0.1 N iodine. Mix, and allow to stand for 5 min. Add 2 mL of sodium thiosulfate solution (1 in 40). **Acceptance criteria:** A deep red color is produced.

### ASSAY

#### PROCEDURE

**Sample:** 300 mg

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid, warming slightly if needed to dissolve. Add crystal violet TS to the *Sample*, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.32 mg of epinephrine ( $C_9H_{13}NO_3$ ).



Acceptance criteria: 97.0%–100.5% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): Negligible, from 100 mg
- **LIMIT OF ADRENALONE**

**Sample solution:** 2 mg/mL of Epinephrine in dilute hydrochloric acid (1 in 200)

**Analysis:** Determine the absorptivity of the *Sample solution* at 310 nm (see *Spectrophotometry and Light-Scattering* (851)).

Acceptance criteria: NMT 0.2

- **LIMIT OF NOREPINEPHRINE**

**Standard stock solution A:** 364 mg/mL of USP Epinephrine Bitartrate RS in formic acid

**Standard solution A:** 20 mg/mL of epinephrine in methanol, from *Standard stock solution A*

**Standard stock solution B:** 16 mg/mL of USP Norepinephrine Bitartrate RS in formic acid

**Standard solution B:** 1.6 mg/mL of USP Norepinephrine Bitartrate RS in methanol from *Standard stock solution B*

**Sample solution:** 20 mg/mL of Epinephrine. Dissolve 200 mg of Epinephrine in 1.0 mL of formic acid, and dilute with methanol to 10.0 mL.

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** *n*-Butanol, water, and formic acid (7:2:1)

**Spray reagent:** Folin-Ciocalteu phenol TS

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

In an unsaturated chamber, develop the plate in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate in warm circulating air. Spray with *Spray reagent*, followed by sodium carbonate solution (1 in 10).

**Acceptance criteria:** The  $R_f$  value of the principal spot from the *Sample solution* corresponds to that of *Standard solution A*. Any spot from the *Sample solution* is not larger or more intense than the spot with the same  $R_f$  value from *Standard solution B*, corresponding to NMT 4.0% norepinephrine.

#### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* (781S):  $-50.0^\circ$  to  $-54.0^\circ$   
**Sample solution:** 20 mg/mL, in 0.6 N hydrochloric acid
- **LOSS ON DRYING** (731): Dry it in a vacuum over silica gel for 18 h: it loses NMT 2.0% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Epinephrine Bitartrate RS  
USP Norepinephrine Bitartrate RS

not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

#### USP Reference standards (11)—

USP Epinephrine Bitartrate RS

**Identification**—Place 10 mL of water in a small beaker, and deliver 2 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To 5 mL of the solution add 1 drop of dilute sulfuric acid (1 in 200), add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

**Delivered dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

#### PROCEDURE FOR DOSE UNIFORMITY—

*Ferro-citrate solution* and *Buffer solution*—Prepare as directed under *Epinephrine Assay* (391).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 18  $\mu$ g per mL.

*Test preparation*—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

*Procedure*—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100  $\mu$ L of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in  $\mu$ g, of  $C_9H_{13}NO_3$  contained in the minimum dose taken by the formula:

$$(183.20 / 333.29)(20CN)(A_U / A_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; *N* is the number of sprays discharged to obtain the minimum recommended dose; 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay**—Weigh the Inhalation Aerosol, chill to a temperature below  $-30^\circ$ , remove the valve by suitable means, and allow the Inhalation Aerosol to warm slowly to room temperature to expel the more volatile propellant fractions. Transfer the residues in the aerosol container and valve to a 125-mL separator with the aid of six 5-mL portions of dilute sulfuric acid (1 in 1000), and extract the solution with three 25-mL portions of chloroform. Proceed as directed in the *Assay* under *Epinephrine Nasal Solution*, beginning with "Rinse the stopper and mouth of the separator," but use 10.0 mL instead of 5.0 mL of chloroform in the determination of the specific rotation. Dry the empty aerosol container and valve, weigh them, and determine the net weight of the Inhalation

## Epinephrine Inhalation Aerosol

» Epinephrine Inhalation Aerosol is a solution of Epinephrine in propellants and Alcohol prepared with the aid of mineral acid in a pressurized container. It contains not less than 90.0 percent and

Aerosol. Calculate the quantity, in mg, of  $C_9H_{13}NO_3$  in the Inhalation Aerosol taken by the formula:

$$(183.20 / 309.32)(W)(0.5 + 0.5R / 93)$$

in which 183.20 and 309.32 are the molecular weights of epinephrine and triacetylepinephrine, respectively, and  $W$  is the weight, in mg, and  $R$  is the specific rotation (in degrees, without regard to the sign), of the isolated triacetylepinephrine.

## Epinephrine Injection

» Epinephrine Injection is a sterile solution of Epinephrine in Water for Injection prepared with the aid of Hydrochloric Acid or other suitable buffers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I glass.

**Labeling**—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Epinephrine Bitartrate RS

**Color and clarity**—

**Standard solution**—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Visually examine a portion of the Injection (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

**Identification**—

**A:** It responds to the *Identification* test under *Epinephrine Nasal Solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 357.0 USP Endotoxin Units per mg of epinephrine.

**pH** (791): between 2.2 and 5.0.

**Total acidity**—Transfer 5.0 mL of Injection to a flask, add 10 mL of water, and titrate with 0.01 N sodium hydroxide VS to a pH of 7.40. Perform a blank determination, and make any necessary correction. Not more than 25.0 mL of 0.01 N sodium hydroxide is required.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—To 1 L of 0.05 M monobasic sodium phosphate add about 519 mg of sodium 1-octanesulfonate and about 45 mg of edetate disodium, and mix. Adjust by the dropwise addition of phosphoric acid, if necessary, to a pH of 3.8. Mix 85 volumes of this solution with 15 volumes of methanol. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg of epinephrine per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of epinephrine, to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability preparation**—Dissolve 10 mg of dopamine hydrochloride in 100 mL of the *Standard preparation*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for epinephrine and 2.0 for dopamine hydrochloride; the resolution,  $R$ , between epinephrine and dopamine hydrochloride is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of epinephrine ( $C_9H_{13}NO_3$ ) in each mL of the Injection taken by the formula:

$$(183.20/333.29)(10)(C/V)(r_U / r_S)$$

in which 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively;  $C$  is the concentration, in mg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Epinephrine Inhalation Solution

» Epinephrine Inhalation Solution is a sterile solution of Epinephrine in Purified Water prepared with the aid of Hydrochloric Acid. It contains, in each 100 mL, not less than 0.9 g and not more than 1.15 g of  $C_9H_{13}NO_3$ .

**Packaging and storage**—Preserve in small, well-filled, tight, light-resistant containers.

**Labeling**—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**Color and clarity**—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

**Identification**—It meets the requirements for the *Identification* test under *Epinephrine Nasal Solution*.

**Sterility** (71): meets the requirements.

**Assay**—Pipet 10 mL of Inhalation Solution into a 125-mL separator, and extract the solution with two 10-mL portions of chloroform. Proceed as directed in the *Assay* under *Epinephrine Nasal Solution*, beginning with "Rinse the stopper and mouth of the separator," but use for the acetylation 1.05 g of sodium bicarbonate and 0.50 mL of acetic anhydride, and extract the acetylated product with six 15-mL portions of chloroform instead of the 25-mL portions speci-

fied therein, and use 15.0 mL of chloroform instead of 5.0 mL in the determination of the specific rotation.

## Epinephrine Nasal Solution

» Epinephrine Nasal Solution is a solution of Epinephrine in Purified Water prepared with the aid of Hydrochloric Acid. It contains, in each 100 mL, not less than 90 mg and not more than 115 mg of  $C_9H_{13}NO_3$ .

**Packaging and storage**—Preserve in small, well-filled, tight, light-resistant containers.

**Labeling**—The label indicates that the Nasal Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**Color and clarity**—Using the Nasal Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

**Identification**—To 5 mL of pH 4.0 acid phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) add 0.5 mL of Nasal Solution and 1.0 mL of 0.1 N iodine. Mix, and allow to stand for 5 minutes. Add 2 mL of sodium thiosulfate solution (1 in 40): a deep red color is produced.

**Assay**—Pipet 30 mL of Nasal Solution into a 125-mL separator, add 25 mL of chloroform, shake vigorously for 1 minute, allow the liquids to separate, and discard the chloroform. Wash twice more with chloroform, separating and discarding the lower layer as completely as possible each time. Rinse the stopper and mouth of the separator with a few drops of water. Add 0.2 mL of starch TS, then while swirling the separator add iodine and potassium iodide TS 1 dropwise until the blue color formed persists, and immediately add just sufficient 0.1 N sodium thiosulfate to discharge the blue color. [NOTE—Proceed with the assay from this point without delay.]

Add to the liquid in the separator 2.10 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe that is not fitted with a needle, rapidly inject 1.0 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with six 25-mL portions of chloroform, filtering each extract through a small pledget of cotton, previously washed with chloroform, into a beaker.

Evaporate the combined chloroform extracts on a steam bath in a current of air to about 3 mL, transfer the residue by means of small portions of chloroform to a tared 50-mL beaker, and heat again to evaporate the solvent completely. Heat further at  $105^\circ$  for 30 minutes, cool in a desiccator, and weigh the residue of triacetylepinephrine. Add 5.0 mL of chloroform, cover the beaker, gently swirl the contents until the residue has completely dissolved, and determine the specific rotation,  $R$ , using a 200-mm semimicro polarimeter tube.

Calculate the quantity, in mg, of  $C_9H_{13}NO_3$  in the volume of Nasal Solution taken by the formula:

$$(183.20/309.32)(W)(0.5 + 0.5R / 93)$$

in which 183.20 and 309.32 are the molecular weights of epinephrine and triacetylepinephrine, respectively; and  $W$  is the weight, in mg, and  $R$  is the specific rotation (in degrees, without regard to the sign), of the isolated triacetylepinephrine.

## Epinephrine Ophthalmic Solution

» Epinephrine Ophthalmic Solution is a sterile, aqueous solution of Epinephrine prepared with the aid of Hydrochloric Acid. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_9H_{13}NO_3$ . It contains a suitable antibacterial agent and may contain an anti-oxidant, suitable buffers, and chelating and tonicity-adjusting agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—

USP Epinephrine Bitartrate RS

**Color and clarity**—Using the Ophthalmic Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

**Identification**—

**A:** The UV absorption spectrum of the *Assay preparation* prepared as directed in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Epinephrine Bitartrate RS.

**B:** A solution (1 in 2) is levorotatory.

**Sterility** (71): meets the requirements.

**pH** (791): between 2.2 and 4.5.

**Assay**—

**pH 5.8 Buffer**—Mix 1 volume of 1 M dibasic potassium phosphate with 9 volumes of 1 M monobasic potassium phosphate. Adjust by the addition of small volumes of either solution to a pH of  $5.80 \pm 0.05$ .

**Standard preparation**—Dissolve a suitable quantity of USP Epinephrine Bitartrate RS, accurately weighed, in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 40  $\mu$ g of epinephrine per mL.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of epinephrine, to a 250-mL beaker containing 2.0 mL of pH 5.8 Buffer. Add 9 g of chromatographic siliceous earth, and mix. Carefully transfer the mixture to a 45- $\times$  2.2-cm chromatographic tube containing a pledget of glass wool at the bottom, and tap the column gently to effect packing. Dry-wash the beaker with about 1 g of chromatographic siliceous earth, add to the column, and plug the top with a pledget of glass wool. Wash the column with 100 mL of water-washed ether, and discard the eluant. Add 10.0 mL of 0.1 N hydrochloric acid to a 125-mL separator, and place the separator under the column. To about 100 mL of water-washed ether add 1 mL of bis(2-ethylhexyl) phosphoric acid, and elute the column with this solution, collecting the eluate in the separator. Extract the epinephrine into the aqueous acid layer, and carefully transfer the aqueous layer to a 500-mL volumetric flask. Shake the ether layer with two 50-mL portions of 0.1 N hydrochloric acid, add the acidic aqueous extracts to the volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid

as the blank. Calculate the quantity, in mg, of  $C_9H_{13}NO_3$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C/V)(A_U/A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of epinephrine in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Epinephrine Bitartrate

$C_9H_{13}NO_3 \cdot C_4H_6O_6$  333.29

1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (R)-, [R-(R\*,R\*)]-2,3-dihydroxybutanedioate (1:1) (salt).  
(-)-3,4-Dihydroxy- $\alpha$ -[(methylamino)methyl]benzyl alcohol  
(+)-tartrate (1:1) salt [51-42-3].

» Epinephrine Bitartrate contains not less than 97.0 percent and not more than 102.0 percent of  $C_9H_{13}NO_3 \cdot C_4H_6O_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Epinephrine Bitartrate RS

USP Norepinephrine Bitartrate RS

**Identification**—Dissolve about 500 mg in 20 mL of water containing about 100 mg of sodium bisulfite. Add 6 N ammonium hydroxide until the solution has a distinct odor of ammonia, and allow to stand in a refrigerator for 1 hour. Filter the precipitate, wash it with three 2-mL portions of cold water, then with 5 mL of cold alcohol, and finally with 5 mL of cold ether, and dry in vacuum over silica gel for 3 hours. The epinephrine so obtained responds to the *Identification* test under *Epinephrine*, and its specific rotation (see *Optical Rotation* (781)), determined by dissolving 200 mg, accurately weighed, in sufficient dilute hydrochloric acid (1 in 20) to make 10.0 mL, is between  $-50^\circ$  and  $-53.5^\circ$ .

**Melting range** (741): between  $147^\circ$  and  $152^\circ$ , with decomposition.

**Loss on drying** (731)—Dry it in vacuum over silica gel for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): negligible, from 100 mg.

**Limit of adrenalone**—Its absorptivity (see *Spectrophotometry and Light-scattering* (851)) at 310 nm, determined in a solution in dilute hydrochloric acid (1 in 200) containing 4 mg per mL, is not more than 0.2.

**Limit of norepinephrine bitartrate**—

*Epinephrine standard solution*—Dilute with methanol an accurately measured volume of an aqueous solution of USP Epinephrine Bitartrate RS containing about 200 mg per mL to obtain a solution having a known concentration of about 20 mg per mL.

*Norepinephrine standard solution*—Dilute with methanol an accurately measured volume of an aqueous solution of USP Norepinephrine Bitartrate RS containing 8.0 mg per mL to obtain a solution having a known concentration of 0.80 mg per mL.

*Test solution*—Dissolve 200 mg of Epinephrine Bitartrate in 1.0 mL of water, dilute with methanol to 10.0 mL, and mix.

*Procedure*—Apply 5- $\mu\text{L}$  portions of *Epinephrine standard solution*, *Norepinephrine standard solution*, and *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in an unsaturated tank using a

solvent system consisting of *n*-butanol, water, and formic acid (7:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm circulating air. Spray with Folin-Ciocalteu Phenol TS, followed by sodium carbonate solution (1 to 10): the  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Epinephrine standard solution*. Any spot obtained from the *Test solution* is not larger nor more intense than the spot with the same  $R_f$  value obtained from *Norepinephrine standard solution*, corresponding to not more than 4.0% of norepinephrine bitartrate.

**Assay**—Dissolve about 500 mg of Epinephrine Bitartrate, accurately weighed, in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.33 mg of  $C_9H_{13}NO_3 \cdot C_4H_6O_6$ .

## Epinephrine Bitartrate Inhalation Aerosol

» Epinephrine Bitartrate Inhalation Aerosol is a suspension of microfine Epinephrine Bitartrate in propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of epinephrine bitartrate ( $C_9H_{13}NO_3 \cdot C_4H_6O_6$ ).

**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

**USP Reference standards** (11)—

USP Epinephrine Bitartrate RS

**Identification**—

**A:** Place 10 mL of water in a small beaker, and deliver 3 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. Filter, and to 5 mL of the filtrate add 1 drop of dilute hydrochloric acid (1 in 120). Add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

**B:** Actuate the valve of the Aerosol by pressing the tip against a station of a white porcelain spot plate. Cover the spot with 2 or 3 drops of a mixture of 3 volumes of pyridine and 1 volume of acetic anhydride: an emerald-green color is produced.

**Delivered dose uniformity over the entire contents:**

meets the requirements for *Metered-Dose Inhalers under Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

PROCEDURE FOR DOSE UNIFORMITY—

*Ferro-citrate solution* and *Buffer solution*—Prepare as directed under *Epinephrine Assay* (391).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 15  $\mu\text{g}$  per mL.

*Test preparation*—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform,

insert the stopper, shake vigorously for 1 minute and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

**Procedure**—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100  $\mu$ L of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in  $\mu$ g of  $C_9H_{13}NO_3 \cdot C_4H_6O_6$  contained in the minimum dose taken by the formula:

$$(20CN)(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Particle size**—Proceed with Epinephrine Bitartrate Inhalation Aerosol as directed in the test for *Particle size* under *Isoproterenol Sulfate Inhalation Aerosol*. It meets the limits of the test.

#### Assay—

*Ferro-citrate solution* and *Buffer solution*—Prepare as directed under *Epinephrine Assay* <391>.

*Standard preparation*—Prepare as directed under *Delivered dose uniformity over the entire contents*.

*Assay preparation*—[NOTE—A suitable specimen beaker is one having a small indentation formed on its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the delivery of the specimen.] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of Epinephrine Bitartrate Inhalation Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the chloroform, and shake it gently preparatory to delivering another spray similarly under the surface of the chloroform. Deliver a total of 3 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the specimen in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 3 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500). Insert the stopper, shake vigorously for 1 minute, centrifuge for 5 minutes, and use the clear supernatant as the *Assay preparation*.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 100  $\mu$ L of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_9H_{13}NO_3 \cdot C_4H_6O_6$  in each mL of the Aerosol taken by the formula:

$$(0.01Cd / W)(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, d is the density, in g per mL, of the Aerosol, determined as directed for d in the *Procedure* in the Assay under *Isoproterenol Sulfate*

*Inhalation Aerosol*, W is the weight, in g, of the specimen taken, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Epinephrine Bitartrate Ophthalmic Solution

» Epinephrine Bitartrate Ophthalmic Solution is a sterile, buffered, aqueous solution of Epinephrine Bitartrate. It contains an amount of epinephrine bitartrate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ). It contains a suitable antibacterial agent and may contain suitable preservatives.

**Packaging and storage**—Preserve in small, well-filled, tight, light-resistant containers.

**Labeling**—The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** <11>—

USP Epinephrine Bitartrate RS

**Color and clarity**—Using the Ophthalmic Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

**pH** <791>: between 3.0 and 3.8.

**Other requirements**—It responds to the *Identification* test under *Epinephrine Nasal Solution*, and meets the requirements under *Sterility Tests* <71>.

#### Assay—

*pH 2.5 Buffer*—Transfer 6.8 g of monobasic potassium phosphate and 1.1 g of sodium 1-octanesulfonate to a 1-liter volumetric flask. Dissolve in water, dilute with water to volume, and mix. Adjust the solution with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 2.5 Buffer* and acetonitrile (850:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, freshly mixed and free from air bubbles, equivalent to about 50 mg of epinephrine bitartrate, to a 500-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.2-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2000 theoretical plates, the tailing factor for the analyte peak is not more than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of epinephrine ( $C_9H_{13}NO_3$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$(183.20 / 333.29)(500C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, V is the volume, in mL, of Ophthalmic Solution taken, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Epinephrine Bitartrate for Ophthalmic Solution

» Epinephrine Bitartrate for Ophthalmic Solution is a sterile, dry mixture of Epinephrine Bitartrate and suitable antioxidants, prepared by freeze-drying. It contains an amount of epinephrine bitartrate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

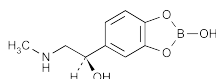
**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**Completeness of solution** (641)—A 100-mg portion dissolves in 5 mL of water to yield a clear solution.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Other requirements**—A solution of it responds to the *Identification* test under *Epinephrine Nasal Solution*, and meets the requirements of the *Assay* under *Epinephrine Bitartrate Ophthalmic Solution*. It meets also the requirements under *Sterility Tests* (71) and *Uniformity of Dosage Units* (905).

## Epinephryl Borate Ophthalmic Solution



$C_9H_{12}BNO_4$  209.01

1,3,2-Benzodioxaborole-5-methanol, 2-hydroxy- $\alpha$ -[(methylamino)methyl]-, (R)-.

(-)-3,4-Dihydroxy- $\alpha$ -[(methylamino)methyl]benzyl alcohol, cyclic 3,4-ester with boric acid [5579-16-8].

» Epinephryl Borate Ophthalmic Solution is a sterile solution in water of Epinephrine as a borate complex. It contains an amount of epinephryl borate ( $C_9H_{12}BNO_4$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ). It contains a suitable antibacterial agent and one or more suitable preservatives and buffering agents.

**Packaging and storage**—Preserve in small, well-filled, tight, light-resistant containers.

**Labeling**—The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

### Color and clarity—

**Standard solution**—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Visually examine a portion of the Ophthalmic Solution (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish, and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

### Identification—

**A:** To 5 mL of pH 4.0 acid phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) add 0.5 mL of Ophthalmic Solution and 1 mL of 0.1 N iodine. Mix, allow to stand for 5 minutes, and add 2 mL of 0.1 N sodium thiosulfate: a deep red color is produced.

**B:** To 5 mL in a porcelain evaporating dish add 5 drops of sulfuric acid and 5 mL of methanol: the ignited mixture burns with a green-bordered flame.

**Sterility** (71): meets the requirements.

**pH** (791): between 5.5 and 7.6.

**Assay**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 100 mg of epinephrine, to a 250-mL separator. Dilute with water to 30 mL, and adjust with dilute hydrochloric acid (1 in 12) to a pH of  $4.0 \pm 0.2$ . Add 25 mL of carbon tetrachloride, shake vigorously for 1 minute, allow the phases to separate, and discard the carbon tetrachloride washing. In the same manner, wash with two additional 25-mL portions of carbon tetrachloride, and discard the washings. Rinse the stopper and the mouth of the separator with 2 to 3 mL of water such that the rinsings enter the separator and combine with the solution under assay. Add 0.2 mL of starch TS, and, while swirling the separator, add iodine and potassium iodide TS 1 dropwise until the blue color persists. Immediately add a volume of 0.1 N sodium thiosulfate just sufficient to discharge the blue color. [NOTE—Proceed with the assay from this point without delay.]

Add 2.10 g of sodium bicarbonate through a dry powder funnel to prevent the powder from coming in contact with the mouth of the separator, and swirl to dissolve most of the solid. By means of a syringe fitted with a suitable pipet, rapidly inject 1.0 mL of acetic anhydride directly into the contents of the separator. Swirl the unstoppered separator gently for 3 minutes to allow carbon dioxide to escape. Insert the stopper, and shake gently until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure through the stopcock as necessary. Allow to stand for 5 minutes. Extract with six 25-mL portions of chloroform, shaking for 1 minute each time, filtering each extract through a small pledget of chloroform-saturated cotton and collecting the extracts in a 400-mL beaker. Add several glass beads, and evaporate on a steam bath to about 3 mL. With the aid of 15 to 20 mL of chloroform, transfer the residue to a tared 50-mL beaker, and evaporate on the steam bath to dryness. Dry the residue at  $105^\circ$  for 30 minutes, cool in a desiccator, and weigh the triacetylepinephrine so obtained. Transfer 10.0 mL of chloroform to the beaker, and gently swirl to dissolve the residue, dislodging the semisolid residue from the glass surface, if necessary, with a small metal spatula. Determine the angular rotation of the solution in a 100-mm polarimeter tube.

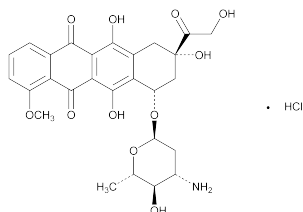
Calculate the quantity, in mg, of epinephrine ( $C_9H_{13}NO_3$ ) in the volume of Ophthalmic Solution taken by the formula:

$$(183.20/309.32)(W)(0.5 + 0.5R/93)$$

in which 183.20 and 309.32 are the molecular weights of epinephrine and triacetylepinephrine, respectively; W is the weight, in mg, of the isolated triacetylepinephrine; and R is

the specific rotation, in degrees, of the triacetylepinephrine solution.

## Epirubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$  579.98  
 5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy- $\alpha$ -L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-, hydrochloride, (8*S*-*cis*)-;  
 (1*S*,3*S*)-3-Glycoloyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl-3-amino-2,3,6-trideoxy- $\alpha$ -L-arabino-hexopyranoside hydrochloride. [56390-09-1].

### DEFINITION

Epirubicin Hydrochloride contains NLT 97.0% and NMT 102.0% of  $C_{27}H_{29}NO_{11} \cdot HCl$ , calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** <197M>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Chloride** <191>: A 10 mg/mL solution in a mixture of nitric acid and water (1:1) meets the requirements.

### ASSAY

#### PROCEDURE

[NOTE—Allow the *System suitability solution*, *Standard solution*, and the *Sample solution* to stand for 3 h before use.]

**Solution A:** Dissolve 3.7 g of sodium lauryl sulfate in 950 mL of water. To the resulting solution, add 28 mL of 10% phosphoric acid, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (29:17:54)

**System suitability solution:** 0.1 mg/mL each of USP Epirubicin Hydrochloride RS and USP Doxorubicin Hydrochloride RS in *Mobile phase*

**Standard solution:** 1 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Epirubicin Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 6- $\mu$ m packing L13

**Temperature:** 35°

**Flow rate:** 2.5 mL/min

**Injection size:** 10  $\mu$ L

**Run time:** About 3.5 times the retention time of the epirubicin peak

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between doxorubicin and epirubicin

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{27}H_{29}NO_{11} \cdot HCl$  in the portion of Epirubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Organic Impurities

##### PROCEDURE 1

[NOTE—Allow the *System suitability solution*, *Sample solution*, and *Standard solution* to stand for 3 h before use.]

**Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 0.01 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*

**Peak identification solution:** Dissolve 10 mg of USP Doxorubicin Hydrochloride RS in 10 mL of a mixture of water and phosphoric acid (1:1). Allow to stand for 30 min. Adjust with 2 N sodium hydroxide solution to a pH of 2.6. Add 15 mL of acetonitrile and 10 mL of methanol, and mix.

#### Analysis

**Samples:** *Sample solution*, *Standard solution*, and *Peak identification solution*

[NOTE—Use the *Peak identification solution* to identify peaks using the relative retention times in *Impurity Table 1*.]

Calculate the percentage of each impurity in the portion of Epirubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity peak from the *Sample solution*

$r_S$  = peak response of the epirubicin peak from the *Standard solution*

$C_S$  = concentration of USP Epirubicin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Epirubicin Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 3.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxorubicinone	0.3	1.4	1.0
Daunorubicinone	0.4	1.0	0.5
Doxorubicin	0.8	1.0	1.0
Epirubicin	1.0	—	—
Dihydrodaunorubicin	1.1	1.0	0.5
Daunorubicin	1.5	1.0	0.5
Epidaunorubicin	1.7	1.0	1.0
Epirubicin dimer	2.1	1.0	1.0
Individual unspecified impurity	—	1.0	0.5

• **PROCEDURE 2: LIMIT OF ACETONE**

Analysis: See *Residual Solvents* (467).

Acceptance criteria: NMT 1.5%

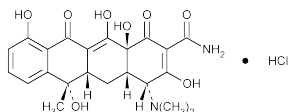
**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method 1c* (921): NMT 4.0%
- **PH** (791): 4.0–5.5 for a 5 mg/mL solution
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 1.1 USP Endotoxin Units/mg, where the label states that Epirubicin Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store in airtight containers, protected from light, at a temperature between 2° and 8°. If the substance is sterile, store in a sterile, airtight, tamper-proof container.
- **LABELING:** Where applicable, the label states that the substance is free from bacterial endotoxins.
- **USP REFERENCE STANDARDS** (11)
  - USP Endotoxin RS
  - USP Doxorubicin Hydrochloride RS
  - USP Epirubicin Hydrochloride RS

## Epitetracycline Hydrochloride



$C_{22}H_{24}N_2O_8 \cdot HCl$  480.90

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4R-(4 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,6 $\alpha$ ,12 $\alpha$ )]-

(4R,4aS,5aS,6S,12aS) 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [23313-80-6].

» Epitetracycline Hydrochloride contains not less than 70.0 percent of  $C_{22}H_{24}N_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Tetracycline Hydrochloride RS

**pH** (791): between 2.3 and 4.0, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

**4-Epianhydrotetracycline** (226)—Dissolve about 250 mg, accurately weighed, in 10 mL of 0.1 N hydrochloric acid, and adjust with 6 N ammonium hydroxide to a pH of 7.8. Transfer this solution with the aid of *EDTA buffer* to a 50-mL volumetric flask, dilute with *EDTA buffer* to volume, and mix. Use this solution, without delay, as the *Test solution*: not more than 2.0% is found.

**Assay**—

*Edetate disodium solution*, *Stationary phase*, *Alkaline methanol solution*, *Column support*, *Chromatographic column*, and *Standard preparation*—Prepare as directed in the section *Epitetracycline hydrochloride content and Assay for tetracycline hydrochloride* under *Tetracycline Hydrochloride for Topical Solution*.

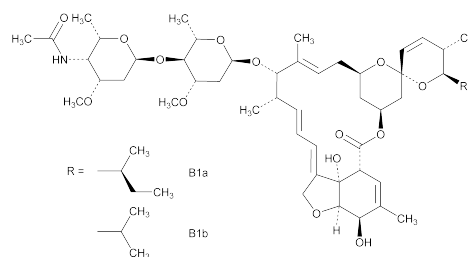
**Assay preparation**—Transfer about 22 mg of Epitetracycline Hydrochloride, accurately weighed, to a 25-mL volumetric flask, add 1 mL of methanol, and swirl to dissolve. Dilute with *Stationary phase* to volume, and mix. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, dilute with *Stationary phase* to volume, and mix. Pipet 2.0 mL of this solution into the *Chromatographic column*, and allow it to penetrate the *Column support*. Add 20 mL of benzene to the solvent reservoir, and collect the eluate at the rate of about 1 mL per minute. When the benzene level reaches the top of the *Column support*, add 60 mL of chloroform to the solvent reservoir, and continue collecting eluate. When the chloroform level reaches the top of the *Column support*, discard the collected eluate, add 50 mL of a mixture of butyl alcohol and methanol (1:1), and collect 8 mL of eluate in a 10-mL graduated cylinder. Replace the 10-mL graduated cylinder with a low-actinic 50-mL volumetric flask, and continue collecting eluate until the column runs dry. The eluate in the 50-mL volumetric flask is the *Assay preparation*.

**Procedure**—Add 2.0 mL of *Alkaline methanol solution* to the *Standard preparation* and to the *Assay preparation*, dilute each with chloroform to volume, and mix. Concomitantly, within 10 minutes of preparation, determine the absorbances of these solutions at the wavelength of maximum absorbance at about 366 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of  $C_{22}H_{24}N_2O_8 \cdot HCl$  in the Epitetracycline Hydrochloride taken by the formula:

$$0.001(WP)(A_U / A_S)$$

in which *W* is the weight, in mg, of USP Tetracycline Hydrochloride RS taken, *P* is the potency, in  $\mu$ g per mg, of the USP Tetracycline Hydrochloride RS, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Eprinomectin



$C_{50}H_{75}NO_{14}$  (Component B<sub>1a</sub>) 914.13.

$C_{49}H_{73}NO_{14}$  (Component B<sub>1b</sub>) 900.10.

Component B<sub>1a</sub>

Avermectin A<sub>1a</sub>, 4''-(acetylamino)-5-O-demethyl-4''-deoxy-, (4''R)-.

(2aE,4E,5'S,6S,6'R,7S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-6'-(S)-sec-Butyl-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-7-yl-4-O-(4-acetamido-2,4,6-trideoxy-3-O-methyl- $\alpha$ -L-lyxohexopyranosyl)-2,6-dideoxy-3-O-methyl- $\alpha$ -L-arabinohexopyranoside (or (4''R)-4''-(acetylamino)-5-O-demethyl-4''-deoxyavermectin A<sub>1a</sub>) [133305-88-1].

Component B<sub>1b</sub>

Avermectin A<sub>1a</sub>, 4''-(acetylamino)-5-O-demethyl-25-de(1-methylpropyl)-4''-deoxy-25-(1-methylethyl)-, (4''R)-.

(2aE,4E,5'S,6S,6'R,7S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6'-isopropyl-5',6,8,19-tetramethyl-



17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl- $\alpha$ -*L*-lyxo-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl- $\alpha$ -*L*-arabino-hexopyranoside (or (4''*R*)-4''-(acetylamino)-5-*O*-demethyl-25-de(1-methyl-propyl)-4''-deoxy-25-(1-methylethyl) avermectin A<sub>1a</sub>) [133305-89-2].

» Eprinomectin is a mixture of Component B<sub>1a</sub> and Component B<sub>1b</sub>. It contains not less than 90.0 percent of Component B<sub>1a</sub> and not less than 95.0 percent of Components B<sub>1a</sub> and B<sub>1b</sub>, calculated on the anhydrous, solvent-free, and antioxidant-free basis. It may contain small amounts of a suitable antioxidant.

**Packaging and storage**—Preserve in tight containers, and store between 2° and 8° at ambient humidity.

**Labeling**—Label it to state the name(s) and amount(s) of any added substance(s). Label to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Eprinomectin RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** The retention times of the Component B<sub>1a</sub> peak and the Component B<sub>1b</sub> peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between +132° and +140°, determined at 405 nm on the anhydrous, solvent-free, and antioxidant-free basis.

*Test solution:* 5 mg per mL, in chloroform.

**Water, Method Ia** (921): not more than 2.0%, determined on 0.250 g.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 10 ppm.

**Limit of residual solvents**—

*Standard solution I*—Transfer 3.0 mL of acetonitrile, 3.0 mL of methanol, 3.0 mL of isopropyl acetate, and 3.0 mL of heptane to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume, and mix well.

*Standard solution II*—Transfer 1.0 mL of *Standard solution I* to a 100-mL volumetric flask, dilute with dimethylacetamide to volume, and mix well. Further dilute 10.0 mL of this solution with dimethylacetamide to 50.0 mL, and mix well.

*Test solution*—Transfer 1 g of Eprinomectin in dimethylacetamide to a 10-mL volumetric flask. Dilute with dimethylacetamide to volume, and mix well.

*Sensitivity solution I*—Transfer 3.0 mL each of methanol, isopropyl acetate, and heptane to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume, and mix well. Further dilute 50  $\mu$ L of this solution with dimethylacetamide to 25 mL, and mix well.

*Sensitivity solution II*—Transfer 3.0 mL of acetonitrile to a 50-mL volumetric flask, dilute with dimethylacetamide to volume, and mix well. Further dilute 50  $\mu$ L of this solution with dimethylacetamide to 25 mL.

*Sensitivity solution III*—Transfer 5.0 mL of *Sensitivity solution I* and 1.0 mL of *Sensitivity solution II* to a 50-mL volumetric flask, dilute with dimethylacetamide to volume, and mix well. [NOTE—This solution contains 100 ppm (m/m) of methanol, isopropyl acetate, and heptane and 20 ppm (m/m) of acetonitrile.]

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm  $\times$  25-m fused-silica analytical column coated with a 20- $\mu$ m S3 stationary phase. The carrier gas is

helium with a flow rate of 20 mL per minute. The chromatograph is programmed as follows: the column temperature is increased from 110° at a rate of 5° per minute to 160° and maintained at 160° for 5 minutes. The column temperature is then increased at a rate of 30° per minute to 220° and maintained at 220° for 25 minutes. The injection port temperature is maintained at 200°, and the detector temperature is maintained at 220°. Chromatograph *Sensitivity solution III* and *Standard solution II* as directed for *Procedure*: in the chromatogram obtained from *Sensitivity solution III*, the peaks for methanol, acetonitrile, isopropyl acetate, and heptane are detectable and elute at relative retention times of 1, 2.1, 7.6, and 8.6, respectively. In the chromatogram obtained from *Standard solution II*, the relative standard deviations for the areas of the solvent peaks are not more than 5.0% for six injections.

*Procedure*—Separately inject equal volumes (about 1  $\mu$ L) of *Standard solution II* and the *Test solution*. Reinject *Standard solution II* in duplicate after every six sample injections. The individual values for the area response of the two injections agree within  $\pm 5\%$  of their corresponding average response. Calculate the percentage of each solvent present using the following formula:

$$0.12D(r_U / r_S)$$

in which *D* is the density, in mg per mL, of acetonitrile (0.787), isopropyl acetate (0.870), methanol (0.796), and heptane (0.684); *r<sub>U</sub>* is the solvent peak area in the chromatogram obtained from the *Test solution*; and *r<sub>S</sub>* is the solvent peak area in the chromatogram obtained from *Standard solution II*. Not more than 0.005% of acetonitrile is found, and the sum of all solvents is not more than 0.5%.

**Limit of 8a-oxo-B<sub>1a</sub>**—

*Solution A, Solution B, Diluent, and System suitability solution*—Proceed as directed in the *Assay*.

*System suitability mobile phase*—Use the *Mobile phase* as directed in the *Assay*.

*Mobile phase*—Use a mixture of acetonitrile and *Solution A* (13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Butylated hydroxytoluene solution*—Transfer 50 mg of butylated hydroxytoluene to a 100-mL volumetric flask, and dilute with methanol to volume. Sonicate, if necessary, and mix well. Dilute 2 mL of the resulting solution with *Diluent* to 100 mL.

*System suitability determination*—Use the conditions as directed for *Chromatographic system* in the *Assay*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L7. The flow rate is 1.5 mL per minute, and the column temperature is 40°. Chromatograph the *Butylated hydroxytoluene solution* and the *Test solution*, and record the peak responses as directed for *Procedure*: the retention time for the peak corresponding to butylated hydroxytoluene is approximately 12 to 17 minutes, and the relative standard deviation of the peak area is not more than 3.0% for six injections. In the chromatogram obtained from the *Test solution*, the retention time for the peak corresponding to 8a-oxo-B<sub>1a</sub> is approximately 4 to 9 minutes.

*Procedure*—Separately inject equal volumes (about 15  $\mu$ L) of the *Butylated hydroxytoluene solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the con-

tent of 8a-oxo-B<sub>1a</sub>, in mg, on the anhydrous, solvent-free, and antioxidant-free basis taken by the formula:

$$DCPF(r_U / r_S)$$

in which *D* is the dilution factor, in mL, used to prepare the *Test solution*; *C* is the concentration, in mg per mL, of butylated hydroxytoluene in the *Butylated hydroxytoluene solution*; *P* is the purity of butylated hydroxytoluene used to prepare the *Butylated hydroxytoluene solution*; *F* is equal to 0.4 and is the relative response factor for butylated hydroxytoluene with respect to 8a-oxo-B<sub>1a</sub>; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas for 8a-oxo-B<sub>1a</sub> and butylated hydroxytoluene in the chromatograms obtained from the *Test solution* and the *Butylated hydroxytoluene solution*, respectively. Not more than 0.5% of 8a-oxo-B<sub>1a</sub> is found.

#### Related compounds—

*Solution A*, *Solution B*, *Mobile phase*, *Diluent*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Procedure*—Inject a volume (about 15 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of eprinomectin related compounds in the portion of Eprinomectin taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the peak area of each individual related substance obtained from the *Test solution*, and *r<sub>s</sub>* is the sum of the responses of all the peaks: for related compounds with relative retentions of 0.23, 0.93, and 1.16 with respect to the B<sub>1a</sub> peak, not more than 1.0%; for impurity A, not more than 1.0%; for impurity E, not more than 1.0%; for all other known impurities, not more than 0.5%; for total unknown impurities, not more than 1.0%; and for total impurities, not more than 5.0% is found.

#### Assay—

*Solution A*: 0.1% (v/v) solution of perchloric acid in water.

*Solution B*: acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a solution of four volumes of methanol and one volume of water.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Eprinomectin RS in *Diluent* to prepare a solution having a known concentration of about 0.500 mg per mL.

*System suitability solution*—Transfer 4 mL of *Standard preparation* to an HPLC vial. Add 2 drops of 1 M sodium hydroxide and let stand for 20 minutes prior to injecting into the chromatograph.

*Assay preparation*—Dissolve an accurately weighed quantity of Eprinomectin in *Diluent* to prepare a solution having a known concentration of about 0.500 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is 1.5 mL per minute, and the column temperature is 40°. The chromatograph is programmed as follows:

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–15	45	55	isocratic
15–25	45→5	55→95	linear gradient
25–30	5→45	95→55	linear gradient
30–35	45	55	isocratic

Chromatograph the *System suitability solution* and the *Standard preparation* as directed for *Procedure*: the relative retention times are about 0.55, 0.77, 1.00, 1.05, and 1.28 for impurity A, component B<sub>1b</sub>, component B<sub>1a</sub>, impurities C + D, and impurity E, respectively; the resolution, *R*, between components B<sub>1b</sub> and B<sub>1a</sub> is not less than 3; the resolution, *R*, between component B<sub>1a</sub> and impurities C + D is at least 1; the symmetry factor for the B<sub>1a</sub> peak is not more than 1.5; and the theoretical plate count for the B<sub>1a</sub> peak is greater than 4,500. In the chromatogram of the *Standard preparation*, the relative standard deviation for the peak corresponding to B<sub>1a</sub> is not more than 1.0% for five injections.

*Procedure*—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of component B<sub>1a</sub> by the formula:

$$100(r_U / r_T)$$

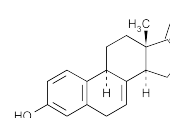
in which *r<sub>U</sub>* is the peak area of component B<sub>1a</sub> in the chromatogram obtained from the *Assay preparation*; and *r<sub>T</sub>* is the sum of the peak areas of components B<sub>1a</sub> and B<sub>1b</sub>.

Calculate the quantity, in mg, of C<sub>50</sub>H<sub>75</sub>NO<sub>14</sub> (component B<sub>1a</sub>) and C<sub>49</sub>H<sub>73</sub>NO<sub>14</sub> (component B<sub>1b</sub>) in the portion of Eprinomectin taken by the formula:

$$DC(r_U / r_S)$$

in which *D* is the dilution factor, in mL, used to prepare the *Assay preparation*; *C* is the concentration, in mg per mL, of component B<sub>1a</sub> or component B<sub>1b</sub> in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas of component B<sub>1a</sub> or component B<sub>1b</sub> in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Equilin



C<sub>18</sub>H<sub>20</sub>O<sub>2</sub> 268.35

Estra-1,3,5(10),7-tetraen-17-one, 3-hydroxy-

3-Hydroxyestra-1,3,5(10),7-tetraen-17-one [474-86-2].

» Equilin contains not less than 97.0 percent and not more than 103.0 percent of C<sub>18</sub>H<sub>20</sub>O<sub>2</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Equilin RS

**Clarity of solution**—Add 100 mg to 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, heat on a steam bath until solution is complete, then cool, and transfer to a 100-mL color-comparison tube: the solution is clear.

**Identification—**

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 50 µg per mL.

*Medium:* alcohol.

**Specific rotation** (781S): between +300° and +316°.

*Test solution:* 20 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it in vacuum at 105° for 1 hour; it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Assay—**

*Mobile phase*—Prepare a suitable and degassed solution containing 35 volumes of acetonitrile and 65 volumes of water.

*Internal standard solution*—Dissolve phenol in acetonitrile to obtain a solution having a concentration of about 35 µg per mL.

*Standard preparation*—Dissolve a suitable quantity of USP Equilin RS, accurately weighed, in *Internal standard solution* to obtain a solution having a known concentration of about 0.2 mg per mL.

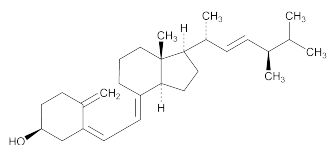
*Assay preparation*—Transfer about 10 mg of Equilin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%, and the resolution factor between equilin and phenol is not less than 5. Adjust the operating parameters such that the peak obtained from the *Standard preparation* is about 0.7 full-scale.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks: the retention times for equilin and phenol are about 14 and 3 minutes, respectively. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>20</sub>O<sub>2</sub> in the portion of Equilin taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Equilin RS in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of the peak responses of the equilin peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Ergocalciferol**

C<sub>28</sub>H<sub>44</sub>O 396.65  
9,10-Secoergosta-5,7,10 (19),22-tetraen-3-ol, (3β,5Z,7E,  
22E)-;  
Ergocalciferol [50-14-6].

**DEFINITION**

Ergocalciferol contains NLT 97.0% and NMT 103.0% of ergocalciferol (C<sub>28</sub>H<sub>44</sub>O).

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** (197K)

*Wavelength range:* 2–12 µm

• **B. ULTRAVIOLET ABSORPTION** (197U)

*Analytical wavelength:* 265 nm

*Sample solution:* 10 µg/mL in alcohol

*Acceptance criteria:* Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.

• **C.**

*Sample solution:* 0.5 mg in 5 mL of chloroform

*Analysis:* Add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid to the *Sample solution*, and shake vigorously.

*Acceptance criteria:* A bright red color is produced and rapidly changes through violet and blue to green.

• **D. THIN-LAYER CHROMATOGRAPHY**

[NOTE—For the *Standard solutions* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]

*Diluent:* 10 mg/mL of squalane in chloroform

*Standard solution A:* 50 mg/mL of USP Ergocalciferol RS in *Diluent*

*Standard solution B:* 100 µg/mL of USP Ergosterol RS in *Diluent*

*Sample solution:* 50 mg/mL of Ergocalciferol in *Diluent*

*Chromatographic system* (See *Chromatography* (621), *Thin-Layer Chromatography*.)

*Mode:* TLC

*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture

*Application volume:* 10 µL

*Developing solvent system:* Cyclohexane and ether (1:1)

*Spray reagent:* 20 mg/mL of acetyl chloride in antimony trichloride TS

**Analysis**

*Samples:* *Standard solution A*, *Standard solution B*, and *Sample solution*

[NOTE—Perform the development and subsequent operations in the dark.]

Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.

*Acceptance criteria:* The *Sample solution* shows a yellowish-orange area (ergocalciferol) having the same  $R_f$  value as the area of *Standard solution A* and may show a violet area below the ergocalciferol area. The color of the violet area is not more intense than that of the violet area from *Standard solution B*.

**ASSAY**

• **PROCEDURE**

*Dehydrated hexane:* Prepare a chromatographic column by packing a chromatographic tube, 8 cm × 60 cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* (621), *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.

*Mobile phase:* *n*-amyl alcohol in *Dehydrated hexane* (3 in 1000)

*System suitability solution:* 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]

[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]

**Standard stock solution:** 0.6 mg/mL of USP Ergocalciferol RS in toluene

**Standard solution:** 120 µg/mL of USP Ergocalciferol RS in *Mobile phase*, prepared from *Standard stock solution*

**Sample stock solution:** 0.6 mg/mL of Ergocalciferol in toluene

**Sample solution:** 120 µg/mL of Ergocalciferol in *Mobile phase*, prepared from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Injection size:** 5–10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between *trans*-cholecalciferol and precholecalciferol

**Relative standard deviation:** NMT 2.0% for the peak response of cholecalciferol

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of ergocalciferol (C<sub>28</sub>H<sub>44</sub>O) in the portion of Ergocalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ergocalciferol RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Ergocalciferol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–103.0%

#### IMPURITIES

##### • REDUCING SUBSTANCES

**Standard solution:** 0.2 µg/mL of hydroquinone in dehydrated alcohol

**Sample solution:** 10 mg/mL of Ergocalciferol in dehydrated alcohol

**Blank:** dehydrated alcohol

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

To 10 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 0.5 mL of 5 mg/mL blue tetrazolium in methanol. Then add 0.5 mL of tetramethylammonium hydroxide TS in dehydrated alcohol (1 in 10). Allow the mixture to stand for 5 min, accurately timed, then add 1 mL of glacial acetic acid. Determine the absorbance of the solution at 525 nm, with a suitable spectrometer, against the *Blank*.

**Acceptance criteria:** The absorbance of the *Sample solution* is NMT that of the *Standard solution*.

#### SPECIFIC TESTS

##### • MELTING RANGE OR TEMPERATURE, *Class 1b* <741>:

115°–119°

##### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 15 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Ergocalciferol from a container opened not longer than 30 min.]

**Acceptance criteria:** +103° to +106°

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.

#### • USP REFERENCE STANDARDS <11>

USP Ergocalciferol RS

USP Ergosterol RS

C<sub>28</sub>H<sub>44</sub>O 396.66

USP Vitamin D Assay System Suitability RS

## Ergocalciferol Capsules

#### DEFINITION

Ergocalciferol Capsules usually consist of an edible vegetable oil solution of Ergocalciferol, encapsulated with Gelatin.

Ergocalciferol Capsules contain NLT 100.0% and NMT 120.0% of the labeled amount of vitamin D as ergocalciferol (C<sub>28</sub>H<sub>44</sub>O).

#### ASSAY

##### • PROCEDURE

[NOTE—Throughout this Assay, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.]

**Dehydrated hexane:** Prepare a chromatographic column by packing a chromatographic tube, 60-cm × 8-cm in diameter, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* <621>, *Column Adsorption Chromatography*.) Pass 500 mL of hexanes through the column, and collect the eluate in a glass-stoppered flask.

**Butylated hydroxytoluene solution:** 10 mg/mL of butylated hydroxytoluene in chromatographic hexane

**Aqueous potassium hydroxide solution:** 1 g/mL of potassium hydroxide in freshly boiled water. [NOTE—Prepare this solution fresh daily.]

**Alcoholic potassium hydroxide solution:** 3 g of potassium hydroxide in 50 mL of freshly boiled water. Add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare this solution fresh daily.]

**Sodium ascorbate solution:** 175 mg/mL of ascorbic acid in 1 N sodium hydroxide. [NOTE—Prepare this solution fresh daily.]

**Sodium sulfide solution:** 12 g of sodium sulfide in 20 mL of water. Dilute with glycerin to 100 mL.

**Mobile phase:** *Dehydrated hexane* and *n*-amyl alcohol (997:3). The ratio of components and the flow rate may be varied to meet the *System suitability requirements*.

**Standard stock solution:** 0.5 mg/mL of USP Ergocalciferol RS in toluene. [NOTE—Prepare solution fresh daily.]

**Standard solution A:** 20 µg/mL from the *Standard stock solution* in *Mobile phase*. [NOTE—Store this solution at a temperature not above 0°.]

**Standard solution B:** Pipet 4 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser, and add 2 or 3 crystals of butylated hydroxytoluene. Displace the air with nitrogen, and heat in a water bath maintained at a temperature of 90° in subdued light under a nitrogen atmosphere for 45 min to obtain a solution containing vitamin D and pre-vitamin D. Cool, transfer with the aid of several portions of *Mobile phase* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**System suitability solution:** 100 mg of USP Vitamin D Assay System Suitability RS to a 10-mL volumetric flask. Add a mixture (1 in 5) of toluene and *Mobile phase* to volume, and mix. Heat a portion of this solution under reflux, at 90° for 45 min, and cool.

**Sample solution:** Reflux NLT 10 Capsules with a mixture of 10 mL of *Sodium ascorbate solution* and 2 drops of *Sodium sulfide solution* on a steam bath for 10 min, crush any remaining solids with a blunt glass rod, and

continue heating for 5 min. Cool, and add 25 mL of alcohol and 3 mL of *Aqueous potassium hydroxide solution*. Reflux the mixture on a steam bath for 30 min. Cool rapidly under running water, and transfer the saponified mixture to a conical separator, rinsing the saponification flask with two 15-mL portions of water, 10 mL of alcohol, and two 50-mL portions of ether. [NOTE—Use ether within 24 h after opening the container.] Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Transfer the aqueous phase to a second conical separator, add a mixture of 10 mL of alcohol and 50 mL of solvent hexane, and shake vigorously. Allow to separate, transfer the aqueous phase to a third conical separator, and transfer the solvent hexane phase to the first separator, rinsing the second separator with two 10-mL portions of solvent hexane and adding the rinsings to the first separator. Shake the aqueous phase in the third separator with 50 mL of solvent hexane, and add the solvent hexane phase to the first separator. Wash the combined ether-solvent hexane extracts by shaking vigorously with three 50-mL portions of *Alcoholic potassium hydroxide solution*, and wash with 50-mL portions of water vigorously until the last washing is neutral to phenolphthalein. Drain any remaining drops of water from the combined ether-solvent hexane extracts, add 2 sheets of 9-cm filter paper, in strips, to the separator, and shake. Transfer the washed ether-solvent hexane extracts to a round-bottomed flask, rinsing the separator and paper with solvent hexane. Combine the solvent hexane rinsings with the ether-solvent hexane extracts, add 100  $\mu$ L of *Butylated hydroxytoluene solution*, and mix. Evaporate in a vacuum to dryness by swirling in a water bath maintained at a temperature not higher than 40°. Cool under running water, and introduce nitrogen sufficient to restore atmospheric pressure. Without delay, dissolve and dilute the residue in an accurately measured volume of a mixture (1 in 5) of toluene and *Mobile phase*, until the concentration of vitamin D is about 25  $\mu$ g/mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 25-cm  $\times$  4.6-mm; packing L3

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for pre-cholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 is between *trans*-cholecalciferol and pre-cholecalciferol

**Relative standard deviation:** NMT 2.0% for the cholecalciferol peak

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

#### Ergocalciferol response factor

Calculate the *Ergocalciferol response factor*,  $F_D$ :

$$F_D = C_S/r_S$$

$C_S$  = concentration of USP Ergocalciferol RS in the *Standard solution A* ( $\mu$ g/mL)

$r_S$  = peak response of ergocalciferol from *Standard solution A*

#### Pre-ergocalciferol response factor

Calculate the concentration of ergocalciferol,  $C'_S$ , in  $\mu$ g/mL, in the *Standard solution B*:

$$C'_S = F_D \times r'_S$$

$F_D$  = ergocalciferol response factor

$r'_S$  = peak area of ergocalciferol from *Standard solution B*

Calculate the concentration of pre-ergocalciferol,  $C'_{pre}$ , in  $\mu$ g/mL, in the *Standard solution B*:

$$C'_{pre} = C_S - C'_S$$

$C_S$  = concentration of USP Ergocalciferol RS in the *Standard solution A* ( $\mu$ g/mL)

$C'_S$  = concentration of ergocalciferol in the *Standard solution B* ( $\mu$ g/mL)

Calculate the response factor,  $F_{pre}$ , for pre-ergocalciferol:

$$F_{pre} = C'_{pre}/r'_{pre}$$

$C'_{pre}$  = concentration of pre-ergocalciferol ( $\mu$ g/mL)

$r'_{pre}$  = peak response of pre-ergocalciferol from *Standard solution B*

[NOTE—The value of  $F_{pre}$  determined in duplicate, on different days, can be used during the entire procedure.]

#### Vitamin D content

Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ( $C_{28}H_{44}O$ ) in the portion of Capsules taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r'_{pre})]/C_U\} \times 100$$

$F_D$  = ergocalciferol response factor

$r_C$  = peak area of ergocalciferol from the *Sample solution*

$F_{pre}$  = pre-ergocalciferol response factor

$r'_{pre}$  = peak area of pre-ergocalciferol from the *Sample solution*

$C_U$  = nominal concentration of ergocalciferol in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 100.0%–120.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION <701>

**Buffer solution:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.5 \pm 0.05$

**Immersion fluid:** *Buffer solution*

**Time:** 45 min

**Acceptance criteria:** Meet the requirements

##### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers.

##### • LABELING: Label the Capsules to indicate the content of ergocalciferol in mg. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 $\mu$ g.

##### • USP REFERENCE STANDARDS <11>

USP Ergocalciferol RS

USP Vitamin D Assay System Suitability RS

## Ergocalciferol Oral Solution

#### DEFINITION

Ergocalciferol Oral Solution is a solution of Ergocalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 100.0% and NMT 120.0% of the labeled amount of vitamin D as ergocalciferol ( $C_{28}H_{44}O$ ).

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Chloroform containing alcohol as a preservative

**Standard stock solution:** 50 µg/mL of USP Ergocalciferol RS in chloroform. [NOTE—Prepare this solution fresh daily.]

**Standard solution A:** 5 µg/mL of USP Ergocalciferol RS from the *Standard stock solution* in chloroform. [NOTE—Store this *Standard solution A* at a temperature not above 0°.]

**Standard solution B:** Transfer 5.0 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing ergocalciferol and pre-ergocalciferol. Cool, transfer, with the aid of several portions of chloroform, to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

**Sample solution:** Equivalent to 5 µg/mL of ergocalciferol from an accurately measured volume of Oral Solution in chloroform

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L3

**Flow rate:** 1 mL/min

**Injection size:** 10–20 µL

#### System suitability

**Sample:** *Standard solution B*

[NOTE—The relative retention times for pre-ergocalciferol and for ergocalciferol are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between the pre-ergocalciferol peak and the ergocalciferol peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

#### Ergocalciferol response factor

Calculate the *Ergocalciferol response factor*,  $F_D$ :

$$F_D = C_S/r_S$$

$C_S$  = concentration of USP Ergocalciferol RS in the *Standard solution A* (µg/mL)

$r_S$  = peak response of ergocalciferol from *Standard solution A*

#### Pre-ergocalciferol response factor

Calculate the concentration,  $C'_S$ , in µg/mL, of ergocalciferol in *Standard solution B*:

$$C'_S = F_D \times r'_S$$

$F_D$  = Ergocalciferol response factor

$r'_S$  = peak area of ergocalciferol from *Standard solution B*

Calculate the concentration,  $C'_{pre}$ , in µg/mL, of pre-ergocalciferol:

$$C'_{pre} = C_S - C'_S$$

$C_S$  = concentration of USP Ergocalciferol RS in the *Standard solution A* (µg/mL)

$C'_S$  = concentration of ergocalciferol in the *Standard solution B* (µg/mL)

Calculate the *Pre-ergocalciferol response factor*,  $F_{pre}$ :

$$F_{pre} = C'_{pre}/r_p$$

$C'_{pre}$  = concentration of pre-ergocalciferol (µg/mL)

$r_p$  = peak response of pre-ergocalciferol from *Standard solution B*

#### Vitamin D content

Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ( $C_{28}H_{44}O$ ) in the portion of Oral Solution taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

$F_D$  = ergocalciferol response factor

$r_C$  = peak area of ergocalciferol from the *Sample solution*

$F_{pre}$  = response factor for pre-ergocalciferol

$r_{pre}$  = peak area of pre-ergocalciferol from the *Sample solution*

$C_U$  = nominal concentration of ergocalciferol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 100.0%–120.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** Label the Oral Solution to indicate the concentration of ergocalciferol, in mg per dosage unit. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 µg.

• **USP REFERENCE STANDARDS** <11>

USP Ergocalciferol RS

## Ergocalciferol Tablets

#### DEFINITION

Ergocalciferol Tablets contain NLT 100.0% and NMT 120.0% of the labeled amount of ergocalciferol ( $C_{28}H_{44}O$ ).

#### IDENTIFICATION

##### A. ULTRAVIOLET ABSORPTION

**Standard solution:** 0.01 mg/mL of USP Ergocalciferol RS in alcohol

**Sample solution:** Evaporate 1 mL of the *Sample solution* obtained from *Identification test B* under a stream of nitrogen to dryness. Dissolve the residue in 50 mL of alcohol.

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured.

##### B. COLOR REACTION

**Sample solution:** Triturate a quantity of powdered Tablets, equivalent to 5 mg of ergocalciferol, with 10 mL of chloroform, and filter.

**Analysis:** To 5 mL of the *Sample solution* add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake vigorously.

**Acceptance criteria:** A bright red color is produced and rapidly changes through violet and blue to green.

#### ASSAY

• **PROCEDURE:** Proceed with Tablets as directed in *Vitamin D Assay* <581>, *Chemical Method*.

**Acceptance criteria:** 100.0%–120.0%

#### PERFORMANCE TESTS

##### DISINTEGRATION <701>

Time: 30 min

• **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

#### ADDITIONAL REQUIREMENTS

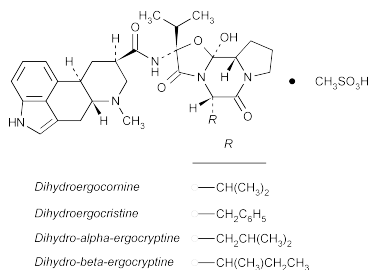
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** Label the Tablets to indicate the content of ergocalciferol, in mg. The activity may be expressed also

in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 µg.

- **USP REFERENCE STANDARDS** (11)  
USP Ergocalciferol RS

## Ergoloid Mesylates



C<sub>31</sub>H<sub>41</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S (dihydroergocornine mesylate) 659.79

C<sub>35</sub>H<sub>41</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S (dihydroergocristine mesylate) 707.84

C<sub>32</sub>H<sub>43</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S (dihydro- $\alpha$ -ergocryptine mesylate) 673.82

C<sub>32</sub>H<sub>43</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S (dihydro- $\beta$ -ergocryptine mesylate) 673.82

Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2',5'-bis(1-methylethyl)-, (5' $\alpha$ ,10 $\alpha$ )-, monomethanesulfonate (salt) mixture with 9,10 $\alpha$ -dihydro-12'-hydroxy-2'-(1-methylethyl)-5' $\alpha$ -(phenylmethyl)ergotaman-3',6',18-trione monomethanesulfonate (salt), 9,10 $\alpha$ -dihydro-12'-hydroxy-2'-(1-methylethyl)-5' $\alpha$ -(2-methylpropyl)ergotaman-3',6',18-trione monomethanesulfonate (salt), and 9,10 $\alpha$ -dihydro-12'-hydroxy-2'-(1-methylethyl)-5' $\alpha$ -(1-methylpropyl)ergotaman-3',6',18-trione monomethanesulfonate (salt).

Dihydroergotoxine monomethanesulfonate (salt).

An equiproportional mixture of dihydroergocornine mesylate, dihydroergocristine mesylate, and ratio of dihydro- $\alpha$ -ergocryptine mesylate to dihydro- $\beta$ -ergocryptine mesylate is (1.5-2.5:1) [8067-24-1].

» Ergoloid Mesylates is a mixture of the methanesulfonate salts of the three hydrogenated alkaloids, dihydroergocristine (C<sub>35</sub>H<sub>41</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S), dihydroergocornine (C<sub>31</sub>H<sub>41</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S), and dihydroergocryptine (C<sub>32</sub>H<sub>43</sub>N<sub>5</sub>O<sub>5</sub> · CH<sub>4</sub>O<sub>3</sub>S), in an approximate weight ratio of 1:1:1. Ergoloid Mesylates contains not less than 97.0 percent and not more than 103.0 percent of the alkaloid methanesulfonate mixture, calculated on the anhydrous basis, and not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids. Dihydroergocryptine mesylate exists as a mixture of *alpha*- and *beta*- isomers. The ratio of *alpha*- to *beta*- isomers is not less than 1.5:1.0 and not more than 2.5:1.0.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Ergoloid Mesylates RS

### Identification—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wave-

lengths as that of a similar, undried preparation of USP Ergoloid Mesylates RS.

**B:** In a suitable chromatographic chamber, arranged for thin-layer chromatography, place a volume of a solvent system consisting of a mixture of acetone, *n*-butyl alcohol, ammonium hydroxide, and water (65:20:10:5) sufficient to develop the chromatogram. Prepare a test solution of Ergoloid Mesylates in a mixture of chloroform and methanol (9:1) containing 40 mg per mL. Apply 10 µL of this solution and 10 µL of a reference solution of methanesulfonic acid containing 0.4 mL in 100 mL of a mixture of chloroform and methanol (9:1) to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram until the solvent front has moved 10 cm. Remove the plate from the developing chamber, mark the solvent front, and dry in a current of cold air. Spray the plate with a 1 in 1000 solution of bromocresol purple in alcohol that previously has been adjusted to the purple color with 6 N ammonium hydroxide, then place in a stream of warm air until the spots appear: the *R<sub>F</sub>* value of the methanesulfonic acid spot obtained from the test solution corresponds to that obtained from the reference solution.

**Specific rotation** (781S): between +11.0° and +15.0°.

**Test solution:** 10 mg per mL, in dilute alcohol (1 in 2).

**pH** (791): between 4.2 and 5.2 in a solution (1 in 200).

**Water, Method I** (921): not more than 5.0%.

**Limit of ergotamine**—Prepare three solutions in a mixture of chloroform and methanol (9:1) containing 5 mg of Ergoloid Mesylates per mL, 5 mg of USP Ergoloid Mesylates RS per mL, and 5 mg of Ergotamine Tartrate per mL. Apply 5-µL volumes of the solutions at points about 2 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Add the solvent system consisting of a mixture of chloroform and methanol (9:1) and a small beaker of ammonium hydroxide to a suitable chamber, seal, and allow to equilibrate for 30 minutes. Develop the chromatogram in the equilibrated chamber until the solvent front has moved about 15 cm from the points of application. Remove the plate, air-dry, and locate the spots, first by viewing under long-wavelength UV light, and then by spraying with a reagent prepared by dissolving 800 mg of *p*-(dimethylamino)-benzaldehyde in a mixture of 80 mL of alcohol and 11 mL of sulfuric acid: the chromatogram from Ergoloid Mesylates shows primary spots that correspond in size and color to the spots obtained from the USP Ergoloid Mesylates RS solution, and shows no spot corresponding to the principal spot in the chromatogram of Ergotamine Tartrate.

**Limit of nonhydrogenated alkaloids**—Prepare a solution in alcohol containing 0.4 mg of Ergoloid Mesylates per mL, and prepare a 1 in 10 dilution of the first solution. Determine the absorbances in 1-cm cells of the first solution at 317.5 nm and the dilution at 280 nm, using alcohol as the blank: the absorbance of the first solution is not more than 0.15 times that of the dilution.

### Assay—

**Mobile phase**—Prepare a degassed solution containing a mixture of water, acetonitrile, and triethylamine (80:20:2.5). Adjust the ratio as necessary.

**Standard preparation**—Transfer about 10 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 10-mL volumetric flask. Dissolve in a mixture of acetonitrile and water (1:1), dilute with the same solvent to volume, and mix. Prepare this solution fresh.

**Assay preparation**—Using about 10 mg of Ergoloid Mesylates, accurately weighed, proceed as directed for *Standard preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1.

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between dihydro- $\alpha$ -ergocryptine mesylate and dihydroergocristine mesylate is not less than 1.35; the resolution,  $R$ , between dihydroergocristine and dihydro- $\beta$ -ergocryptine is not less than 1.0; the column efficiency determined for the dihydro- $\beta$ -ergocryptine mesylate peak is not less than 950 theoretical plates; the tailing factor for dihydro- $\beta$ -ergocryptine mesylate is not more than 2.5; and the relative standard deviation of the sum of the four peaks for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The order of elution is dihydroergocornine, dihydro- $\alpha$ -ergocryptine, dihydroergocristine, and dihydro- $\beta$ -ergocryptine. Calculate the total quantity, in mg, of these alkaloids in the portion of Ergoloid Mesylates taken by the formula:

$$10C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation* and  $r_U$  and  $r_S$  are the sums of the responses of the four major peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the percentage of each alkaloid taken by the formula:

$$100r_i(MW)_i / \Sigma[r_i(MW)_i]$$

in which  $r_i$  is the peak response of an individual alkaloid;  $(MW)_i$  is the molecular weight of that alkaloid; and  $\Sigma[r_i(MW)_i]$  is the summation of the products of peak responses and molecular weights calculated for the four alkaloids.

## Ergoloid Mesylates Capsules

» Ergoloid Mesylates Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methane-sulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha*- to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

**Packaging and storage**—Preserve in tight, light-resistant containers between 15° and 25°. Do not freeze.

### USP Reference standards (11)—

USP Ergoloid Mesylates RS

### Identification—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard as obtained in the *Assay*.

**B:** Using a sharp blade, carefully open 1 Capsule and transfer the entire contents into a 250-mL flask. Inspect the encapsulated liquid to ensure that crystallization or agglomeration of the drug substance has not taken place. Add 5 mL of water to the flask, and swirl to dissolve. Add 10 mL of *p*-dimethylaminobenzaldehyde TS: a blue color develops within 2 minutes and persists for not less than 10 minutes.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—The total bacterial count does not exceed 1000 per g, and the total combined molds and yeasts count does not exceed 200 per g. Capsules meet also the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The test specimen shows an absence of the members of the *Enterobacteriaceae* family and *Pseudomonadaceae* family at levels greater than 100 per g of each.

### Dissolution (711)—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and note if there is membrane formation. Record the time that each Capsule ruptures.

**Tolerances**—All of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 Capsules fail to rupture in 15 minutes but rupture in not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units (905):** meet the requirements, chloroform being used as the solvent in the procedure for *Weight Variation*.

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and triethylamine (32:18:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve an accurately weighed quantity of *m*-chloroacetanilide in acetonitrile to obtain a solution having a known concentration of about 0.12 mg per mL.

**Tartaric acid solution**—Dissolve an accurately weighed quantity of tartaric acid in water to obtain a solution having a known concentration of about 5.6 mg per mL.

**Standard preparation**—Transfer about 25.0 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix. Transfer 40.0 mL of this solution to a 200-mL volumetric flask, add 40.0 mL of *Tartaric acid solution*, and mix.

**Assay preparation**—Pipet 40.0 mL of *Tartaric acid solution* into a 200-mL volumetric flask, and heat in a water bath maintained at 50°. Add 10 Capsules (or the equivalent of 10 mg of ergoloid mesylates), and shake the flask by mechanical means for 10 minutes or until the gelatin has dissolved. Pipet 40.0 mL of *Internal standard solution* into the flask, and shake for an additional 10 minutes. Remove the flask from the bath and cool to room temperature. Transfer about 20 mL of the solution to a 30-mL centrifuge tube, and centrifuge at 10,000 rpm for 60 minutes. Filter a portion of the supernatant through a filter having a porosity of 0.45  $\mu$ m, discarding the first 5 mL of the filtrate. Use the remainder of the filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and an 8-mm  $\times$  10-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times for *m*-chloroacetanilide, dihydroergocornine, dihydro- $\alpha$ -ergocryptine, dihydroergocristine, and dihydro- $\beta$ -ergocryptine are about 1.0, 1.4, 1.8, 2.2, and 2.8, respectively; the column efficiency determined for the dihydro- $\beta$ -ergocryptine peak is not less than 950 theoretical plates; the tailing factor for dihydro- $\beta$ -ergocryptine is not more than 2.5 and that for dihydroergocornine is not more than 2.0; the resolution,  $R$ ,



between dihydro- $\alpha$ -ergocryptine and dihydroergocristine is not less than 1.35; and the relative standard deviation of the sum of the four ergoloid mesylate peaks for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the total quantity, in mg, of ergoloid mesylates in the portion of Capsules taken by the formula:

$$80C(\Sigma R_U / \Sigma R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation*, and  $\Sigma R_U$  and  $\Sigma R_S$  are sums of the ratios of the peak responses of the individual alkaloids to the peak response of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of each of the individual alkaloids in the portion of Capsules taken by the formula:

$$100R_U(MW)_U / \Sigma[R_U(MW)_U]$$

in which  $(MW)_U$  is the molecular weight of the individual alkaloid.

### Ergoloid Mesylates Oral Solution

» Ergoloid Mesylates Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Ergoloid Mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha*- to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at a temperature not exceeding 30°.

**USP Reference standards** <11>—

USP Ergoloid Mesylates RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** <905>—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Alcohol content, Method II** <611>: between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Assay**—

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay* under *Ergoloid Mesylates*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ergoloid Mesylates RS in acetonitrile solution (1 in 5) to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 25 mg of ergoloid mesylates, to a 100-mL volumetric flask. Rinse the pipet with small portions of acetonitrile solution (1 in 5), collect-

ing the rinsings in the volumetric flask. Dilute with acetonitrile solution (1 in 5) to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Ergoloid Mesylates*, except to inject about 60- $\mu$ L volumes of the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of ergoloid mesylates in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Oral Solution taken, and the other terms are as defined therein.

Calculate the percentage of each of the individual alkaloids in the Oral Solution taken by the formula given in the *Assay* under *Ergoloid Mesylates*.

### Ergoloid Mesylates Tablets

» Ergoloid Mesylates Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha*- to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label the Tablets to indicate that they are intended for swallowing intact.

**USP Reference standards** <11>—

USP Ergoloid Mesylates RS

**Identification**—Mix a small amount of powdered Tablets, equivalent to about 5 mg of ergoloid mesylates, with 5 mL of water and 5 mL of a mixture of equal volumes of glacial acetic acid and sulfuric acid, and add 1 drop of freshly prepared ferric chloride solution (1 in 20): a violet-blue color develops within 5 minutes.

**Dissolution** <711>—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm, the distance between the paddle blade and the inside bottom of the vessel being maintained at  $4.5 \pm 0.2$  cm during the test.

**Time:** 30 minutes.

Determine the amount of ergoloid mesylates dissolved using the following procedure.

**Mobile phase**—Prepare as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Ergoloid Mesylates RS in water to obtain a solution having a known concentration of about 50  $\mu$ g per mL. Transfer 4 mL of this solution for every 0.5 mg of ergoloid mesylates contained in the Tablets to a 200-mL volumetric flask, add 1 mL of 0.1 N hydrochloric acid and 100 mL of water, mix, and dilute with water to volume.

**Test solution**—Transfer a 20-mL portion of the solution under test to a suitable container, add 100  $\mu$ L of 0.1 N hydrochloric acid, and mix.

**Chromatographic system**—Proceed as directed in the *Assay* except that the sum of the relative standard deviation values for the four ergoloid mesylates peaks for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 500  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of ergoloid mesylates as directed in the *Assay*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of ergoloid mesylates is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed solution containing a mixture of water, acetonitrile, and triethylamine (700:300:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 113 mg of papaverine hydrochloride to a 1-L flask. Add a mixture of 0.01 M tartaric acid and acetonitrile (2:1) to volume, and mix.

**Standard preparation**—Transfer about 33 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume, and mix. Use a freshly prepared solution.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of ergoloid mesylates, to a 50-mL centrifuge tube. Add 15.0 mL of *Internal standard solution*, insert the stopper into the tube, and shake for about 15 minutes. Centrifuge, filter if necessary, and use the clear supernatant.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. [NOTE—Use an L1 column capable of handling pH values greater than 11.] The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined for the dihydro- $\beta$ -ergocryptine mesylate peak is not less than 1000 theoretical plates; the tailing factor for the dihydro- $\beta$ -ergocryptine mesylate peak is not more than 2.0; the resolution,  $R$ , between the dihydro- $\alpha$ -ergocryptine mesylate and dihydroergocristine mesylate is not less than 2.0, and between dihydroergocristine and dihydro- $\beta$ -ergocryptine peaks is not less than 2.0; and the relative standard deviation of the ratio of the sum of the four peaks to the internal standard for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The order of elution is papaverine, dihydroergocornine, dihydro- $\alpha$ -ergocryptine, dihydroergocristine, and dihydro- $\beta$ -ergocryptine. Calculate the quantity, in mg, of ergoloid mesylates in the portion of Tablets taken by the formula:

$$15C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the sums of the ratios of responses of the four major peaks to the response of the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of each of the individual alkaloids taken by the formula:

$$100R_i(MW)_i / \sum[R_i(MW)_i]$$

in which  $R_i$  is the peak response ratio of the individual alkaloid to the internal standard;  $(MW)_i$  is the molecular weight of the individual alkaloid; and  $\sum[R_i(MW)_i]$  is the summation of the products of peak response ratios and molecular weights for the four alkaloids.

## Ergoloid Mesylates Sublingual Tablets

» Ergoloid Mesylates Sublingual Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha*- to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label the Tablets to indicate that they are intended for sublingual administration.

**USP Reference standards** (11)—  
USP Ergoloid Mesylates RS

**Identification**—Mix a small amount of powdered Sublingual Tablets, equivalent to about 5 mg of ergoloid mesylates, with 5 mL of water and 5 mL of a mixture of equal volumes of glacial acetic acid and sulfuric acid, and add 1 drop of freshly prepared ferric chloride solution (1 in 20): a violet-blue color develops within 5 minutes.

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed solution containing a mixture of water, acetonitrile, and triethylamine (700:300:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 113 mg of papaverine hydrochloride to a 1-L flask. Add a mixture of 0.01 M tartaric acid and acetonitrile (2:1) to volume, and mix.

**Standard preparation**—Transfer about 33 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume, and mix. Use a freshly prepared solution.

**Assay preparation**—Weigh and finely powder not fewer than 20 Sublingual Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of ergoloid mesylates, to a 50-mL centrifuge tube. Add 15.0 mL of *Internal standard solution*, insert the stopper into the tube, and shake for about 15 minutes. Centrifuge, filter if necessary, and use the clear supernatant.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. [NOTE—Use an L1 column capable of handling pH values greater than 11.] The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined for the dihydro- $\beta$ -ergocryptine mesylate peak is not less than 1000 theoretical plates; the tailing factor for the dihydro- $\beta$ -ergocryptine mesylate peak is not more than 2.0; the resolution,  $R$ , between the dihydro- $\alpha$ -ergocryptine mesylate and dihydroergocristine mesylate peaks is not less than 2.0, and between dihydroergocristine and dihydro- $\beta$ -ergocryptine peaks is not less than 2.0; and the relative standard deviation of the ratio of the sum of the four peaks to the internal standard for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The order of elution is papaverine, dihydroergocornine, dihydro- $\alpha$ -ergocryptine, dihydroergocristine, and dihydro- $\beta$ -ergocryptine. Calculate the quantity, in mg, of ergoloid mesylates in the portion of Sublingual Tablets taken by the formula:

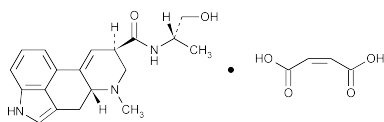
$$15C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the sums of the ratios of responses of the four major peaks to the response of the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of each of the individual alkaloids taken by the formula:

$$100R_i(MW)_i / \Sigma[R_i(MW)_i]$$

in which  $R_i$  is the peak response ratio of the individual alkaloid to the internal standard;  $(MW)_i$  is the molecular weight of the individual alkaloid; and  $\Sigma[R_i(MW)_i]$  is the summation of the products of peak response ratios and molecular weights for the four alkaloids.

## Ergonovine Maleate



$C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  441.48  
Ergoline-8-carboxamide, 9,10-didehydro-*N*-(2-hydroxy-1-methylethyl)-6-methyl-, 8 $\beta$ (*S*)-, (*Z*)-2-butenedioate (1:1) (salt).  
9,10-Didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8 $\beta$ -carboxamide maleate (1:1) (salt) [129-51-1].

» Ergonovine Maleate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, in a cold place.

### USP Reference standards (11)—

USP Ergonovine Maleate RS

#### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 20  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 311 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** The  $R_f$  value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation* in the chromatogram prepared as directed in the test for *Related alkaloids*.

**Specific rotation** (781S): between +51° and +56°.

*Test solution:* 5 mg per mL, in water.

**Loss on drying** (731)—Dry it in vacuum at 80° for 3 hours; it loses not more than 2.0% of its weight.

**Related alkaloids**—[NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Solvent mixture**—Prepare a mixture of alcohol and ammonium hydroxide (9:1).

**Standard preparation**—Prepare a solution of USP Ergonovine Maleate RS in *Solvent mixture* having a known concentration of about 10 mg per mL.

**Standard dilutions**—Prepare a series of dilutions of the *Standard preparation* in *Solvent mixture* having known concentrations of about 0.20, 0.10, and 0.05 mg per mL. Use immediately after preparation.

**Test preparation**—Immediately prior to use, prepare a solution of Ergonovine Maleate in *Solvent mixture* having a concentration of about 10 mg per mL.

**Application volume:** 5  $\mu$ L.

**Developing solvent system:** a mixture of chloroform, methanol, and water (75:25:3), equilibrated for 30 minutes.

**Procedure**—Apply 5- $\mu$ L portions of the *Standard preparation*, each of the three *Standard dilutions*, and the *Test preparation*, and proceed as directed for *Thin-Layer Chromatography* (621). Locate the spots on the plate by spraying thoroughly and evenly with a solution prepared by dissolving 1 g of *p*-dimethylaminobenzaldehyde in a cooled mixture of 50 mL of alcohol and 50 mL of hydrochloric acid. Immediately dry in a stream of nitrogen for about 2 minutes: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation*. Estimate the concentration of any other spots observed in the chromatogram of the *Test preparation* by comparison with the *Standard dilutions*. The spots from the 0.20, 0.10, and 0.05 mg per mL dilutions are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is not greater than 2.0%.

#### Assay—

**Standard preparation**—Using a suitable quantity of USP Ergonovine Maleate RS, accurately weighed, prepare a solution in water having a known concentration of about 40  $\mu$ g per mL.

**Assay preparation**—Transfer about 40 mg of Ergonovine Maleate, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute 10.0 mL of this solution with water to 100.0 mL.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide a blank, to separate conical flasks. Add 10.0 mL of *p*-dimethylaminobenzaldehyde TS with constant swirling to each, and allow to stand for 20 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 555 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Ergonovine Maleate RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ergonovine Maleate Injection

» Ergonovine Maleate Injection is a sterile solution of Ergonovine Maleate in Water for Injection. It contains not less than 90.0 percent and not

more than 110.0 percent of the labeled amount of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in single-dose, light-resistant containers, preferably of Type I glass, and store in a cold place.

**USP Reference standards** (11)—

USP Ergonovine Maleate RS

USP Endotoxin RS

**Identification**—The  $R_f$  value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from *Standard preparation A* in the chromatogram prepared as directed in the test for *Related alkaloids*.

**Bacterial endotoxins** (85)—It contains not more than 700.0 USP Endotoxin Units per mg of ergonovine maleate.

**pH** (791): between 2.7 and 3.5.

**Related alkaloids**—[NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

*Solvent mixture*, *Standard preparation*, and *Standard dilutions*—Prepare as directed in the test for *Related alkaloids* under *Ergonovine Maleate*.

*Test preparation*—Immediately prior to use, transfer a volume of Injection, equivalent to about 5 mg of ergonovine maleate, to a separator, and extract with three 5-mL portions of chloroform. Discard the chloroform extracts. Render alkaline to litmus with 6 N ammonium hydroxide, and extract with three 5-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve the residue so obtained in 0.5 mL of *Solvent mixture*.

*Procedure*—Proceed as directed for *Procedure* in the test for *Related alkaloids* under *Ergonovine Maleate*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*0.05 M Phosphate buffer*—Dissolve 6.8 g of monobasic potassium phosphate in 600 mL of water and adjust with phosphoric acid to a pH of 2.1. Dilute with water to 1000 mL, and mix.

*Mobile phase*—Prepare a suitable and degassed solution of *0.05 M Phosphate buffer* and acetonitrile (80:20) such that the retention time is approximately 3 minutes with a flow rate of 1 mL per minute.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ergonovine Maleate RS in *Mobile phase*, adding sufficient water to equal 10% of the final volume, to obtain a solution having a known concentration of about 0.02 mg per mL.

*Assay preparation*—Quantitatively dilute an accurately measured volume of the Injection, equivalent to about 2 mg of ergonovine maleate, with *Mobile phase* and water, if necessary, to obtain a solution having a concentration of about 0.02 mg per mL in which the Injection volume plus any added water constitutes 10% of the final volume.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 312-nm detector and a 3-mm  $\times$  30-cm column that contains packing L1. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 3.0%.

*Procedure*—By means of a suitable sampling valve, introduce equal volumes (about 100  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph. Measure the peak responses of Ergonovine Maleate, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*. Calculate the quantity,

in mg, of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  in each mL of the Injection taken by the formula:

$$(CD / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ergonovine Maleate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, D is the dilution factor, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ergonovine Maleate Tablets

» Ergonovine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Ergonovine Maleate RS

**Identification**—The  $R_f$  value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation* in the chromatogram prepared as directed in the test for *Related alkaloids*.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  dissolved from fluorometric measurements, using 322 nm as the excitation wavelength and 428 nm as the emission wavelength, of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ergonovine Maleate RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related alkaloids**—[NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

*Solvent mixture*—Mix 75 volumes of chloroform, 25 volumes of methanol, and 1 volume of ammonium hydroxide.

*Detecting reagent*—Cautiously dissolve 800 mg of *p*-dimethylaminobenzaldehyde in a mixture of alcohol and sulfuric acid (101:11).

*Standard stock solution*—Transfer 25 mg of USP Ergonovine Maleate RS, accurately weighed, to a separator, shake with 10 mL of water, render the mixture alkaline to litmus with 6 N ammonium hydroxide, and extract with three 10-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve and dilute the residue to 10.0 mL with the *Solvent mixture*.

*Standard preparations A, B, C, and D*—Dilute accurately measured volumes of *Standard stock solution* quantitatively with *Solvent mixture* (designated below as parts by volume of *Standard stock solution* in total parts by volume of the finished *Standard preparation*) to obtain *Standard preparations*, designated below by letter, having the following compositions:

Standard Solution	Dilution	Concentration (μg RS per mL)	Percentage (% for comparison with test specimen)
A	(1 in 20)	125	5.0
B	(1 in 33)	75	3.0
C	(1 in 100)	25	1.0
D	(1 in 200)	12.5	0.5

**Test preparation**—Immediately prior to use, transfer a quantity of finely powdered Tablets, equivalent to about 5 mg of ergonovine maleate, to a separator, shake with 10 mL of water, render the mixture alkaline to litmus with 6 N ammonium hydroxide, and extract with three 10-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve the residue obtained in 2.0 mL of *Solvent mixture*.

**Procedure**—Apply separately 20 μL of the *Test preparation* and 20 μL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the plate with the aid of a current of cool air. Position the plate in a chromatographic chamber, and develop the chromatograms in *Solvent mixture* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in a current of cool air. Examine the plate under long-wave-length UV light. Mark the principal and any secondary fluorescent spots. Spray the plate with *Detecting reagent*, and mark the principal and secondary blue spots. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: the sum of the intensities of secondary spots obtained from the *Test preparation* corresponds to not more than 5.0% of related compounds.

#### Assay—

**0.05 M Phosphate buffer, Mobile phase, and Chromatographic system**—Proceed as directed in the Assay under *Ergonovine Maleate Injection*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ergonovine Maleate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

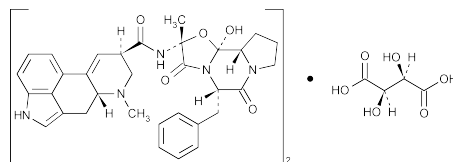
**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of ergonovine maleate, to a 50-mL volumetric flask, add 25 mL of *Mobile phase*, place in a sonic bath for 5 minutes, cool to room temperature, dilute with *Mobile phase* to volume, mix, and centrifuge. Use the supernatant as directed in the *Procedure*.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Ergonovine Maleate Injection*. Calculate the quantity, in mg, of  $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$(50C)(r_U / r_S)$$

in which the terms are as defined therein.

## Ergotamine Tartrate



$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  1313.41

Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'α)-, [R-(R\*,R\*)]-2,3-dihydroxy-butanedioate (2:1) (salt).

Ergotamine tartrate (2:1) (salt) [379-79-3].

» Ergotamine Tartrate contains not less than 97.0 percent and not more than 100.5 percent of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers in a cold place.

#### USP Reference standards (11)—

USP Ergotamine Tartrate RS

**Identification**—The chromatogram of the test solution prepared as directed in the test for *Related alkaloids* exhibits its principal fluorescent spot and principal blue spot at the same  $R_f$  value as the principal spot of *Standard solution A*.

**Specific rotation** (781S)—[NOTE—For this test, use chloroform from which any alcohol present has been removed by prior washing with water.] Dissolve about 350 mg in 25 mL of tartaric acid solution (1 in 100) contained in a separator, then add 500 mg of sodium bicarbonate, and mix gently but thoroughly. Add 10 mL of chloroform, shake vigorously, and after the layers have separated draw off the chloroform phase through a small filter, previously moistened with chloroform, into a 50-mL volumetric flask. Rapidly continue the extraction with three 10-mL portions of chloroform, passing the extracts through the same filter. Place the flask in a bath at 20° for 10 minutes. Adjust the volume of extract to 50.0 mL at 20° by the addition of chloroform. Mix the solution, and determine the angular rotation at 20°. Determine the concentration of ergotamine in the chloroform solution by evaporating a 25.0-mL aliquot of the solution on a rotary evaporator to dryness, maintaining the temperature of the bath below 45°. Dissolve the residue in 25 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.05 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 29.08 mg of  $C_{33}H_{35}N_5O_5$ . From the angular rotation of the solution and the concentration of ergotamine base, calculate the specific rotation of the base: between −155° and −165°.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

**Related alkaloids**—[NOTE—Conduct this test without exposure to daylight and with the minimum necessary exposure to artificial light.]

**Solvent mixture**—Mix 9 volumes of chloroform with 1 volume of methanol.

**Standard preparation and Standard dilutions**—Prepare a solution of USP Ergotamine Tartrate RS in *Solvent mixture* to contain 10.0 mg per mL (*Standard preparation*). Prepare a series of dilutions of the *Standard preparation* in *Solvent mixture* to contain 0.2 mg, 0.1 mg, 0.05 mg, and 0.025 mg per mL (*Standard dilutions*) corresponding to 2.0%, 1.0%, 0.5%, and 0.25% of the *Standard preparation*, respectively.

**Test preparation**—Dissolve 50.0 mg of Ergotamine Tartrate in 5.0 mL of *Solvent mixture*.

**Procedure**—In a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* (621)) place a volume of a solvent system consisting of ether, dimethylformamide, chloroform, and dehydrated alcohol (70:15:10:5). Line the chamber with filter paper, and allow it to equilibrate for 15 minutes. Apply 5- $\mu$ L portions of the *Test preparation*, the *Standard preparation*, and each of the *Standard dilutions* to a suitable chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Place each spot over an opened bottle of ammonium hydroxide for 20 seconds, then allow the plate to dry in a current of cold air for 20 seconds. Develop the chromatogram until the solvent front has moved about 17 cm. Remove the plate from the developing chamber, allow the solvent to evaporate in a current of cold air for approximately 2 minutes, and spray with a freshly prepared solution of 200 mg of *p*-(dimethylamino)benzaldehyde in a mixture of 5.5 mL of hydrochloric acid and 4.5 mL of water. Dry the plate at 60° for about 5 minutes and compare the chromatograms: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation*, the sum of the intensities of any secondary spots in the chromatogram from the *Test preparation* is not greater than the intensity of the principal spot from the 2.0% *Standard dilution*, and the intensity of not more than one of the secondary spots is greater than that of the principal spot from the 1.0% *Standard dilution*.

**Assay**—Transfer about 200 mg of Ergotamine Tartrate, accurately weighed, to a small conical flask, and dissolve in 15 mL of a mixture of 6 volumes of acetic anhydride and 100 volumes of glacial acetic acid. Add 1 drop of crystal violet TS, and titrate with 0.05 N perchloric acid VS from a 10-mL buret. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 32.84 mg of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ .

## Ergotamine Tartrate Inhalation Aerosol

» Ergotamine Tartrate Inhalation Aerosol is a suspension of microfine Ergotamine Tartrate in propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergotamine tartrate  $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ .

**Packaging and storage**—Preserve in small, non-reactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

### USP Reference standards (11)—

USP Ergotamine Tartrate RS

**Identification**—Place 10 mL of water in a small beaker, and deliver 2 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To one part of the resulting solution in a test tube add 2 parts of *p*-(dimethylamino)-benzaldehyde TS, and mix: a blue color is produced.

**Delivered dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

### PROCEDURE FOR DOSE UNIFORMITY—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ergotamine Tartrate RS in a tartaric acid solution (1 in 500), and dilute quantitatively and stepwise with the same tartaric acid solution as necessary to obtain a solution having a known concentration of about 18  $\mu$ g per mL.

**Test preparation**—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a tartaric acid solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of trichlorotrifluoroethane, insert the stopper, shake vigorously for 1 minute and centrifuge for 5 minutes. Use the clear supernatant as the *Test preparation*.

**Procedure**—Into three separate flasks transfer 6.0 mL each of the *Test preparation*, the *Standard preparation*, and a tartaric acid solution (1 in 500) to provide the blank. To each flask add 10.0 mL of *p*-dimethylaminobenzaldehyde TS, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 546 nm, against the blank. Calculate the quantity, in  $\mu$ g, of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  contained in the minimum dose taken by the formula:

$$(20CN)(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Particle size**—Prime the valve of the Inhalation Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of the spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450 $\times$  magnification. Focus on the particles of 25 fields of view near the center of the test specimen pattern, and note the size of the great majority of individual particles: they are less than 5  $\mu$ m in diameter. Record the number and size of all individual crystalline particles (not agglomerates) more than 10  $\mu$ m in length measured along the longest axis: not more than 10 such particles are observed.

### Assay—

**Standard preparation**—Prepare as directed under *Unit spray content*.

**Assay preparation**—[NOTE—A suitable specimen beaker is one having a small indentation formed on its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the delivery of the specimen.] Place 10 mL of trichlorotrifluoroethane in a suitable 100-mL beaker. Prime the valve of Ergotamine Tartrate Inhalation Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the trichlorotrifluoroethane, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the trichlorotrifluoroethane, and shake it gently preparatory to delivering another spray similarly under the surface of the trichlorotrifluoroethane. Deliver a total of 2 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of trichlorotrifluoroethane, collecting the rinsing with the specimen in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 2 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of trichlorotrifluoroethane, and add 10.0 mL of tartaric acid solution (1 in 500). Insert the stopper, shake vigorously for 15 minutes, centrifuge for 5 minutes, and use the clear supernatant as the *Assay preparation*.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and a blank consisting of tartaric acid solution (1 in 500) to separate test tubes. To each tube add 10.0 mL of *p*-dimethylaminobenzaldehyde TS, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions against the blank in 1-cm cells at the wavelength of maximum absorbance at about 546 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  in each mL of the Aerosol taken by the formula:

$$(0.01Cd / W)(A_U / A_S)$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*; *d* is the density, in g per mL, of Aerosol determined as directed for *d* in the *Procedure* in the *Assay under Isoproterenol Sulfate Inhalation Aerosol*; *W* is the weight, in g, of the specimen taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ergotamine Tartrate Injection

» Ergotamine Tartrate Injection is a sterile solution of Ergotamine Tartrate and the tartrates of its epimer, ergotamine, and of other related alkaloids, in Water for Injection to which Tartaric Acid or suitable stabilizers have been added. The total alkaloid content, in each mL, is not less than 450  $\mu\text{g}$  and not more than 550  $\mu\text{g}$ . The content of ergotamine tartrate  $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$  is not less than 52.0 percent and not more than 74.0 percent of the content of total alkaloid; the content of ergotamine tartrate is not more than 45.0 percent of the content of total alkaloid.

**Packaging and storage**—Preserve in single-dose, light-resistant containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Endotoxin RS

USP Ergotamine Tartrate RS

**Bacterial endotoxins** (85)—It contains not more than 357.0 USP Endotoxin Units per mg of ergotamine tartrate.

**pH** (791): between 3.5 and 4.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Chloroform**—Use chloroform that recently has been saturated with water.

**Standard preparation**—Dissolve about 10 mg of USP Ergotamine Tartrate RS, accurately weighed, with warming if necessary, in 50 mL of diluted alcohol and sufficient water to give a concentration of 50.0  $\mu\text{g}$  per mL.

**Ergotamine preparation and Ergotamine preparation**—Pipet a volume of Injection, equivalent to about 5 mg of ergotamine tartrate, into a beaker. Add 5 mL of *Chloroform* and a portion of sodium bicarbonate approximately equivalent in weight to one-tenth that of the portion of Injection taken. Mix, and add sufficient chromatographic siliceous earth to make a fluffy mixture (about 1 g for each mL of the Injection taken plus 3 g in addition). Pack the mixture in a chromatographic tube about 2.5 cm in diameter and about 30 cm in length. Rinse the sides of the beaker with 2 mL of

*Chloroform*. Add sufficient chromatographic siliceous earth to make a fluffy mixture, and transfer it to the column.

Prepare a second column using a mixture of 9 g of the siliceous earth with 7 mL of citric acid solution (1 in 4). Place a mixture of 2 g of the siliceous earth and 2 mL of water on top of the second column. Insert a pledget of glass wool, and mount the tube containing the specimen so that the eluate from it will drain into the tube containing the citric acid solution. Add a total of 90 mL of *Chloroform* to the upper tube, and receive the eluate from the lower tube in a 200-mL volumetric flask. Rinse the tip of the upper tube with *Chloroform*. Pass sufficient *Chloroform* through the lower tube to dilute the eluate to volume. This eluate is the *Ergotamine preparation*.

Extrude the adsorbent from the second column by means of slight air pressure into a 600-mL beaker containing 10 g of sodium bicarbonate, and mix. Cautiously add 50 mL of water, with continuous stirring. Wash the mixture with water into a 250-mL separator, and extract the ergotamine with four 15-mL portions of *Chloroform*. Pass the extracts through a glass wool filter, combining them in a 100-mL volumetric flask, wash the filter, and dilute with *Chloroform* to volume. This is the *Ergotamine preparation*.

Pipet 10 mL of the *Ergotamine preparation* and 20 mL of the *Ergotamine preparation* into separate, small conical flasks, and evaporate with the aid of a current of air to dryness.

**Total alkaloid preparation**—Pipet a volume of Injection, equivalent to about 2.5 mg of ergotamine tartrate, into a 50-mL volumetric flask, add 25 mL of alcohol, then add tartaric acid solution (1 in 100) to volume.

**Procedure**—Pipet 5 mL each of the *Standard preparation* and the *Total alkaloid preparation* into separate, small conical flasks. To the dried residues of *Ergotamine preparation* and *Ergotamine preparation* add 5.0 mL of a freshly prepared solution of equal volumes of alcohol and tartaric acid solution (1 in 100). In turn, place each flask in an ice bath, and swirl continuously while adding, dropwise, 10.0 mL of *p*-dimethylaminobenzaldehyde TS. Allow to stand in subdued light at room temperature for not less than 90 minutes and not more than 2 hours. Concomitantly determine the absorbances of the four solutions at the wavelength of maximum absorbance at about 545 nm, with a suitable spectrophotometer, against a reagent blank.

**Calculation**—Calculate the quantity of total alkaloids in terms of mg of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  in the volume of Injection taken by the formula:

$$0.05C(A_U / A_S)$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of *Ergotamine preparation* in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Total alkaloid preparation* and the *Standard preparation*, respectively.

Calculate the percentage of ergotamine tartrate taken by the formula:

$$(A' / A_U)50$$

in which *A'* represents the absorbance of the solution from the *Ergotamine preparation* and  $A_U$  is as defined in the preceding paragraph. Calculate the percentage of ergotamine tartrate taken by the formula:

$$(A'' / A_U)50$$

in which *A''* represents the absorbance of the solution from the *Ergotamine preparation*.

## Ergotamine Tartrate Tablets

» Ergotamine Tartrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label the Tablets to indicate that they are intended for swallowing intact.

**USP Reference standards** (11)—  
USP Ergotamine Tartrate RS

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 5 mg of ergotamine tartrate, with 10 mL of solvent hexane for a few minutes, allow to settle, and discard the solvent hexane extract. Add to the residue 10 mL of chloroform saturated with ammonia (prepared by shaking chloroform with ammonium hydroxide, then drawing off the chloroform layer), triturate for a few minutes, filter, and evaporate the filtrate on a steam bath to dryness. Dissolve the residue in a mixture of 4 mL of glacial acetic acid and 4 mL of ethyl acetate. To 1 mL of this solution add slowly, with continuous agitation and cooling, 1 mL of sulfuric acid: a blue color with a red tinge develops. Add 0.1 mL of ferric chloride TS, previously diluted with an equal volume of water: the red tinge becomes less apparent and the blue color more pronounced.

**Dissolution** (711)—

*Medium*: tartaric acid solution (1 in 100); 1000 mL.

*Apparatus* 2: 75 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  dissolved from fluorescence intensities, using the maximum excitation wavelength at about 327 nm and the maximum emission wavelength at about 427 nm, on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ergotamine Tartrate RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.01 M monobasic potassium phosphate (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 40 mg of ergonovine maleate to a 250-mL volumetric flask, add a mixture of acetonitrile and water (55:45) to volume, and mix.

**Standard preparation**—Transfer about 10 mg of USP Ergotamine Tartrate RS, accurately weighed, to a 50-mL volumetric flask, add a mixture of acetonitrile and water (55:45) to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with the mixture of acetonitrile and water (55:45) to volume, and mix to obtain a solution having a known concentration of about 0.02 mg of USP Ergotamine Tartrate RS per mL.

**Assay preparation**—Transfer a number of whole Tablets, equivalent to about 10 mg of ergotamine tartrate, to a 500-mL volumetric flask. Add 50.0 mL of *Internal standard solution*, 300 mL of a mixture of acetonitrile and water (55:45), and sonicate for about 10 minutes. Dilute with the mixture of acetonitrile and water (55:45) to volume, and

mix. Filter through a 0.45-μm membrane disk, discarding the first 25 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for ergonovine maleate and 1.0 for ergotamine tartrate; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0; the column efficiency determined from the analyte peak is not less than 3000 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  in the portion of Ergotamine Tartrate Tablets taken by the formula:

$$500C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ergotamine Tartrate Sublingual Tablets

» Ergotamine Tartrate Sublingual Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label Tablets to indicate that they are intended for sublingual administration.

**USP Reference standards** (11)—  
USP Ergotamine Tartrate RS

**Identification**—Triturate a quantity of finely powdered Sublingual Tablets, equivalent to about 5 mg of ergotamine tartrate, with 10 mL of solvent hexane for a few minutes, allow to settle, and discard the solvent hexane extract. Add to the residue 10 mL of chloroform saturated with ammonia (prepared by shaking chloroform with ammonium hydroxide, then drawing off the chloroform layer), triturate for a few minutes, filter, and evaporate the filtrate on a steam bath to dryness. Dissolve the residue in a mixture of 4 mL of glacial acetic acid and 4 mL of ethyl acetate. To 1 mL of this solution add slowly, with continuous agitation and cooling, 1 mL of sulfuric acid: a blue color with a red tinge develops. Add 0.1 mL of ferric chloride TS, previously diluted with an equal volume of water: the red tinge becomes less apparent and the blue color more pronounced.

**Disintegration** (701): 5 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.01 M monobasic potassium phosphate (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).



**Internal standard solution**—Transfer about 40 mg of ergonovine maleate to a 250-mL volumetric flask, add a mixture of acetonitrile and water (55:45) to volume, and mix.

**Standard preparation**—Transfer about 10 mg of USP Ergotamine Tartrate RS, accurately weighed, to a 50-mL volumetric flask, add a mixture of acetonitrile and water (55:45) to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with the mixture of acetonitrile and water (55:45) to volume, and mix to obtain a solution having a known concentration of about 0.02 mg of USP Ergotamine Tartrate RS per mL.

**Assay preparation**—Transfer a number of whole Sublingual Tablets, equivalent to about 10 mg of ergotamine tartrate, to a 500-mL volumetric flask. Add 50.0 mL of *Internal standard solution*, 300 mL of a mixture of acetonitrile and water (55:45), and sonicate for about 10 minutes. Dilute with the mixture of acetonitrile and water (55:45) to volume, and mix. Filter through a 0.45- $\mu$ m membrane disk, discarding the first 25 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for ergonovine maleate and 1.0 for ergotamine tartrate; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0; the column efficiency determined from the analyte peak is not less than 3000 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$  in the portion of Sublingual Tablets taken by the formula:

$$500C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*, and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ergotamine Tartrate and Caffeine Suppositories

» Ergotamine Tartrate and Caffeine Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of ergotamine tartrate  $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$  and of caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers, at a temperature not above 25°. Do not expose unwrapped Suppositories to sunlight.

### USP Reference standards <11>—

USP Caffeine RS  
USP Ergotamine Tartrate RS  
USP Ergotaminine RS

**Identification**—Melt 1 Suppository in 10 mL of hot tartaric acid solution (1 in 100), and mix. Chill the mixture until the layer of oil has hardened, then filter, divide the filtrate into two parts, and use this filtrate for the following tests.

**A:** To one part of the filtrate add 10 mL of *p*-dimethylaminobenzaldehyde TS: a blue color develops (*presence of ergotamine*).

**B:** Transfer the remaining part of the filtrate to a small evaporating dish, evaporate on a steam bath to dryness, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, and evaporate. Invert the dish over a vessel containing ammonium hydroxide: the residue acquires a purple color, which disappears upon the addition of 1 N sodium hydroxide (*presence of caffeine*).

**Assay**—[NOTE—Protect all solutions from light.]

**Mobile phase A**—Prepare a degassed mixture of water, acetonitrile, and triethylamine (850:150:0.5), and adjust by the dropwise addition of fluorometric grade sulfuric acid to a pH of  $3.1 \pm 0.1$ .

**Mobile phase B**—Prepare a degassed mixture of water, acetonitrile, and triethylamine (1380:620:1), and adjust by the dropwise addition of fluorometric grade sulfuric acid to a pH of  $3.1 \pm 0.1$ . Make any necessary adjustments in *pH* to meet relative retention times, and make other adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Solvent mixture**—Mix equal volumes of methanol and tartaric acid solution (1 in 100).

**Ergotamine tartrate standard solution**—Using an accurately weighed amount of USP Ergotamine Tartrate RS, prepare a solution in *Solvent mixture* having a known concentration of about 40  $\mu$ g per mL.

**Mixed standard preparation**—Into a 10-mL volumetric flask pipet 5 mL of *Ergotamine tartrate standard solution*, and add about 10 mg of USP Caffeine RS, accurately weighed. Dilute with *Solvent mixture* to volume, and mix to obtain a solution having known concentrations of about 20  $\mu$ g of USP Ergotamine Tartrate RS per mL and about 1 mg of USP Caffeine RS per mL.

**System suitability preparation**—Pipet 5 mL of the *Mixed standard preparation* and 1 mL of a solution containing about 20  $\mu$ g of USP Ergotaminine RS per mL into a 10-mL volumetric flask. Dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh not fewer than 20 Suppositories, and grind to a fine mesh. Transfer a portion of the ground mass, equivalent to about 2 mg of ergotamine tartrate, to a suitable glass-stoppered flask. Add 100.0 mL of *Solvent mixture*, insert the stopper in the flask, and place it in a water bath maintained at 40°. Shake vigorously for 5 minutes, or longer if necessary, until the specimen is completely melted. Sonicate for 30 minutes, and transfer to a freezer for 45 minutes. Pass through a 0.7- $\mu$ m glass fiber filter, discarding the first 5 to 10 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 4.6-mm  $\times$  25-cm column that contains packing L7. The chromatograph is equipped with a 244-nm detector in series with a fluorometric detector operating at an excitation wavelength of 229 nm and an emission wavelength of 435 nm. Equilibrate the system with *Mobile phase A*. The flow rate is about 2 mL per minute. At 3 minutes after injection of a specimen, or after caffeine has eluted, whichever occurs last, switch to *Mobile phase B*, and at 23 minutes after the initial injection, return to *Mobile phase A*. Allow not less than 2 minutes to elapse between injections. Chromatograph the *Mixed standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between ergotamine and ergotaminine is not less than 3.0, and the relative standard deviation for replicate injections of the *Mixed standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Mixed standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative re-

tention times are about 4 for ergotamine, 4.5 for ergotamine, and 1.0 for caffeine. Calculate the quantity, in mg, of caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Suppositories taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Caffeine RS in the *Mixed standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Mixed standard preparation*, respectively. Calculate the quantity, in mg, of ergotamine tartrate [ $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ ] in the portion of Suppositories taken by the formula:

$$0.1C(I_U / I_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Ergotamine Tartrate RS in the *Mixed standard preparation*; and  $I_U$  and  $I_S$  are the fluorometric responses obtained from the *Assay preparation* and the *Mixed standard preparation*, respectively.

## Ergotamine Tartrate and Caffeine Tablets

» Ergotamine Tartrate and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of ergotamine tartrate [ $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ ] and of caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Caffeine RS  
USP Ergotamine Tartrate RS  
USP Ergotamine RS

**Identification**—Crush 1 Tablet, shake with 10 mL of chloroform and 3 drops of ammonium hydroxide, and filter. Divide the filtrate into two parts in small evaporating dishes, evaporate on a steam bath to dryness, and use the residues for the following tests.

**A:** Mix one of the residues with 5 mL of tartaric acid solution (1 in 100), and add 10 mL of *p*-dimethylaminobenzaldehyde TS: a blue color develops (*presence of ergotamine*).

**B:** To the other residue add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, and evaporate on a steam bath to dryness. Invert the dish over a vessel containing ammonium hydroxide: the residue acquires a purple color, which disappears upon the addition of 1 N sodium hydroxide (*presence of caffeine*).

### Dissolution (711)—

**Medium:** tartaric acid solution (1 in 100); 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 30 minutes.

**Standard preparation**—Using suitable quantities of USP Ergotamine Tartrate RS and USP Caffeine RS, accurately weighed, prepare a solution, using the *Medium*, having known concentrations of about 1  $\mu$ g of ergotamine tartrate and 100  $\mu$ g of caffeine in each mL.

**Procedure**—Measure the amount of caffeine in solution on filtered portions of the *Medium*, suitably diluted, at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, in comparison with the *Standard preparation*. Similarly, measure the amount of ergotamine tartrate in solution on filtered portions of the *Me-*

*dium*, suitably diluted, in a suitable fluorometer, using 327 nm as the excitation wavelength and 427 nm as the emission wavelength, in comparison with the *Standard preparation*.

**Tolerances**—Not less than 70% (Q) of the labeled amount of ergotamine tartrate [ $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ ] and not less than 75% (Q) of the labeled amount of caffeine ( $C_8H_{10}N_4O_2$ ) is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Assay**—[NOTE—Protect all solutions from light.]

**Mobile phase A**—Prepare a degassed mixture of water, acetonitrile, and triethylamine (850:150:0.5), and adjust by the dropwise addition of fluorometric grade sulfuric acid to a pH of  $2.7 \pm 0.1$ .

**Mobile phase B**—Prepare a degassed mixture of water, acetonitrile, and triethylamine (1380:620:1), and adjust by the dropwise addition of fluorometric grade sulfuric acid to a pH of  $2.7 \pm 0.1$ . Make necessary adjustments in pH with sodium hydroxide solution (1 in 20) or with fluorometric grade sulfuric acid to meet relative retention times, and make other adjustments if necessary (see *System Suitability under Chromatography (621)*).

**Solvent mixture**—Transfer 10 g of tartaric acid to a 1-L volumetric flask, add 500 mL of water, and mix with shaking. Add 330 mL of alcohol, and mix. Dilute with water to volume, and mix. Use a freshly prepared mixture.

**Ergotamine tartrate standard solution**—Using an accurately weighed amount of USP Ergotamine Tartrate RS, prepare a solution in *Solvent mixture* having a known concentration of about 40  $\mu$ g per mL.

**Caffeine standard solution**—Using an accurately weighed amount of USP Caffeine RS, prepare a solution in *Solvent mixture* having a known concentration of about 4 mg per mL.

**Mixed standard preparation**—Pipet 10 mL of *Ergotamine tartrate standard solution* and 10 mL of *Caffeine standard solution* into a 100-mL volumetric flask. Dilute with *Solvent mixture* to volume, and mix to obtain a solution having known concentrations of 4  $\mu$ g of USP Ergotamine Tartrate RS per mL and 0.4 mg of USP Caffeine RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of ergotamine tartrate, to a 250-mL volumetric flask. Add 150 mL of *Solvent mixture* and 20 drops of benzalkonium chloride solution (1 in 2). Shake by mechanical means for 45 minutes. [NOTE—Two to 3 mL of methanol may be added, if necessary, to break up bubbles that form during shaking.] Dilute with *Solvent mixture* to volume, and mix. Filter through a 0.5- $\mu$ m membrane, discarding the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate into a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**System suitability preparation**—Pipet 20 mL of *Caffeine standard solution*, 20 mL of *Ergotamine tartrate standard solution*, and 4 mL of a solution containing 20  $\mu$ g of USP Ergotamine RS per mL of *Solvent mixture*, into a 200-mL volumetric flask. Dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system** (see *Chromatography (621)*)—The liquid chromatograph is equipped with a 4.6-mm  $\times$  25-cm column that contains packing L7. The chromatograph is equipped with a 254-nm detector in series with a fluorometric detector operating at an excitation wavelength of 325 nm and an emission wavelength of 435 nm. Equilibrate the system with *Mobile phase A*. The flow rate is about 2 mL per minute. At 3 minutes after injection of a specimen, or after caffeine has eluted, whichever occurs last, switch to *Mobile phase B*, and at 18 minutes after initial injection, return to *Mobile phase A*. Wait not less than 2 minutes between injections. Chromatograph the *Mixed standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the tailing factor

for the ergotamine peak is not more than 2.0; the resolution,  $R$ , between ergotamine and ergotamine is not less than 3.0; and the relative standard deviation for replicate injections of the *Mixed standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Mixed standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 3.5 for ergotamine, 4 for ergotamine, and 1.0 for caffeine. Calculate the quantity, in mg, of caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken by the formula:

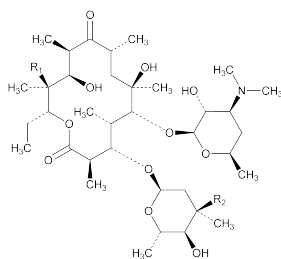
$$2500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Caffeine RS in the *Mixed standard preparation*; and  $r_U$  and  $r_S$  are the 254-nm peak responses obtained from the *Assay preparation* and the *Mixed standard preparation*, respectively. Calculate the quantity, in mg, of ergotamine tartrate [ $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ ] in the portion of Tablets taken by the formula:

$$2.5C(I_U / I_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Ergotamine Tartrate RS in the *Mixed standard preparation*; and  $I_U$  and  $I_S$  are the fluorometric responses obtained from the *Assay preparation* and the *Mixed standard preparation*, respectively.

## Erythromycin



Erythromycin	$R_1$	$R_2$
A	OH	OCH <sub>3</sub>
B	H	OCH <sub>3</sub>
C	OH	OH

$C_{37}H_{67}NO_{13}$  733.93

Erythromycin.

(3*R*\*,4*S*\*,5*S*\*,6*R*\*,7*R*\*,9*R*\*,11*R*\*,12*R*\*,13*S*\*,14*R*\*)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribohexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylohexopyranosyl]oxy]oxacyclotetradecane-2,10-dione [114-07-8].

» Erythromycin consists primarily of erythromycin A ( $C_{37}H_{67}NO_{13}$ ). The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is not less than 85.0 percent and not more than 100.5 percent, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin B RS

USP Erythromycin C RS

USP Erythromycin Related Compound N RS

N-Demethylerythromycin A.

$C_{36}H_{65}NO_{13}$  719.91

**Identification**—The IR absorption spectrum of a solution of it containing 50 mg per mL, previously dried at a pressure not exceeding 5 mm of mercury at 60° for 3 hours, in chloroform, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Erythromycin RS, except in the region between 1980  $cm^{-1}$  and 2050  $cm^{-1}$ .

**Specific rotation** (781S): between  $-71^\circ$  and  $-78^\circ$ , determined after standing for 30 minutes.

**Test solution**: 20 mg per mL, in dehydrated alcohol.

**Crystallinity** (695): meets the requirements.

**Water**, Method I (921): not more than 10.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Residue on ignition** (281): not more than 0.2%.

**Limit of thiocyanate**—

**Standard solutions**—Transfer about 100 mg of potassium thiocyanate, previously dried at 105° for 1 hour, cooled, and accurately weighed, to each of two 50-mL volumetric flasks. Add about 20 mL of methanol to each flask, swirl to dissolve, dilute with methanol to volume, and mix. Transfer 5.0 mL of each of these stock solutions to separate 50-mL volumetric flasks, dilute with methanol to volume, and mix. Transfer 5.0 mL of each of these intermediate solutions to separate 50-mL low-actinic volumetric flasks. To each flask add 1.0 mL of ferric chloride TS, dilute with methanol to volume, and mix. [NOTE—Use these *Standard solutions* within 30 minutes.]

**Test solution**—Transfer about 100 mg of Erythromycin, accurately weighed, to a 50-mL low-actinic volumetric flask. Add 20 mL of methanol, and swirl to dissolve. Add 1.0 mL of ferric chloride TS, dilute with methanol to volume, and mix. [NOTE—Use this *Test solution* within 30 minutes.]

**Blank solution**—Transfer 1.0 mL of ferric chloride TS to a 50-mL low-actinic volumetric flask, dilute with methanol to volume, and mix. [NOTE—Use this *Blank solution* within 30 minutes.]

**Procedure**—Determine the absorbances of each *Standard solution* and the *Test solution* at the wavelength of maximum absorbance at about 492 nm with a spectrophotometer, using the *Blank solution* to zero the instrument. Calculate the suitability value,  $S$ , by the formula:

$$(A_1 / W_1)(W_2 / A_2)$$

in which  $A_1$  and  $A_2$  are the absorbance values obtained from the respective *Standard solutions*; and  $W_1$  and  $W_2$  are the weights, in mg, of the potassium thiocyanate taken to prepare the corresponding *Standard solutions*. In a suitable determination, the value,  $S$ , is not less than 0.985 and not more than 1.015. Calculate the percentage of thiocyanate in the Erythromycin taken by the formula:

$$(58.08/97.18)(A_U / W_U)(0.5)[(W_1 / A_1) + (W_2 / A_2)]$$

in which 58.08 and 97.18 are the molecular weights of the thiocyanate moiety and of potassium thiocyanate, respectively;  $A_U$  is the absorbance of the *Test solution*;  $W_U$  is the weight, in mg, of Erythromycin taken to prepare the *Test solution*; and the other terms are as defined above: not more than 0.3% is found.

**Limit of related substances**—Using the chromatograms of the *Assay preparation* and the *Diluted standard preparation* obtained in the *Assay*, calculate the percentage of any individual related substance observed having the greatest response, other than erythromycin A, erythromycin B, erythromycin C, and erythromycin A enol ether, in the Erythromycin taken by the formula:

$$25(CP / W)(r_i / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Erythromycin RS in the *Diluted standard preparation*;  $P$  is the

designated percentage of erythromycin A in the USP Erythromycin RS;  $W$  is the weight, in mg, of Erythromycin taken to prepare the *Assay preparation*;  $r_i$  is the peak response of an individual related substance, other than erythromycin A, erythromycin B, erythromycin C, or erythromycin A enol ether, observed in the chromatogram obtained from the *Assay preparation*; and  $r_s$  is the erythromycin A peak response in the chromatogram obtained from the *Diluted standard preparation*: not more than 3.0% of any individual related substance is found. Calculate the percentage of erythromycin A enol ether in the Erythromycin taken by the formula:

$$(25 / 11)(CP / W)(r_E / r_s)$$

in which 11 is the response factor for erythromycin A enol ether in relation to that of erythromycin A;  $r_E$  is the peak response of the erythromycin A enol ether peak observed in the chromatogram obtained from the *Assay preparation*; and the other terms are as defined above: not more than 3.0% of erythromycin A enol ether is found. The percentage of erythromycin B obtained in the *Assay* is not more than 12.0%; and the percentage of erythromycin C obtained in the *Assay* is not more than 5.0%.

#### Assay—

**Solution A**—Dissolve 1.75 g of dibasic potassium phosphate in 50 mL of water, adjust with diluted phosphoric acid (1 in 10) or 0.2 N sodium hydroxide to a pH of 9.0, add 400 mL of water, 165 mL of tertiary butyl alcohol, and 30 mL of acetonitrile. Dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a mixture of *Solution A*, acetonitrile, and water (5:2:1). Make any necessary adjustments (see *System Suitability under Chromatography* <621>).

**Diluent**—Prepare a mixture of pH 7.0 buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and methanol (15:1).

**pH 3.5 Buffer**—Adjust 20 mL of pH 7.0 buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) with phosphoric acid to a pH of 3.5.

**NOTE**—Use the following solutions promptly, or within 1 day if stored in a refrigerator.

**Standard preparation**—Transfer about 100 mg of USP Erythromycin RS, accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, dilute with *Diluent* to volume, and mix.

**Diluted standard preparation**—Transfer 3.0 mL of the *Standard preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.12 mg of USP Erythromycin RS per mL.

**Erythromycins B and C standard solution**—Transfer about 5 mg each of USP Erythromycin B RS and USP Erythromycin C RS, both accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, dilute with *Diluent* to volume, and mix.

**Resolution solution**—Transfer about 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of *Standard preparation*, dilute with *Erythromycins B and C standard solution* to volume, and mix.

**Erythromycin A enol ether retention time solution**—Dissolve about 10 mg of USP Erythromycin RS in 2 mL of methanol. Add 10 mL of pH 3.5 Buffer, mix, and allow to stand for about 30 minutes.

**Assay preparation**—Transfer about 100 mg of Erythromycin, accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, and swirl to dissolve. Dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains packing L21 (1000 Å) and is maintained at a constant temperature of about 65°. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak re-

sponses as directed for *Procedure*: the relative retention times are about 0.56 for erythromycin related compound N (N-demethylethromycin A), 0.61 for erythromycin C, 1.0 for erythromycin A, and 1.6 for erythromycin B; and the resolution,  $R$ , between erythromycin related compound N and erythromycin C is not less than 0.8, and between erythromycin related compound N and erythromycin A not less than 5.5. Chromatograph the *Erythromycin A enol ether retention time solution*, and record the peak responses as directed for *Procedure*: the retention time of the erythromycin A enol ether peak is about 3.2 relative to that of the erythromycin A peak as observed in the chromatogram obtained from the *Resolution solution*. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard preparation*, the *Diluted standard preparation*, the *Erythromycins B and C standard solution*, and the *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is adequate to include the erythromycin A enol ether peak, if present, as determined in the chromatogram obtained from the *Erythromycin A enol ether retention time solution* (about five times the retention time of the main erythromycin A peak). Measure the areas of the peak responses. Calculate the percentage of erythromycin A in the portion of Erythromycin taken by the formula:

$$25(C_A P / W)(r_U / r_A)$$

in which  $C_A$  is the concentration, in mg per mL, of USP Erythromycin RS in the *Standard preparation*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in mg, of Erythromycin taken to prepare the *Assay preparation*; and  $r_U$  and  $r_A$  are the erythromycin A peak responses in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentages of erythromycin B and erythromycin C in the portion of Erythromycin taken by the formula:

$$25(C_S P / W)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Erythromycins B and C standard solution*;  $P$  is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard;  $W$  is the quantity, in mg, of Erythromycin taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses of the relevant analyte in the chromatograms obtained from the *Assay preparation* and the *Erythromycins B and C standard solution*, respectively.

## Erythromycin Delayed-Release Capsules

» Erythromycin Delayed-Release Capsules contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Erythromycin RS

**Identification**—Prepare a test solution by mixing a quantity of finely ground Capsule contents with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 2.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see

*Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Dissolution** (711)—Proceed as directed for *Procedure for Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

*Apparatus 1*: 50 rpm.

*Times*: 60 minutes for *Acid Stage*; 60 minutes for *Buffer Stage*.

**Procedure**—Transfer the contents of 1 Capsule to the apparatus. Proceed as directed for *Acid Stage*, 900 mL of 0.06 N hydrochloric acid being placed in the vessel instead of 1000 mL of 0.1 N hydrochloric acid, and the apparatus operated for 60 minutes instead of 2 hours. Do not perform an analysis at the end of the *Acid stage*. Continue as directed for *Buffer Stage*, 900 mL of the pH 6.8 phosphate buffer being used instead of 1000 mL. Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved after 120 minutes by assaying a filtered portion of the solution under test as directed under *Antibiotics—Microbial Assays* (81).

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{37}H_{67}NO_{13}$  is dissolved in 120 minutes.

**Water, Method I** (921): not more than 7.5%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using not less than 5 Capsules blended for about 3 minutes in a high-speed glass blender jar containing 200 mL of methanol. Add 300 mL of *Buffer No. 3*, and blend again for about 3 minutes. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Topical Gel

» Erythromycin Topical Gel is Erythromycin in a suitable gel vehicle. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Prepare a test solution as follows. To 1 g of Topical Gel in a 50-mL screw-capped tube, add 20 mL of 0.01 N hydrochloric acid, and heat in a water bath to reflux. Remove the tube from the water bath and shake it. Place the tube in the water bath again, and heat to reflux. Remove the tube from the water bath, and immediately decant a portion of the hot clear supernatant into a test tube. Allow to cool, add an equal volume of a mixture of methanol, water, and triethylamine (90:9:1), and mix. Concomitantly prepare a Standard solution as directed above, except to use 5 mg of USP Erythromycin RS and 5 mL of 0.01 N hydrochloric acid instead of 1 g of Topical Gel and 20 mL of 0.01 N hydrochloric acid. Separately apply 5  $\mu$ L of the test solution and the Standard solution to a thin-layer chromato-

graphic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol, water, and triethylamine (90:9:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms. The spots due to erythromycin are black to purple in color: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Minimum fill** (755): meets the requirements.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Topical Gel, equivalent to about 20 mg of erythromycin, blended for about 3 minutes in a high-speed glass blender jar containing 200.0 mL of *Buffer No. 3* to which has been added 0.5% of polysorbate 80. Dilute an accurately measured volume of the blended solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Intramammary Infusion

» Erythromycin Intramammary Infusion is a solution of Erythromycin in a suitable vegetable oil vehicle. It contains one or more suitable preservatives. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

**Packaging and storage**—Preserve in single-dose disposable syringes that are well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Transfer a quantity of Intramammary Infusion, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Transfer the contents of a syringe of Intramammary Infusion to a separator containing 50 mL of solvent hexane. Shake until dissolved. Wash with four separate 20-mL portions of a mixture of methanol and water (4:1). Combine the washings in a 100-mL volumetric flask, dilute with the methanol and water solution to volume, and mix. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Reference Standard.

## Erythromycin Injection

» Erythromycin Injection is a sterile solution of Erythromycin in a polyethylene glycol vehicle. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

**Packaging and storage**—Preserve in multiple-dose containers.

**Labeling**—Label it to indicate that it is for veterinary use only. Label it to state that it is for intramuscular administration only.

**USP Reference standards** (11)—  
USP Erythromycin RS

**Identification**—Transfer a quantity of Injection, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Sterility** (71)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Wash with four separate 20-mL portions of a mixture of methanol and water (4:1). Combine the washings in a 100-mL volumetric flask, dilute with the methanol and water solution to volume, and mix. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Standard.

## Erythromycin Ointment

» Erythromycin Ointment is Erythromycin in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in collapsible tubes or in other tight containers, preferably at controlled room temperature.

**USP Reference standards** (11)—

USP Erythromycin RS  
USP Erythromycin B RS  
USP Erythromycin C RS  
USP Erythromycin Related Compound N RS  
N-Demethylerythromycin A.  
 $C_{36}H_{65}NO_{13}$  719.91

### Identification—

**A:** Transfer a quantity of Ointment, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**B:** The retention times of the peaks for erythromycin A, erythromycin B, and erythromycin C in the chromatogram of the *Assay preparation* correspond to those in the chromatograms of the *Standard preparation* and *Erythromycins B and C standard preparation*, as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

### Assay—

**Solution A**—Prepare a degassed mixture of acetonitrile and water (90:10). Store in a reservoir protected from air by sparging with helium.

**Solution B**—To 1000 mL of degassed water add 0.5 mL of sodium hydroxide solution (1 in 2), using a suitable syringe and needle to minimize exposure to air. Degas, and store in a reservoir protected from air by sparging with helium.

**Solution C**—Use degassed water, and store in a reservoir protected from air by sparging with helium.

**Mobile phase**—Using a suitable pumping system, pump *Solution A*, *Solution C*, and *Solution B* from the respective reservoirs in the ratio of 56:37:7. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of methanol and water (50:50).

**Standard preparation**—Quantitatively prepare a solution of USP Erythromycin RS in *Diluent* having a known concentration of about 0.66 mg per mL.

**Erythromycins B and C standard preparation**—Quantitatively prepare a solution in *Diluent* having known concentrations of about 34 µg each of USP Erythromycin B RS and USP Erythromycin C RS per mL.

**System suitability solution**—Transfer about 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of the *Standard preparation* and 6 mL of *Erythromycins B and C standard preparation*, and mix. Dilute with *Erythromycins B and C standard preparation* to volume, and mix.

**Assay preparation**—Transfer an accurately weighed portion of Ointment, equivalent to about 60 mg of erythromycin, to a 125-mL separator. Add 50 mL of solvent hexane, and shake until dissolved. Extract with four separate 20-mL portions of *Diluent*, collecting the extracts in a 100-mL volumetric flask. Dilute the combined extracts with *Diluent* to volume, mix, and pass a portion of the solution through a filter having a 0.45-µm porosity. Use the clear filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with an electrochemical detector, a glassy carbon electrode fitted with three gaskets, a 4-mm × 5-cm guard column that contains 8-µm packing L50, and a 4-mm × 25-cm analytical column that contains 8-µm packing L50. The electrochemical detector is used in the pulsed integrated amperometric mode with a range of 10 nC, an output of 1 V full scale, a rise time of 0.6 second, positive polarity,  $E = 0.9$  V;  $t_1 = 400$  ms;  $E_2 = 0.9$  V;  $t_2 = 100$  ms;  $E_3 = -0.9$  V;  $t_3 = 100$  ms. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for erythromycin related compound N, 0.5 for erythromycin C, 1.0 for

erythromycin A, and 1.6 for erythromycin B; the resolution,  $R$ , between erythromycin related compound N and erythromycin C is not less than 0.6, between erythromycin C and erythromycin A not less than 2.5, and between erythromycin A and erythromycin B not less than 2.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%. [NOTE—Turn off the electrochemical detector before stopping the flow of the *Mobile phase*.]

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation*, *Erythromycins B and C standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of erythromycin A in the portion of Ointment taken by the formula:

$$0.1(CP/W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Erythromycin RS in the *Standard preparation*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in g, of Ointment taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the erythromycin A peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentages of erythromycin B and erythromycin C in the portion of Ointment taken by the formula:

$$0.0001(CP/W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of the relevant USP Reference Standard in the *Erythromycins B and C standard preparation*;  $P$  is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard;  $W$  is the quantity, in g, of Ointment taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak areas for the relevant analyte obtained from the *Assay preparation* and the *Erythromycins B and C standard preparation*, respectively. Calculate the percentage content of erythromycin in the Ointment by adding the percentages of erythromycin A, erythromycin B, and erythromycin C found.

## Erythromycin Ophthalmic Ointment

» Erythromycin Ophthalmic Ointment is a sterile preparation of Erythromycin in a suitable ointment base. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Transfer a quantity of Ophthalmic Ointment, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification test under Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Sterility** (71): It meets the requirements.

**Minimum fill** (755): meets the requirements.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

**Other requirements**—It meets the requirements for *Water and Assay* under *Erythromycin Ointment*.

## Erythromycin Pledgets

» Erythromycin Pledgets are suitable absorbent pads impregnated with Erythromycin Topical Solution. Pledgets contain not less than 90.0 percent of the labeled volume of Erythromycin Topical Solution.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Pledgets to indicate that each Pledget is to be used once and then discarded. Label Pledgets also to indicate the volume, in mL, of Erythromycin Topical Solution contained in each Pledget, and the concentration, in mg of erythromycin per mL, of the Erythromycin Topical Solution.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Prepare a test solution by shaking one Pledget with methanol, and diluting appropriately to obtain a concentration of about 2.5 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 2.5 mg per mL. Apply separately 10  $\mu$ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Other requirements**—The Erythromycin Topical Solution expressed from Pledgets meets the requirements for *Water and Alcohol content* under *Erythromycin Topical Solution*.

**Topical solution assay**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using the solution expressed from not less than 10 Pledgets. Dilute an accurately measured volume of this solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay and minimum volume**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), assaying 10 Pledgets individually. Transfer 1 Pledget to a suitable container, add about 50 mL of *Buffer No. 3*, and shake for 2 minutes. Transfer the solution so obtained to a 100-mL volumetric flask. Wash the Pledget with two 20-mL portions of *Buffer No. 3*, add the washings to the 100-mL volumetric flask, dilute with *Buffer No. 3* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Calculate the volume, in mL, of Erythromycin Topical Solution in each Pledget taken by dividing the number of mg of erythromycin in each individual Pledget by the number of mg of erythromycin in each mL of Erythromycin Topical Solution as obtained in the *Topical Solution assay*. The volume of Erythromycin Topical Solution in each Pledget is not less than 90.0% of the labeled amount. If this requirement is not met, determine the volume of Erythromycin Topical Solution in each of 20 additional Pledgets. The volume of Erythromycin Topical Solution in not more

than 1 of the 30 Pledgets is less than 90.0% of the labeled amount.

## Erythromycin Topical Solution

» Erythromycin Topical Solution is a solution of Erythromycin in a suitable vehicle. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Prepare a test solution by mixing a portion of the Topical Solution with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Water, Method I** (921): not more than 8.0% if it contains 20 mg per mL, or not more than 5.0% if it contains 15 mg per mL, or not more than 2.0% if it contains acetone, 20 mL of a mixture of pyridine and methanol (1:1) being used in place of methanol in the titration vessel.

**Alcohol content, Method II** (611): between 92.5% and 107.5% of the labeled amount of  $C_2H_5OH$ .

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Topical Solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Tablets

» Erythromycin Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{37}H_{67}NO_{13}$ .

NOTE—Tablets that are enteric-coated meet the requirements for *Erythromycin Delayed-Release Tablets*.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Prepare a test solution by mixing a quantity of finely powdered Tablets with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Dissolution** (711)—

**Medium:** 0.05 M pH 6.8 phosphate buffer (see *Buffer solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Test solution**—If necessary, dilute a filtered portion of the solution under test with *Medium* to obtain a solution having a concentration of about 0.28 mg of erythromycin per mL, and mix.

**Standard solution**—Dissolve an accurately weighed quantity of USP Erythromycin RS in methanol (not more than

1 mL of methanol for each 14 mg of the Reference Standard), and dilute with water, quantitatively and with mixing, to obtain a stock solution containing about 0.56 mg per mL. Immediately prior to use, dilute the stock solution quantitatively with water to obtain a *Standard solution* having a known concentration of about 0.28 mg per mL.

**Procedure**—Transfer 5.0-mL portions of the *Test solution* and the *Standard solution* to separate 25-mL volumetric flasks, and treat each as follows: Add 2.0 mL of water, and allow to stand for 5 minutes with intermittent swirling. Add 15.0 mL of 0.25 N sodium hydroxide, dilute with *Medium* to volume, and mix. Heat to 60° for 5 minutes, and allow to cool. Concomitantly determine the absorbances of these solutions at the wavelength of maximum absorbance at about 236 nm, with a suitable spectrophotometer, using blank solutions similarly prepared, except that 2.0 mL of 0.5 N sulfuric acid is substituted for the 2.0 mL of water. Calculate the amount of  $C_{37}H_{67}NO_{13}$  dissolved.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{37}H_{67}NO_{13}$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of powdered Tablets in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Assay**—Place not less than 4 Tablets in a high-speed glass blender jar with 200 mL of methanol, and blend for 3 minutes. Add 300 mL of *Buffer No. 3*, and blend for 3 minutes. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Delayed-Release Tablets

» Erythromycin Delayed-Release Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the Tablets are enteric-coated. The labeling indicates the *Dissolution Test* with which the product complies.

**USP Reference standards** (11)—

USP Erythromycin RS

**Identification**—Prepare a test solution by mixing a quantity of finely powdered Tablets with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

**Dissolution** (711)—Proceed as directed for *Procedure for Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*.

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Apparatus 1:** 100 rpm.

**Times:** 60 minutes, Stage 1; 60 minutes, Stage 2.

**Acid stage**—Using 900 mL of simulated gastric fluid TS (prepared without pepsin) in place of 0.1 N hydrochloric acid, conduct this stage of the test for 1 hour, and do not perform an analysis of the medium.

**Buffer stage**—Using 900 mL of 0.05 M pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), conduct this stage of the test for 60 minutes.



**Test solution**—If necessary, dilute a filtered portion of the solution under test with *Dissolution Medium* to obtain a solution having a concentration of about 0.28 mg of erythromycin per mL, and mix.

**Procedure**—Transfer a 2.0-mL portion of the *Test solution* to a suitable separator. Add 6 mL of pH 1.2 buffer (see *Solutions* in the section *Reagents, Indicators, and Solutions*), and 8 mL of a solution of bromocresol purple, prepared by dissolving 1 g of bromocresol purple in 1 L of pH 4.5 phosphate buffer, and mix. Extract with 40.0 mL of chloroform. Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 410 nm using the chloroform extracts. Similarly prepare a Standard solution, having a known concentration of USP Erythromycin RS, and treat similarly.

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed under *Test 1*, except to use *Apparatus 2* at 75 rpm.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 6.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Assay**—Place not fewer than 4 Tablets in a high-speed glass blender jar with 200 mL of methanol, and blend for 3 minutes. Add 300 mL of *Buffer No. 3*, and blend for 3 minutes. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin and Benzoyl Peroxide Topical Gel

» Erythromycin and Benzoyl Peroxide Topical Gel is a mixture of Erythromycin in a suitable gel vehicle containing benzoyl peroxide and one or more suitable dispersants, stabilizers, and wetting agents. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of erythromycin ( $C_{37}H_{67}NO_{13}$ ) and benzoyl peroxide ( $C_{14}H_{10}O_4$ ).

**Packaging and storage**—Before mixing, preserve the Erythromycin and the vehicle containing benzoyl peroxide in separate, tight containers. After mixing, preserve the mixture in tight containers.

**USP Reference standards** (11)—  
USP Erythromycin RS

**Identification**—Prepare *Standard preparation* and an *Assay preparation* as directed in the *Assay*, except to omit the *Internal standard solution*, and chromatograph as directed in the *Assay*: the *Assay preparation* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard preparation*.

**Minimum fill** (755): meets the requirements.

**Limit of benzoyl peroxide related substances**—

*Mobile phase A, Mobile phase B, Standard preparation A, Standard preparation B, Standard preparation C, Standard preparation D, Resolution solution, and Chromatographic system*—Proceed as directed in the test for *Related compounds* under *Benzoyl Peroxide Gel*.

*Test preparation*—Transfer an accurately weighed quantity of Topical Gel, equivalent to about 100 mg of benzoyl per-

oxide, to a 50-mL volumetric flask, add 25 mL of acetonitrile, shake vigorously to disperse the specimen, sonicate for 5 minutes, dilute with acetonitrile to volume, mix, and filter.

**Procedure**—Proceed as directed for *Procedure* in the test for *Related compounds* under *Benzoyl Peroxide Gel*: it meets the limits stated.

**Assay for erythromycin**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Topical Gel blended for 3 to 5 minutes in a high-speed glass blender jar containing 0.5 mL of polysorbate 80 and an accurately measured volume of *Buffer No. 3* sufficient to obtain a stock solution having a convenient concentration of erythromycin. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Standard.

**Assay for benzoyl peroxide**—

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Benzoyl Peroxide Gel*.

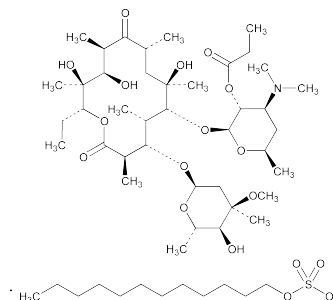
*Assay preparation*—Prepare as directed for *Assay preparation* in the *Assay* under *Benzoyl Peroxide Gel*, using Topical Gel.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Benzoyl Peroxide Gel*. Calculate the quantity, in mg, of benzoyl peroxide ( $C_{14}H_{10}O_4$ ) in the portion of Topical Gel taken by the formula:

$$125C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of benzoyl peroxide in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of benzoyl peroxide peak response to ethyl benzoate peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Erythromycin Estolate



$C_{40}H_{71}NO_{14} \cdot C_{12}H_{25}O_4S$  1056.39

Erythromycin, 2'-propanoate, dodecyl sulfate (salt).

Erythromycin 2'-propionate dodecyl sulfate (salt)  
[3521-62-8].

» Erythromycin Estolate has a potency equivalent to not less than 600 µg of erythromycin ( $C_{37}H_{67}NO_{13}$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

**Identification, Infrared Absorption** (197K).

**Crystallinity** (695): meets the requirements.

**Water, Method I** (921): not more than 4.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Free erythromycin**—Prepare a test solution of it in methanol containing 10.0 mg per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 0.3 mg per mL. [NOTE—Prepare these solutions immediately before use.] Prepare a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Before using, place the plate in an unlined developing chamber containing about 100 mL of methanol, and allow the solvent front to move to the top of the plate, marking the direction of travel. Remove the plate, and allow to dry. Apply separate 1-μL volumes of the test solution and the Standard solution on the plate, allow the spots to dry, and develop the chromatograms in a freshly prepared solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about one-half of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to dry. Place the plate under a hood, and spray uniformly with a solution consisting of 150 mg of xanthidol dissolved in a mixture of hydrochloric acid and glacial acetic acid (92.5:7.5). Heat the sprayed plate in an oven at 100° for 5 minutes. [Caution—Avoid exposure to acid fumes when removing the plate from the oven.] Examine the plate for reddish violet spots: free erythromycin has an  $R_F$  value of about 0.3, and erythromycin estolate has an  $R_F$  value of about 0.7. Any spot corresponding to free erythromycin obtained from the test solution does not exceed in size or intensity that of the principal spot obtained from the Standard solution (3.0%).

**Assay**—Proceed with Erythromycin Estolate as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Erythromycin Estolate dissolved in methanol to obtain a solution containing the equivalent of 1.0 mg of erythromycin per mL. Immediately dilute quantitatively with 9 volumes of *Buffer No. 3*, and allow to stand at room temperature for 18 hours. Dilute a portion of this solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Estolate Capsules

» Erythromycin Estolate Capsules contain the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

**Identification**—Prepare a test solution by mixing a quantity of Capsule contents with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3 μL of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."

**Disintegration** (701): 30 minutes, proceeding as directed for *Hard Gelatin Capsules*, except to use disks and to

use simulated gastric fluid TS as the immersion fluid instead of water.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Assay**—Place not less than 4 Capsules in a high-speed glass blender jar with 200.0 mL of methanol, and blend for 3 minutes. Add 300.0 mL of *Buffer No. 3*, and blend for 3 minutes. Allow this solution to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Estolate Oral Suspension

» Erythromycin Estolate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ). It contains one or more suitable buffers, colors, diluents, dispersants, and flavors.

**Packaging and storage**—Preserve in tight containers, in a cool place.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

**Identification**—Transfer a quantity of Oral Suspension, equivalent to about 20 mg of erythromycin, to a separator. Add 15 mL of 0.02 N sodium hydroxide, and swirl to mix. Add 2 g of sodium chloride and 25 mL of chloroform, and shake for 3 minutes. Drain the chloroform phase through a small amount of chloroform-washed anhydrous sodium sulfate, and collect the chloroform extract in a beaker, rinsing the sodium sulfate with an additional 10 mL of chloroform. Evaporate the chloroform to dryness. Dissolve the residue in 1 mL of methanol (test solution). Prepare a Standard solution by transferring a quantity of USP Erythromycin Estolate RS, equivalent to 20 mg of erythromycin, to a separator and carrying out the extraction procedure described for preparation of the test solution. Apply separately 3 μL of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."

**Uniformity of dosage units** (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 3.5 and 6.5.

**Assay**—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with methanol to obtain a solution containing the equivalent of 2.5 mg of erythromycin per mL. Dilute with 1.5 volumes of *Buffer No. 3*, and allow to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Estolate for Oral Suspension

» Erythromycin Estolate for Oral Suspension is a dry mixture of Erythromycin Estolate with one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

**Identification**—Prepare a test solution by mixing a quantity of Erythromycin Estolate for Oral Suspension with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3  $\mu$ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 7.0 (if pediatric drops, between 5.0 and 5.5), in the suspension constituted as directed in the labeling.

**Water, Method I** (921): not more than 2.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Assay**—Constitute Erythromycin Estolate for Oral Suspension as directed in the labeling, and proceed as directed in the *Assay* under *Erythromycin Estolate Oral Suspension*.

## Erythromycin Estolate Tablets

» Erythromycin Estolate Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent (115.0 percent, if chewable) of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Tablets to indicate whether they are to be chewed before swallowing.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

**Identification**—Prepare a test solution by mixing a quantity of finely powdered Tablets with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3  $\mu$ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification*

test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber." [NOTE—Use the following procedure for chewable Tablets. Transfer a quantity of finely powdered Tablets, equivalent to about 20 mg of erythromycin, to a separator, and proceed as directed in the *Identification* test under *Erythromycin Estolate Oral Suspension*, beginning with "Add 15 mL of 0.02 N sodium hydroxide."]

**Disintegration** (701): 30 minutes, proceeding as directed for *Uncoated Tablets*, except to use disks and to use simulated gastric fluid as the immersion fluid instead of water. [NOTE—Chewable tablets are exempt from this requirement.]

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.0%; or if chewable tablets, not more than 4.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Assay**—Proceed with Tablets as directed in the *Assay* under *Erythromycin Estolate Capsules*.

## Erythromycin Estolate and Sulfisoxazole Acetyl Oral Suspension

» Erythromycin Estolate and Sulfisoxazole Acetyl Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ) and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of sulfisoxazole ( $C_{11}H_{13}N_3O_3S$ ). It contains one or more suitable buffers, colors, diluents, emulsifiers, flavors, preservatives, and suspending agents.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Estolate RS

USP Sulfisoxazole Acetyl RS

**Identification**—To a quantity of the Oral Suspension add a volume of methanol sufficient to yield a solution having a concentration equivalent to about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a solution of USP Erythromycin Estolate RS in methanol containing about 3 mg per mL (*Standard solution A*). Prepare a solution of USP Sulfisoxazole Acetyl RS in methanol containing about 8.7 mg per mL (*Standard solution B*). Apply separately 10  $\mu$ L each of the test solution and the two Standard solutions to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin estolate appears as a black-to-purple spot and the sulfisoxazole acetyl appears as a yellow spot: the  $R_f$  value of the principal black-to-purple spot obtained from the test solution corresponds to that obtained from *Standard solution A*, and the  $R_f$  value of the

principal yellow spot obtained from the test solution corresponds to that obtained from *Standard solution B*.

**Uniformity of dosage units** (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 3.5 and 6.5.

**Assay for erythromycin**—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with methanol to obtain a solution containing the equivalent of 2.5 mg of erythromycin per mL. Dilute with 1.5 volumes of *Buffer No. 3*, and allow to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of erythromycin per mL).

**Assay for sulfisoxazole**—

*Mobile solvent*—Mix 40 volumes of acetonitrile and 60 volumes of water. The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for sulfisoxazole acetyl. Filter the solution through a membrane filter (1-µm or finer porosity).

*Internal standard solution*—Prepare a solution of benzanilide in acetonitrile having a concentration of about 0.33 mg per mL. Filter the solution through a membrane filter (1-µm or finer porosity).

*Standard preparation*—Prepare a solution of USP Sulfisoxazole Acetyl RS in *Internal standard solution* having a known concentration of about 1 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 600 mg of sulfisoxazole, to a 125-mL separator, and extract with three 75-mL portions of chloroform. Collect the chloroform extracts in a 250-mL volumetric flask, dilute with chloroform to volume, and mix. Filter a portion of this solution through a membrane filter (1-µm or finer porosity). Pipet 4 mL of the filtrate into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a current of dry air to dryness. Add 10.0 mL of *Internal standard solution*, and mix.

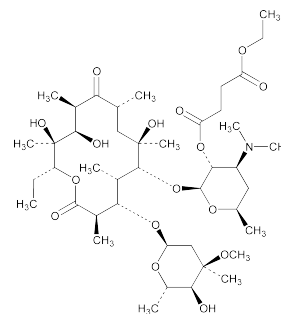
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*; the resolution factor between sulfisoxazole acetyl and benzanilide is not less than 3.0.

*Procedure*—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sulfisoxazole (C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S) in each mL of the Oral Suspension taken by the formula:

$$(267.31 / 309.35)(625C / V)(R_U / R_S)$$

in which 267.31 and 309.35 are the molecular weights of sulfisoxazole and sulfisoxazole acetyl, respectively; *C* is the concentration, in mg, of USP Sulfisoxazole Acetyl RS in each mL of the *Standard preparation*; *V* is the volume, in mL, of Oral Suspension taken; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of peak responses of sulfisoxazole acetyl peak to benzanilide peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Erythromycin Ethylsuccinate



C<sub>43</sub>H<sub>75</sub>NO<sub>16</sub> 862.05

Erythromycin 2'-(ethyl butanedioate).

Erythromycin 2'-(ethyl succinate). [1264-62-6].

» Erythromycin Ethylsuccinate consists primarily of the 2'-ethylsuccinate ester of erythromycin A. The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is not less than 76.5 percent, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Erythromycin Ethylsuccinate that is noncrystalline is labeled to indicate that it is amorphous. Any preparation containing the amorphous form of Erythromycin Ethylsuccinate is so labeled.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin B RS

USP Erythromycin C RS

USP Erythromycin Related Compound N RS

N-demethylethylerythromycin A.

C<sub>36</sub>H<sub>65</sub>NO<sub>13</sub> 719.91

USP Erythromycin Ethylsuccinate RS

**Identification, Infrared Absorption** (197S)—

*Solution:* 1 in 100.

*Medium:* chloroform.

*Cell size:* 1.0-mm.

**Crystallinity** (695): meets the requirements, except that when it is labeled as being in the amorphous state it does not meet the requirements.

**X-ray diffraction** (941)—Where labeled as being in the amorphous state, its X-ray diffraction pattern performed at high sensitivity for angles of diffraction between 2° and 20° exhibits no reflection, and between 7° and 10° exhibits a more intense hachured baseline, creating a halo.

**Water, Method I** (921): not more than 3.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Residue on ignition** (281): not more than 1.0% after ignition at 550 ± 50°, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Related compounds**—Using the chromatograms obtained for the *Assay preparation* and *Standard preparation 2* in the *Assay*, begin peak integration after the two peaks for succinates that elute just after the solvent front, and calculate the percentage of each related compound having the greatest response, other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin N-ethylsuccinate (retention time relative to erythromycin A

peak is about 1.3), in the portion of Erythromycin Ethylsuccinate taken by the formula:

$$50(C_{S2}P / W)(r_i / r_{S2})$$

in which  $C_{S2}$  is the concentration, in mg per mL, of USP Erythromycin RS in *Standard preparation 2*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in mg, of Erythromycin Ethylsuccinate taken to prepare the *Assay preparation*;  $r_i$  is the peak response of each related compound, other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin N-ethylsuccinate, in the chromatogram obtained from the *Assay preparation*; and  $r_{S2}$  is the erythromycin A peak response in the chromatogram obtained from *Standard preparation 2*: not more than 3.0% of any individual related compound is found.

Calculate the percentage of erythromycin A enol ether in the portion of Erythromycin Ethylsuccinate taken by the formula:

$$(50 / 11)(C_{S2}P / W)(r_E / r_{S2})$$

in which 11 is the response factor for erythromycin A enol ether in relation to that of erythromycin A;  $r_E$  is the peak response of the erythromycin A enol ether observed in the chromatogram obtained from the *Assay preparation*; and the other terms are as defined above: not more than 3.0% of erythromycin A enol ether is found.

Calculate the percentage of erythromycin N-ethylsuccinate in the portion of Erythromycin Ethylsuccinate taken by the formula:

$$(50 / 7.4)(C_{S2}P / W)(r_N / r_{S2})$$

in which 7.4 is the response factor for erythromycin N-ethylsuccinate in relation to that of erythromycin A;  $r_N$  is the peak response of the erythromycin N-ethylsuccinate (retention time relative to the erythromycin A peak is about 1.3) observed in the chromatogram obtained from the *Assay preparation*; and the other terms are as defined above: not more than 3.0% of erythromycin N-ethylsuccinate is found.

#### Assay—

*Hydrolysis reagent*—Prepare a solution of dibasic potassium phosphate (2 in 100), and adjust with phosphoric acid to a pH of 8.0.

*pH 8.0 Buffer*—Prepare a solution of dibasic potassium phosphate (3.5 in 100), and adjust with phosphoric acid to a pH of 8.0.

*pH 3.5 Buffer*—Adjust 20 mL of *pH 8.0 Buffer* with phosphoric acid to a pH of 3.5.

*Mobile phase*—Mix 50 mL of *pH 8.0 Buffer* with 400 mL of water, add 175 mL of tertiary butyl alcohol and 30 mL of acetonitrile, dilute with water to 1000 mL, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

NOTE—Use the following solutions promptly, or within 1 day if stored in a refrigerator.

*Standard preparation 1*—Transfer about 50 mg of USP Erythromycin RS, accurately weighed, to a 25-mL volumetric flask, add 12.5 mL of methanol, and swirl to dissolve. Dilute with *Hydrolysis reagent* to volume, and mix.

*Standard preparation 2*—Transfer about 5 mg each of USP Erythromycin B RS and USP Erythromycin C RS, both accurately weighed, to a 50-mL volumetric flask, add 25.0 mL of methanol, and swirl to dissolve. Add 2.5 mL of *Standard preparation 1*, dilute with *Hydrolysis reagent* to volume, and mix.

*System suitability solution*—Dissolve about 2 mg of USP Erythromycin Related Compound N RS in about 20 mL of *Standard preparation 2*, and mix.

*Erythromycin A enol ether solution*—Dissolve about 10 mg of USP Erythromycin RS in 2 mL of methanol. Add 10 mL of

*pH 3.5 Buffer*, mix, and allow to stand for about 30 minutes. Refrigerate this solution until used, and discard 8 hours after preparation.

*Assay preparation*—Transfer about 115 mg of Erythromycin Ethylsuccinate, accurately weighed, to a 50-mL volumetric flask, add 25.0 mL of methanol, and swirl to dissolve. Add about 20 mL of *Hydrolysis reagent*, mix, and allow to stand at room temperature for about 12 hours to effect hydrolysis. Dilute with *Hydrolysis reagent* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains packing L21 (1000 Å) and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the order of elution of the components is erythromycin related compound N, erythromycin C, erythromycin A, and erythromycin B; and the resolution,  $R$ , between erythromycin related compound N and erythromycin C is not less than 0.8 and between erythromycin related compound N and erythromycin A not less than 5.5. Chromatograph the *Erythromycin A enol ether solution*, and record the peak responses as directed for *Procedure*: adjust the duration of chromatography to include the erythromycin A enol ether peak, which has a retention time of about 4.3 to 4.7 times that of erythromycin A. Chromatograph *Standard preparation 1*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 200 µL) of *Standard preparation 1*, *Standard preparation 2*, and the *Assay preparation* into the chromatograph; record the chromatograms for a period of time that is adequate to include the erythromycin A enol ether peak, if present; and measure the areas of the peak responses. Calculate the percentage of erythromycin A in the portion of Erythromycin Ethylsuccinate taken by the formula:

$$50(C_{S1}P / W)(r_U / r_{S1})$$

in which  $C_{S1}$  is the concentration, in mg per mL, of USP Erythromycin RS in *Standard preparation 1*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in mg, of Erythromycin Ethylsuccinate taken to prepare the *Assay preparation*; and  $r_U$  and  $r_{S1}$  are the erythromycin A peak responses in the chromatograms obtained from the *Assay preparation* and *Standard preparation 1*, respectively.

Calculate the percentage of erythromycin B and erythromycin C in the portion of Erythromycin Ethylsuccinate taken by the formula:

$$50(C_{S2}P / W)(r_U / r_{S2})$$

in which  $C_{S2}$  is the concentration, in mg per mL, of the relevant USP Reference Standard in *Standard preparation 2*;  $P$  is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard;  $W$  is the quantity, in mg, of Erythromycin Ethylsuccinate taken to prepare the *Assay preparation* and  $r_U$  and  $r_{S2}$  are the peak responses of the relevant analyte in the chromatograms obtained from the *Assay preparation* and *Standard preparation 2*, respectively. The percentage of erythromycin B is not more than 12.0%, and the percentage of erythromycin C is not more than 5.0%.

## Erythromycin Ethylsuccinate Injection

» Erythromycin Ethylsuccinate Injection is a sterile solution of Erythromycin Ethylsuccinate in Poly-

ethylene Glycol 400, and contains 2 percent of butyl aminobenzoate and a suitable preservative. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Erythromycin RS

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, using a membrane filter resistant to the solvent effect of polyethylene glycol 400.

**Water**, *Method I* (921): not more than 1.5%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Injection diluted quantitatively with methanol to yield a solution containing the equivalent of about 1 mg of erythromycin per mL. Dilute a portion of this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Sterile Erythromycin Ethylsuccinate

» Sterile Erythromycin Ethylsuccinate is Erythromycin Ethylsuccinate suitable for parenteral use. It has a potency equivalent to not less than 765  $\mu$ g of erythromycin ( $C_{37}H_{67}NO_{13}$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Ethylsuccinate RS

**Identification**, *Infrared Absorption* (197S)—

*Solution*: 1 in 100.

*Medium*: chloroform.

*Cell size*: 1.0 mm.

**Sterility** (71)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*.

**Heavy metals** (231)—Dissolve 1 g in 25 mL of water, and proceed as directed for *Method I*: the limit is 0.002%.

**Other requirements**—It conforms to the Definition and meets the requirements for *Water*, *Residue on ignition*, *CrySTALLINITY*, and the *Assay* under *Erythromycin Ethylsuccinate*.

## Erythromycin Ethylsuccinate Oral Suspension

» Erythromycin Ethylsuccinate Oral Suspension is a suspension of Erythromycin Ethylsuccinate containing one or more suitable buffers, colors, dispersants, flavors, and preservatives. It contains

the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers, and store in a cold place.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Ethylsuccinate RS

**Identification**—To a quantity of the Oral Suspension add a volume of methanol sufficient to yield a solution having a concentration equivalent to about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a Standard solution of USP Erythromycin Ethylsuccinate RS in methanol containing about 3 mg per mL. Apply separately 10  $\mu$ L each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin and succinic acid moieties appear as black-to-purple spots: the  $R_f$  values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Uniformity of dosage units** (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 6.5 and 8.5.

**Assay**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, blended for  $4 \pm 1$  minutes in a high-speed glass blender jar with sufficient methanol to give a stock solution containing the equivalent of about 1 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Ethylsuccinate for Oral Suspension

» Erythromycin Ethylsuccinate for Oral Suspension is a dry mixture of Erythromycin Ethylsuccinate with one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Ethylsuccinate RS

**Identification**—To a quantity of Erythromycin Ethylsuccinate for Oral Suspension add a volume of methanol sufficient to yield a solution containing the equivalent of about

2.5 mg of erythromycin per mL, and stir for 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Proceed as directed in the Identification test under *Erythromycin Ethylsuccinate Oral Suspension*, beginning with "Prepare a Standard solution."

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 7.0 and 9.0, in the suspension constituted as directed in the labeling.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Assay**—Constitute Erythromycin Ethylsuccinate for Oral Suspension as directed in the labeling, and proceed as directed in the Assay under *Erythromycin Ethylsuccinate Oral Suspension*.

## Erythromycin Ethylsuccinate Tablets

» Erythromycin Ethylsuccinate Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the chewable Tablets to indicate that they are to be chewed before swallowing.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Ethylsuccinate RS

**Identification**—To a quantity of powdered Tablets add a volume of methanol sufficient to yield a solution containing the equivalent of about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Proceed as directed in the Identification test under *Erythromycin Ethylsuccinate Oral Suspension*, beginning with "Prepare a Standard solution."

**Dissolution** (711)—

FOR NONCHEWABLE TABLETS—

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved by employing the following method.

**Color reagent**—Slowly, and with constant mechanical stirring, add 325 mL of sulfuric acid to 173 mL of cold water. Allow the solution to cool, add 2 mL of ferric chloride solution (1 in 40) and 1 g of *p*-dimethylaminobenzaldehyde, and stir to dissolve. Store in a low actinic flask. [NOTE—Prepare this *Color reagent* on the day of use.]

**Standard solution**—Dissolve an accurately weighed quantity of USP Erythromycin RS in *Medium* to obtain a solution having a known concentration of about 0.44 mg per mL, using sonication if necessary to dissolve. [NOTE—Use this *Standard solution* within 5.5 hours.]

**Test solution**—Pass a portion of the solution under test through a filter having a porosity of 0.5  $\mu$ m or less, discarding the first 5 mL of the filtrate.

**Procedure**—To three separate 50-mL glass-stoppered conical flasks add 2.0 mL of *Standard solution*, 2.0 mL of *Test solution*, and 2.0 mL of *Medium* (to serve as the *Blank*), respectively. Place the flasks in an ice bath for about 15 minutes. At precise 1-minute intervals add 10.0 mL of *Color reagent*

to the *Standard solution*, the *Test solution*, and the *Blank*, in turn. Immediately after adding the *Color reagent*, remove each flask from the ice bath, insert the stopper, mix, and allow to stand at room temperature for exactly 30 minutes. Sequentially determine the absorbance at 480 nm of the *Standard solution* and the *Test solution* at precise 1-minute intervals, using the *Blank* to set the spectrophotometer to zero. Calculate the quantity, in mg, of  $C_{37}H_{67}NO_{13}$  equivalent dissolved by the formula:

$$0.9CP(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Erythromycin RS in the *Standard solution*, *P* is the designated content, in  $\mu$ g per mg, of erythromycin in the USP Erythromycin RS, and  $A_U$  and  $A_S$  are the absorbances of the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 75% (*Q*) of the labeled amount of  $C_{37}H_{67}NO_{13}$  equivalent is dissolved in 45 minutes.

FOR TABLETS LABELED AS CHEWABLE—

**Arsenomolybdate stock solution**—Dissolve 100 g of ammonium molybdate in 1.7 liters of water in a 2-liter volumetric flask. Slowly add, with mixing, 84 mL of sulfuric acid. Add 12 g of sodium arsenate dissolved in 100 mL of water. Dilute with water to volume, and mix. Store in an amber bottle for 24 hours before using. [NOTE—This solution should not come in contact with rubber.]

**Arsenomolybdate solution**—Use a mixture of water and *Arsenomolybdate stock solution* (2:1). [NOTE—Prepare fresh on the day of use.]

**pH 4.8 Acetate buffer**—Dissolve 133 g of sodium acetate in about 3.5 liters of water. Adjust with glacial acetic acid to a pH of  $4.8 \pm 0.1$ , dilute with water to 4 liters, and mix.

**4.5 M Sulfuric acid**—[NOTE—This reagent is called out in the *Diagram of Dissolution Test Method for Erythromycin Ethylsuccinate Tablets Labeled as Chewable under Automated Methods of Analysis* (16).] Add 1.5 liters of water to a 2-liter volumetric flask, and place the flask in an ice bath. Slowly add, with stirring, 300 mL of sulfuric acid. Allow the solution to cool, dilute with water to volume, and mix. At the time of use, add 0.5 g of sodium dodecyl sulfate to each L.

**Medium:** 0.1 M acetate buffer, pH 5.0; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 60 minutes.

Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved by employing the following method.

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Erythromycin RS in *pH 4.8 Acetate buffer* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Standard solutions**—Transfer 8.0-, 4.0-, and 1.0-mL volumes of the *Standard stock solution* to separate 100-mL volumetric flasks, add 6.0 mL of pH 7.6 phosphate buffer (see *Solutions* under *Reagents, Indicators, and Solutions*) and 6.0 mL of *Medium* to each flask, dilute with *pH 4.8 Acetate buffer* to volume, and mix to obtain solutions having known concentrations of about 40, 20, and 5  $\mu$ g per mL of USP Erythromycin RS, respectively.

**Procedure**—Transfer 6.0 mL of the solution under test to a 50-mL volumetric flask, add 6.0 mL of pH 7.6 phosphate buffer, and heat in boiling water for 30 minutes. Cool to room temperature, and dilute with *pH 4.8 Acetate buffer* to volume. To determine the absorbance values, use an automated analyzer (illustrated in the *Diagram of Dissolution Test Method for Erythromycin Ethylsuccinate Tablets Labeled as Chewable under Automated Methods of Analysis* (16)) consisting of a liquid sampler; a proportioning pump; a manifold; and a spectrophotometer equipped with matched flow cells, suitable recording devices, and analysis capability at 660 nm. Adjust the system until a steady baseline is achieved. Start the sampler, and conduct determinations at a rate of about 40 to 60 per hour with a 3:1 (sample/wash)

ratio. [NOTE—Adjust the analyzer flow rates if necessary to optimize system performance.] Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved from the absorbance values of the *Test solution* and the *Standard solutions*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{37}H_{67}NO_{13}$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—[NOTE—Chewable Tablets are exempt from this requirement.] Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 4.0% of its weight.

**Water**, Method I (921) (*Chewable Tablets only*): not more than 5.0%.

**Assay**—Proceed with Tablets as directed under *Antibiotics—Microbial Assays* (81), using not less than 4 Tablets, accurately counted, blended for  $4 \pm 1$  minutes in a high-speed glass blender jar with a sufficient accurately measured volume of methanol to give a stock solution containing the equivalent of not more than 5 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension

» Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension is a dry mixture of Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl with one or more suitable buffers, colors, flavors, surfactants, and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ) and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of sulfisoxazole ( $C_{11}H_{13}N_3O_3S$ ).

NOTE—Where Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension is prescribed, without reference to the quantity of erythromycin or sulfisoxazole contained therein, a product containing 40 mg of erythromycin and 120 mg of sulfisoxazole per mL when constituted as directed in the labeling shall be dispensed.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin Ethylsuccinate RS

USP Sulfisoxazole Acetyl RS

**Identification**—To a quantity of the Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension add a volume of methanol sufficient to yield a solution having a concentration equivalent to about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a solution of USP Erythromycin Ethylsuccinate RS in methanol containing about 3 mg per mL (*Standard solution A*). Prepare a solution of USP Sulfisoxazole Acetyl RS in methanol containing about 8.7 mg per mL (*Standard solution B*). Apply separately 10  $\mu$ L each of the test solution and the two Standard solutions to a suitable thin-layer chromatographic plate (see

*Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin and succinic acid moieties appear as black-to-purple spots and the sulfisoxazole acetyl appears as a yellow spot: the  $R_f$  values of the principal black-to-purple spots obtained from the test solution correspond to those obtained from *Standard solution A*; and the  $R_f$  value of the principal yellow spot obtained from the test solution corresponds to that obtained from *Standard solution B*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements for *Content Uniformity* with respect to erythromycin and sulfisoxazole.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 7.2, in the suspension constituted as directed in the labeling.

**Loss on drying** (731): Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Assay for erythromycin**—Constitute Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension as directed in the labeling, and allow to stand for 1 hour. Gently shake the suspension, transfer 5.0 mL to a high-speed blender jar containing 195.0 mL of methanol, and blend for  $4 \pm 1$  minutes. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock test solution diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0  $\mu$ g of erythromycin per mL).

**Assay for sulfisoxazole**—

**Mobile solvent**—Mix 40 volumes of acetonitrile and 60 volumes of water. The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for sulfisoxazole acetyl. Pass the solution through a membrane filter having a 1- $\mu$ m or finer porosity.

**Internal standard solution**—Prepare a solution of benzanilide in acetonitrile having a concentration of about 0.33 mg per mL. Pass the solution through a membrane filter having a 1- $\mu$ m or finer porosity.

**Standard preparation**—Prepare a solution of USP Sulfisoxazole Acetyl RS in *Internal standard solution* having a known concentration of about 1 mg per mL.

**Assay preparation**—Constitute Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension as directed in the labeling, and allow to stand for 1 hour. Gently shake the suspension, transfer to a 125-mL separator an accurately measured volume of it, equivalent to about 600 mg of sulfisoxazole, and extract with three 75-mL portions of chloroform. Collect the chloroform extracts in a 250-mL volumetric flask, dilute with chloroform to volume, and mix. Pass a portion of this solution through a membrane filter having a 1- $\mu$ m or finer porosity. Pipet 4 mL of the filtrate into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a current of dry air to dryness. Add 10.0 mL of *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution fac-



tor between sulfoxazole acetyl and benzanilide is not less than 3.0.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sulfoxazole ( $C_{11}H_{13}N_3O_3S$ ) in each mL of the constituted suspension taken by the formula:

$$(267.31/309.35)(625C/V)(R_U / R_S)$$

in which 267.31 and 309.35 are the molecular weights of sulfoxazole and sulfoxazole acetyl, respectively; C is the concentration, in mg, of USP Sulfoxazole Acetyl RS in each mL of the *Standard preparation*; V is the volume, in mL of constituted suspension taken; and  $R_U$  and  $R_S$  are the ratios of peak responses of sulfoxazole acetyl peak to benzanilide peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Sterile Erythromycin Gluceptate

$C_{37}H_{67}NO_{13} \cdot C_7H_{14}O_8$  960.11

Erythromycin monoglucuheptonate (salt).

Erythromycin gluceptate (1:1) (salt). [23067-13-2].

» Sterile Erythromycin Gluceptate is erythromycin gluceptate suitable for parenteral use. It has a potency equivalent to not less than 600  $\mu$ g of erythromycin ( $C_{37}H_{67}NO_{13}$ ) per mg, calculated on the anhydrous basis. In addition, where packaged for dispensing, it contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Erythromycin RS

USP Erythromycin Gluceptate RS

**Identification, Infrared Absorption** (197M).

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.0 and 8.0, in a solution containing 25 mg per mL.

**Water, Method I** (921): not more than 5.0%.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—Where packaged for dispensing it meets the requirements for *Uniformity of Dosage Units* (905) and *Constituted Solutions and Labeling* under *Injections* (1).

**Assay**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Sterile Erythromycin Gluceptate dissolved in methanol to yield a solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this solution quantitatively with 9 volumes of *Buffer No. 3* to obtain a stock solution containing the equivalent of about 1 mg of erythromycin per mL. Where it is packaged for dispensing, constitute Sterile Erythromycin Gluceptate as directed in the labeling. Withdraw all of the withdrawable contents where

the package is represented as being a single-dose container; or where the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured volume. Dilute quantitatively with *Buffer No. 3* to obtain a stock solution having a convenient concentration. Dilute a portion of the stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Erythromycin Lactobionate for Injection

$C_{37}H_{67}NO_{13} \cdot C_{12}H_{22}O_{12}$  1092.22

Erythromycin mono(4-O- $\beta$ -D-galactopyranosyl-D-gluconate) (salt).

Erythromycin lactobionate (1:1) (salt) [3847-29-8].

» Erythromycin Lactobionate for Injection is a sterile, dry mixture of erythromycin lactobionate and a suitable preservative. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Erythromycin RS

USP Erythromycin Lactobionate RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification, Infrared Absorption** (197M): the specimen and the Reference Standard being previously dried in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.

**pH** (791): between 6.5 and 7.5, in a solution containing the equivalent of 50 mg of erythromycin per mL.

**Water, Method I** (921): not more than 5.0%.

**Particulate matter** (788): meets the requirements for small-volume injections when the constituted solution is diluted with filtered water to a concentration of not more than 5 mg of erythromycin base per mL before the test is performed.

**Heavy metals, Method II** (231): 0.005%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81), using Erythromycin Lactobionate for Injection constituted as directed in the labeling. Withdraw all of the withdrawable contents where the package is represented as being a single-dose container; or, where the labeling specifies the amount of erythromycin equivalent in a given volume of the resultant preparation, withdraw an accurately measured volume. Dilute quantitatively with water to obtain a stock solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Sterile Erythromycin Lactobionate

» Sterile Erythromycin Lactobionate has a potency equivalent to not less than 525 µg of erythromycin ( $C_{37}H_{67}NO_{13}$ ) per mg, calculated on the anhydrous basis. In addition, where packaged for dispensing, it contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Erythromycin RS

USP Erythromycin Lactobionate RS

**Identification, Infrared Absorption** (197M): the specimen and the Reference Standard being previously dried in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.

**Bacterial endotoxins** (85)—It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.5 and 7.5, in a solution containing the equivalent of 50 mg of erythromycin per mL.

**Water, Method I** (921): not more than 5.0%.

**Particulate matter** (788): meets the requirements for small-volume injections when it is diluted with filtered water to a concentration of not more than 5 mg of erythromycin per mL before the test is performed.

**Residue on ignition** (281): not more than 2.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Heavy metals, Method II** (231): 0.005%.

**Other requirements**—Where packaged for dispensing, it meets the requirements for *Uniformity of Dosage Units* (905) and for *Constituted Solutions and Labeling* under *Injections* (1).

**Assay**—

*Standard preparation*—Prepare as directed for erythromycin under *Antibiotics—Microbial Assays* (81).

*Assay preparation 1*—Dissolve an accurately weighed quantity of Sterile Erythromycin Lactobionate quantitatively in methanol to obtain a stock solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer No. 3* (see *Media and Diluents* under *Antibiotics—Microbial Assays* (81)) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

*Assay preparation 2* (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Sterile Erythromycin Lactobionate in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

*Assay preparation 3* (where the label states the quantity of erythromycin in a given volume of constituted solution)—Constitute 1 container of Sterile Erythromycin Lactobionate in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer No. 3* to obtain a *Test Dilution* hav-

ing a concentration assumed to be equal to the median dose level of the Standard.

*Procedure*—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* (81).

## Erythromycin Stearate

$C_{37}H_{67}NO_{13} \cdot C_{18}H_{36}O_2$  1018.40

Erythromycin octadecanoate (salt).

Erythromycin stearate (salt). [643-22-1].

» Erythromycin Stearate is the stearic acid salt of Erythromycin, with an excess of Stearic Acid. The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is not less than 55.0 percent, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Erythromycin RS

USP Erythromycin B RS

USP Erythromycin C RS

USP Erythromycin Related Compound N RS

*N*-demethylethromycin A.

$C_{36}H_{65}NO_{13}$  719.91

USP Erythromycin Stearate RS

**Identification, Infrared Absorption** (197M).

**Crystallinity** (695): meets the requirements.

**Water, Method I** (921): not more than 4.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

**Residue on ignition** (281): not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Related compounds**—Use the chromatograms of the *Assay preparation* and *Standard preparation 2* as obtained in the *Assay*.

Calculate the percentage of each related compound having the greatest response, other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and pseudoerythromycin A enol ether (retention time relative to erythromycin A peak is about 1.5), in the portion of Erythromycin Stearate taken by the formula:

$$30(C_{S2}P/W)(r_i / r_{S2})$$

in which  $C_{S2}$  is the concentration, in mg per mL, of USP Erythromycin RS in *Standard preparation 2*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in mg, of Erythromycin Stearate taken to prepare the *Assay preparation*;  $r_i$  is the peak response of each related compound, other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and pseudoerythromycin A enol ether, observed in the chromatogram obtained from the *Assay preparation*; and  $r_{S2}$  is the erythromycin A peak response in the chromatogram obtained from *Standard preparation 2*; not more than 3.0% of any individual related compound is found.

Calculate the percentage of erythromycin A enol ether in the portion of Erythromycin Stearate taken by the formula:

$$(30 / 11)(C_{S2}P / W)(r_E / r_{S2})$$

in which 11 is the response factor for erythromycin A enol ether in relation to that of erythromycin A;  $r_E$  is the peak response of the erythromycin A enol ether observed in the chromatogram obtained from the *Assay preparation*; and the other terms are as defined above: not more than 3.0% of erythromycin A enol ether is found.

Calculate the percentage of pseudoerythromycin A enol ether in the portion of Erythromycin Stearate taken by the formula:

$$(30 / 6.6)(C_{S2}P / W)(r_p / r_{S2})$$

in which 6.6 is the response factor for pseudoerythromycin A enol ether in relation to that of erythromycin A;  $r_p$  is the peak response of the pseudoerythromycin A enol ether (retention time relative to the erythromycin A peak is about 1.5) observed in the chromatogram obtained from the *Assay preparation*; and the other terms are as defined above: not more than 3.0% of pseudoerythromycin A enol ether is found.

#### Assay—

**pH 8.0 Buffer**—Prepare a solution of dibasic potassium phosphate (2 in 100), and adjust with phosphoric acid to a pH of 8.0.

**pH 9.0 Buffer**—Prepare a solution of dibasic potassium phosphate (3.5 in 100), and adjust with potassium hydroxide TS or diluted phosphoric acid (1 in 10), as appropriate, to a pH of 9.0.

**pH 3.5 Buffer**—Adjust 20 mL of pH 8.0 Buffer with phosphoric acid to a pH of 3.5.

**Mobile phase**—Mix 50 mL of pH 9.0 Buffer with 400 mL of water, add 175 mL of tertiary butyl alcohol and 30 mL of acetonitrile, dilute with water to 1000 mL, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**NOTE**—Use the following solutions promptly, or within 1 day if stored in a refrigerator.

**Standard preparation 1**—Transfer about 40 mg of USP Erythromycin RS, accurately weighed, to a conical flask, add 5.0 mL of methanol, and swirl to dissolve. Add 5.0 mL of pH 8.0 Buffer, and mix.

**Standard preparation 2**—Transfer about 6 mg each of USP Erythromycin RS, USP Erythromycin B RS, USP Erythromycin C RS, and USP Erythromycin Related Compound N RS, all accurately weighed, to a 50-mL conical flask, add 15.0 mL of methanol, and swirl to dissolve. Add 15.0 mL of pH 8.0 Buffer, and mix.

**Erythromycin A enol ether solution**—Dissolve about 5 mg of USP Erythromycin RS in 1 mL of methanol. Add 5 mL of pH 3.5 Buffer, mix, and allow to stand for about 30 minutes.

**Assay preparation**—Transfer about 165 mg of Erythromycin Stearate, accurately weighed, to a 100-mL conical flask, add 15.0 mL of methanol, and swirl to dissolve. Add 15.0 mL of pH 8.0 Buffer, and mix. Allow the resulting suspension to settle, and pass a portion of the supernatant through a filter having a 0.2- $\mu$ m or finer porosity. Use the clear filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L21 (1000 Å) and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph *Standard preparation 2*, and record the peak responses as directed for *Procedure*: the order of elution of the components is erythromycin related compound N, erythromycin C, erythromycin A, and erythromycin B; and the resolution,  $R$ , between erythromycin related compound N and erythromycin C is not less than 0.8 and between erythromycin related compound N and erythromycin A not less than 5.5. Chromatograph the *Erythromycin A enol ether solution*, and adjust the duration of chromatography to include the erythromycin A enol ether peak, which has a retention time of about 4.3 to 4.7 times that of erythromycin A. Chromatograph *Standard preparation 1*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of *Standard preparation 1*, *Standard preparation 2*,

and the *Assay preparation* into the chromatograph; record the chromatograms for a period of time that is adequate to include the erythromycin A enol ether peak, if present; and measure the areas of the peak responses. Calculate the percentage of erythromycin A in the portion of Erythromycin Stearate taken by the formula:

$$30(C_{S1}P / W)(r_U / r_{S1})$$

in which  $C_{S1}$  is the concentration, in mg per mL, of USP Erythromycin RS in *Standard preparation 1*;  $P$  is the designated percentage of erythromycin A in USP Erythromycin RS;  $W$  is the quantity, in mg, of Erythromycin Stearate taken to prepare the *Assay preparation*; and  $r_U$  and  $r_{S1}$  are the erythromycin A peak responses in the chromatograms obtained from the *Assay preparation* and *Standard preparation 1*, respectively.

Calculate the percentage of erythromycin B and erythromycin C in the portion of Erythromycin Stearate taken by the formula:

$$30(C_{S2}P / W)(r_U / r_{S2})$$

in which  $C_S$  is the concentration, in mg per mL, of the relevant USP Reference Standard in *Standard preparation 2*;  $P$  is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard;  $W$  is the quantity, in mg, of Erythromycin Stearate taken to prepare the *Assay preparation*; and  $r_U$  and  $r_{S2}$  are the peak responses of the relevant analyte in the chromatograms obtained from the *Assay preparation* and *Standard preparation 2*, respectively. The percentage of erythromycin B is not more than 12.0%; and the percentage of erythromycin C is not more than 5.0%.

## Erythromycin Stearate Tablets

» Erythromycin Stearate Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ( $C_{37}H_{67}NO_{13}$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Erythromycin RS

USP Erythromycin Stearate RS

**Identification**—To a quantity of powdered Tablets add a volume of methanol sufficient to yield a solution containing the equivalent of about 5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a Standard solution of USP Erythromycin Stearate RS in methanol containing about 8 mg per mL. Apply separately 20  $\mu$ L each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a methanolic solution of 2',7'-dichlorofluorescein (1 in 500), and examine the plate under long-wavelength UV light: the  $R_f$  values of the principal fluorescent spots obtained from the test solution correspond to those obtained from the Standard solution. Then spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and

examine the chromatograms, in which the erythromycin appears as a black-to-purple spot: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

#### Dissolution <711>—

**Medium:** 0.05 M pH 6.8 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 100 rpm.

**Time:** 120 minutes.

**Stock standard solution**—Dissolve an accurately weighed quantity of USP Erythromycin RS in methanol to obtain a solution containing about 14 mg per mL. Dilute quantitatively with water, and mix to obtain a solution having a known concentration of about 0.56 mg of USP Erythromycin RS per mL.

**Working standard solution**—On the day of use, dilute 25.0 mL of *Stock standard solution* with water to 50.0 mL, and mix.

**Test solution**—After 120 minutes, withdraw a portion of the solution under test, filter, and dilute with *Medium*, if necessary, to obtain a solution having an estimated concentration of about 0.28 mg of erythromycin per mL.

**Procedure**—Transfer 5.0-mL portions of the *Working standard solution* to two 25-mL volumetric flasks, one of which serves as a working standard blank. Similarly, transfer 5.0-mL portions of the *Test solution* to two 25-mL volumetric flasks, one of which serves as a blank for that *Test solution*. To each of the flasks designated as a blank add 2.0 mL of 0.5 N sulfuric acid and to the remaining flasks add 2.0 mL of water. Allow to stand for 5 minutes with intermittent swirling. To all flasks add 15.0 mL of 0.25 N sodium hydroxide, dilute with *Medium* to volume, and mix. Heat the flasks in a water bath at  $60 \pm 0.5^\circ$  for 5 minutes, and allow to cool. Using a suitable spectrophotometer, determine the absorbance of each solution, corrected for its blank solution, at the wavelength of maximum absorbance at about 236 nm. Determine the amount of  $C_{37}H_{67}NO_{13}$  dissolved from the *Test solution* in comparison with the solution obtained from the *Working standard solution*.

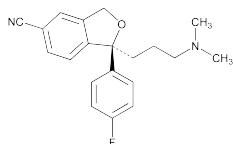
**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{37}H_{67}NO_{13}$  is dissolved in 120 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Loss on drying** <731>—Dry about 100 mg of powdered Tablets in a capillary-stoppered bottle in vacuum at  $60^\circ$  for 3 hours: it loses not more than 5.0% of its weight.

**Assay**—Proceed with Tablets as directed in the Assay under *Erythromycin Tablets*.

## Escitalopram Oxalate



$C_{20}H_{21}FN_2O \cdot C_2H_2O_4$  414.43  
S-(+)-5-Isobenzofurancarboxamide, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-, oxalate;  
S-(+)-1-[3-(Dimethylamino)propyl]-1-(p-fluorophenyl)-5-phthalanocarboxamide oxalate [219861-08-2].

#### DEFINITION

Escitalopram Oxalate contains NLT 98.0% and NMT 102.0% of escitalopram oxalate ( $C_{20}H_{21}FN_2O \cdot C_2H_2O_4$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer:** 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 3.0 prior to final dilution.

**Solution A:** Acetonitrile and *Buffer* (10:90)

**Solution B:** Acetonitrile and *Buffer* (65:35)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	95	5	1
35	65	35	1
45	0	100	1
45.1	0	100	2
60	0	100	2
60.1	95	5	1
68	95	5	1

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 1.6 mL. The injection time can be adjusted relative to the start of a run to accommodate changes in dwell volume from one HPLC system to another to achieve the separation described.]

**System suitability solution:** 2 µg/mL each of USP Escitalopram Oxalate RS and USP Citalopram Related Compound D RS in *Solution A*

**Standard solution:** 0.5 mg/mL of USP Escitalopram Oxalate RS in *Solution A*

**Sample solution:** 0.5 mg/mL of Escitalopram Oxalate in *Solution A*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:**  $45^\circ$

**Flow rate:** See *Table 1*.

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between escitalopram and citalopram related compound D, *System suitability solution*

**Tailing factor:** 0.8–3, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of escitalopram oxalate ( $C_{20}H_{21}FN_2O \cdot C_2H_2O_4$ ) in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, Method II (231): NMT 20 ppm
- **ENANTIOMERIC PURITY**

**Buffer:** Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, add 150 mL of 0.2 N sodium hydroxide, adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile and Buffer (3:17)

**System suitability solution:** 125 µg/mL each of USP R-Citalopram Oxalate RS and USP Escitalopram Oxalate RS in Mobile phase

**Sample solution:** 125 µg/mL of Escitalopram Oxalate in Mobile phase

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L57

**Column temperature:** 30°

**Flow rate:** 0.6 mL/min

**Injection volume:** 15 µL

**System suitability**

**Sample:** System suitability solution

**Suitability requirements**

**Resolution:** NLT 1.3 between R-citalopram and escitalopram

**Tailing factor:** 0.8–2.5 for escitalopram

**Analysis**

**Sample:** Sample solution

Calculate the percentage of R-citalopram oxalate in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = peak response of R-citalopram from the Sample solution

$r_S$  = peak response of escitalopram from the Sample solution

**Acceptance criteria:** NMT 3.0%

**Change to read:**• **ORGANIC IMPURITIES**

**Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 1.5 between escitalopram and citalopram related compound D, System suitability solution

**Tailing factor:** 0.8–3, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

**Analysis**

**Sample:** Sample solution

Calculate the percentage of each impurity in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of escitalopram from the Standard solution

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Oxalic acid*	0.075	—	—▲USP36
5-Dimethylamino-butyryl citalopram <sup>a</sup>	0.40	0.34	0.2
Citalopram related compound A <sup>b</sup>	0.50	0.79	0.1
Citalopram related compound B <sup>c</sup> (3-hydroxycitalopram)	0.74	1.0	0.1
Citalopram related compound C <sup>d</sup> (3-ox-ocitalopram)	0.90	0.79	0.1
Citalopram related compound D <sup>e</sup> (desmethyl citalopram)	0.97	1.0	0.1
Escitalopram	1.0	—	—
Citalopram related compound E <sup>f</sup> (citalopram N-oxide)	1.1	1.0	0.1
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

▲\* Included for identification only. This peak is due to the oxalate counterion and hence is not an impurity.▲USP36

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4'-fluorophenyl)-5-(4-dimethylaminobutyryl)-1,3-dihydroisobenzofuran.

<sup>b</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

<sup>c</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>d</sup> 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.

<sup>e</sup> 1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>f</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-N-oxide.

**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method 1a (921): NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP R-Citalopram Oxalate RS

(R)-1-[3-(Dimethylamino)propyl]-1-(p-fluorophenyl)-5-phthalanarbonitrile oxalate.

C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O · C<sub>2</sub>H<sub>2</sub>O<sub>4</sub> 414.43

USP Citalopram Related Compound D RS

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.

C<sub>19</sub>H<sub>19</sub>FN<sub>2</sub>O · HCl 346.83

USP Escitalopram Oxalate RS

## Escitalopram Tablets

### DEFINITION

Escitalopram Tablets contain an amount of escitalopram oxalate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{20}H_{21}FN_2O$ .

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 1.5 g of anhydrous sodium acetate and 0.4 mL of glacial acetic acid in 1 L of water. Adjust with 1 M sodium hydroxide to a pH of 5.2.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (33:7:60)

**System suitability solution:** 6.2 µg/mL of USP Citalopram Hydrobromide RS (equivalent to 5 µg/mL of citalopram) and 1 µg/mL of USP Citalopram Related Compound C RS in *Mobile phase*

**Standard solution:** 0.62 mg/mL of USP Citalopram Hydrobromide RS in *Mobile phase* (equivalent to 0.5 mg/mL of citalopram)

**Sample solution:** Transfer 10 Tablets to a suitable volumetric flask, add *Buffer* to 10% of the final volume, and shake vigorously for 10 min. Add methanol to 50% of the final volume, shake for 1 additional min, sonicate for 10 min, and dilute with *Mobile phase* to volume to obtain a solution having a concentration of about 0.5 mg/mL of escitalopram.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 10-cm; 3-µm packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between citalopram and citalopram related compound C, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of  $C_{20}H_{21}FN_2O$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution 1:** 3 µg/mL of USP Citalopram Hydrobromide RS in *Medium*

**Standard solution 2:** 15 µg/mL of USP Citalopram Hydrobromide RS in *Medium*

**Standard solution 3:** 30 µg/mL of USP Citalopram Hydrobromide RS in *Medium*

**Sample solution:** Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

#### Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Analytical wavelength:** 239 nm

**Path length:** 0.5 cm

**Blank:** *Medium*

#### System suitability

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

#### Suitability requirements

**Correlation coefficient:** NLT 0.995, determined using *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*, three replicates of each solution

**Relative standard deviation:** NMT 2.0%, determined using *Standard solution 3*, six replicates

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution*

Generate a calibration curve using the data from *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*. Determine the concentration,  $C_U$ , in mg/mL, of citalopram hydrobromide in the *Sample solution* using the calibration curve.

Calculate the percentage of citalopram dissolved:

$$\text{Result} = (C_U/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

$C_U$  = concentration of citalopram hydrobromide in the *Sample solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of escitalopram is dissolved.

- UNIFORMITY OF DOSAGE UNITS <905>:** Meets the requirements

### IMPURITIES

#### ORGANIC IMPURITIES

**Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between citalopram and citalopram related compound C, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of citalopram from the *Standard solution*  
 $C_S$  = concentration of USP Citalopram Hydrobromide RS in the *Standard solution*  
 $C_U$  = nominal concentration of the *Sample solution*  
 $F$  = relative response factor (see *Impurity Table 1*)  
 $M_{r1}$  = molecular weight of citalopram, 324.39  
 $M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 2.0%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A <sup>a</sup>	0.33	0.84	0.3
Citalopram related compound B <sup>b</sup>	0.56	0.78	0.5
Citalopram related compound C <sup>c</sup> (3-oxocitalopram)	0.80	0.51	0.5
Escitalopram	1.0	—	—
Citalopram related compound E <sup>d</sup> (citalopram <i>N</i> -oxide)	1.4	0.94	0.2
Any other individual, unspecified impurity	—	1.0	0.1

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

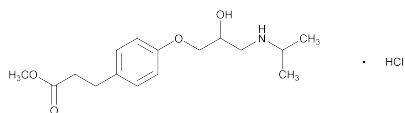
<sup>b</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile; 3-hydroxycitalopram.

<sup>c</sup> 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone.

<sup>d</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Citalopram Hydrobromide RS
  - USP Citalopram Related Compound C RS
  - 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone.
  - $C_{20}H_{19}FN_2O_2$  338.22

**Esmolol Hydrochloride**

$C_{16}H_{25}NO_4 \cdot HCl$  331.83  
 Benzenepropanoic acid, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-, methyl ester, hydrochloride, ( $\pm$ );  
 ( $\pm$ )-Methyl *p*-[2-hydroxy-3-(isopropylamino)propoxy]hydrocinnamate hydrochloride [81161-17-3].

**DEFINITION**

Esmolol Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{16}H_{25}NO_4 \cdot HCl$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 3.0 g of potassium dihydrogen phosphate in 650 mL of water.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (15:20:65)

**System suitability stock solution:** 1 mg/mL of esmolol hydrochloride prepared as follows. Transfer a suitable quantity of USP Esmolol Hydrochloride RS to a suitable volumetric flask, and dissolve in and dilute with 1 N hydrochloric acid to volume. Allow the contents to stand for at least 30 min. [NOTE—This results in the partial degradation of the esmolol resulting in the production of esmolol free acid (see *System suitability* for relative retention time).]

**System suitability solution:** 0.2 mg/mL in water from *System suitability stock solution*

**Standard solution:** 200  $\mu$ g/mL of USP Esmolol Hydrochloride RS in water

**Sample solution:** 200  $\mu$ g/mL of Esmolol Hydrochloride in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 222 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m; L1 packing

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for esmolol free acid and esmolol are 0.41 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4.0 between esmolol free acid and esmolol, *System suitability solution*

**Tailing factor:** NMT 2.0 for the esmolol peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of esmolol hydrochloride ( $C_{16}H_{25}NO_4 \cdot HCl$ ) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of esmolol from the *Sample solution*  
 $r_S$  = peak response of esmolol from the *Standard solution*

$C_S$  = concentration of USP Esmolol Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Esmolol Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**• **HEAVY METALS** (231)

**Standard solution:** Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

**Sample solution:** Into a 50-mL color-comparison tube dissolve 1 g of the sample in water, and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

To each of the tubes add 10 mL of hydrogen sulfide TS, and mix. Allow to stand for 2 min. View downward into the tube over a white background.

**Acceptance criteria:** The color of the *Sample solution* is not darker than the color of the *Standard solution* (NMT 20 ppm).

• **RESIDUE ON IGNITION** (281): NMT 0.1%• **ORGANIC IMPURITIES**

**Buffer and System suitability solution:** Prepare as directed in the *Assay*.

**Solution A:** Methanol

**Solution B:** Prepare as directed for *Mobile phase* in the *Assay*.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	0	100
20	0	100
25	25	75
35	25	75
36	0	100
40	0	100

**Sample solution:** 1 mg/mL of Esmolol Hydrochloride in water

**Chromatographic system:** Prepare as directed in the *Assay*.

**Column temperature:** 30°

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 4.0 between esmolol free acid and esmolol

**Tailing factor:** NMT 2.0 for the esmolol peak

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each individual impurity in the portion of Esmolol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Esmolol free acid <sup>a</sup>	0.43	0.3
Esmolol aminopentanol analog <sup>b</sup>	0.84	0.10
N-Ethyl esmolol <sup>c</sup> (if present)	0.88	0.15
Esmolol	1.0	—
Esmolol dimer <sup>d</sup>	6.5	0.15
Any other individual unidentified impurity	—	0.10
Total impurities <sup>e</sup>	—	0.7

<sup>a</sup> 3-[4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl]propanoic acid.

<sup>b</sup> Methyl 3-[4-(5-amino-2-hydroxypentyloxy)phenyl]propanoate.

<sup>c</sup> Methyl 3-[4-[3-(ethylamino)-2-hydroxypropoxy]phenyl]propanoate.

<sup>d</sup> Methyl 3-[4-[2-hydroxy-3-(3-[4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl)-N-isopropylpropanamido]propoxy]phenyl]propanoate.

<sup>e</sup> Disregard any peak below 0.05%.

**SPECIFIC TESTS**

• **pH** (791): 3.0–5.0

• **WATER DETERMINATION** (921), *Method Ia*: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

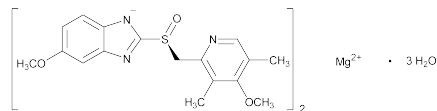
• **PACKAGING AND STORAGE:** Protect from freezing, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Esmolol Hydrochloride RS

Benzenepropanoic acid, 4-[2-hydroxy-3-[(1-methylethyl)-amino]propoxy]-, methyl ester, hydrochloride, (±)-.

C<sub>16</sub>H<sub>25</sub>NO<sub>4</sub> · HCl 331.83

**Esomeprazole Magnesium**

C<sub>34</sub>H<sub>36</sub>MgN<sub>6</sub>O<sub>6</sub>S<sub>2</sub> · 3H<sub>2</sub>O

Trihydrate: 767.17

C<sub>34</sub>H<sub>36</sub>MgN<sub>6</sub>O<sub>6</sub>S<sub>2</sub>

Anhydrous: 713.12

1*H*-Benzimidazole, 5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl], magnesium salt (2:1), trihydrate;

5-Methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl]benzimidazole, magnesium salt (2:1), trihydrate [217087-09-7].

**DEFINITION**

Esomeprazole Magnesium contains NLT 98.0% and NMT 102.0% of C<sub>34</sub>H<sub>36</sub>MgN<sub>6</sub>O<sub>6</sub>S<sub>2</sub>, calculated on the anhydrous basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** (197K)

• **B.** The *Sample solution*, prepared and tested as directed in the test for *Content of Magnesium*, exhibits a significant absorption at 285.2 nm.



**ASSAY****• PROCEDURE**

**Solution A:** Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Solution B:** Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

**Mobile phase:** Acetonitrile and *Solution A* (7:13)

**Standard solution:** Transfer 10 mg of USP Omeprazole RS to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [NOTE—This solution contains 0.05 mg/mL of omeprazole.]

**Sample solution:** Transfer 10 mg of Esomeprazole Magnesium to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [NOTE—This solution contains 0.05 mg/mL of esomeprazole magnesium.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm × 12.5-cm or a 4.6-mm × 15-cm; 5-μm packing L7. [NOTE—Alternatively, a 3.9-mm × 15-cm column that contains 4-μm packing L1 may be used.]

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>34</sub>H<sub>36</sub>MgN<sub>6</sub>O<sub>5</sub>S<sub>2</sub> in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M_{r1}/(2 \times M_{r2})] \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of omeprazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of Esomeprazole Magnesium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of esomeprazole magnesium, 713.12

$M_{r2}$  = molecular weight of omeprazole, 345.42

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**OTHER COMPONENTS****• CONTENT OF MAGNESIUM**

**Lanthanum solution:** Transfer 58.7 g of lanthanum oxide into a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between the additions. Add water while stirring, cool to room temperature, and dilute with water to volume. [NOTE—Store the solution in a plastic bottle.]

**Standard stock solution:** 1000 μg/mL of magnesium in water, from a commercially prepared atomic absorption standard solution. [NOTE—Store the solution in a plastic bottle.]

**Standard solution A:** Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumet-

ric flask, and dilute with water to volume. [NOTE—This solution contains 2 μg/mL of magnesium.]

**Standard solution B:** Combine 5.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.1 μg/mL).

**Standard solution C:** Combine 10.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.2 μg/mL).

**Standard solution D:** Combine 15.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.3 μg/mL).

**Standard solution E:** Combine 20.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.4 μg/mL).

**Standard solution F:** Combine 25.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.5 μg/mL). [NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of *Standard solutions* can be reduced.]

**Blank solution:** Transfer 4.0 mL of *Lanthanum solution* to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 250 mg of Esomeprazole Magnesium to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 min. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution to another 100-mL volumetric flask, add 4.0 mL of *Lanthanum solution*, and dilute with water to volume.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometer

**Flame:** Air-acetylene

**Analytical wavelength:** 285.2 nm

**Analysis**

**Samples:** *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, *Standard solution F*, *Blank solution*, and *Sample solution*

Determine the concentration,  $C_s$ , in μg/mL, of magnesium in the *Sample solution* using the calibration graph.

Calculate the percentage of magnesium in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (C_S/C_U) \times (100/(100 - F)) \times 100$$

$C_S$  = content of magnesium in the *Sample solution* as calculated above (μg/mL)

$C_U$  = concentration of Esomeprazole Magnesium in the *Sample solution* (μg/mL)

$F$  = content of water in Esomeprazole Magnesium, as determined in *Specific Tests, Water Determination* (%)

**Acceptance criteria:** 3.30%–3.55%, on anhydrous basis

**IMPURITIES****Organic Impurities****• PROCEDURE 1**

**Solution A:** 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Mobile phase:** Acetonitrile and *Solution A* (11:29).

[NOTE—To improve the resolution, the composition may be changed to 1:3, if necessary.]

**System suitability solution:** 1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A

RS in 25 mL of *Mobile phase*. [NOTE—Omeprazole Related Compound A is omeprazole sulfone.]

**Sample solution:** 4 mg of Esomeprazole Magnesium in 25 mL of *Mobile phase*. [NOTE—Prepare this solution fresh.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm × 12.5-cm or a 4.6-mm × 15-cm; 5-μm packing L7. [NOTE— Alternatively, a 3.9-mm × 15-cm column that contains 4-μm packing L1 may be used.]

**Flow rate:** 0.8–1 mL/min

**Injection size:** 50 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE— For relative retention times, see *Impurity Table 1*.]

#### Suitability requirements

**Resolution:** NLT 3 between omeprazole related compound A and omeprazole

#### Analysis

**Sample:** *Sample solution*

Record the chromatogram for at least 4.5 times the retention time of the omeprazole peak, and measure the peak responses. Identify the impurities based on the retention times shown in *Impurity Table 1*.

Calculate the percentage of any individual impurity in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of all peak responses

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.5%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole <i>N</i> -oxide <sup>a</sup>	0.45	0.1
Omeprazole sulfone <sup>b</sup> (related compound A)	0.8	0.2
Any other individual impurities	—	0.1
Omeprazole	1.0	—

<sup>a</sup> 4-Methoxy-2-[[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.

<sup>b</sup> 5-Methoxy-2-[[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.

#### • PROCEDURE 2: ENANTIOMERIC PURITY

**Solution A:** Mix 70 mL of 1 M monobasic sodium phosphate with 20 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL.

**Diluent:** Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Solution A* (3:17)

**System suitability solution:** 2 mg of USP Omeprazole RS in 10 mL of *Diluent*. Dilute 1.0 mL of this solution with *Diluent* to 50 mL.

**Sample solution:** 40 mg of Esomeprazole Magnesium in 5 mL of methanol, and dilute with *Diluent* to 25 mL. Dilute 1 mL of this solution with *Diluent* to 50 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 302 nm

**Column:** 4.0-mm × 10-cm; packing L41

**Flow rate:** 0.6 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3 between the enantiomer peaks.

[NOTE— The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of the *R*-enantiomer in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = peak response for the *R*-enantiomer

$r_S$  = sum of the peak responses for esomeprazole and *R*-enantiomer

**Acceptance criteria:** NMT 0.2% of the *R*-enantiomer

#### SPECIFIC TESTS

• **WATER DETERMINATION, Method I** <921>: 6.0%–8.0%

#### • COLOR OF SOLUTION

**Sample solution:** 20 mg/mL of Esomeprazole Magnesium in methanol

**Analysis:** Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank.

**Acceptance criteria:** NMT 0.2

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.

#### • USP REFERENCE STANDARDS <11>

USP Esomeprazole Magnesium RS

USP Omeprazole RS

USP Omeprazole Related Compound A RS

Omeprazole sulfone, 5-methoxy-2-[[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.

C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S 361.42 [CAS-88546-55-8]

## Esomeprazole Magnesium Delayed-Release Capsules

#### DEFINITION

Esomeprazole Magnesium Delayed-Release Capsules contain an amount of Esomeprazole Magnesium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of esomeprazole (C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>S).

#### IDENTIFICATION

##### • A.

**Buffer:** Prepare a pH 6.0 phosphate buffer containing 26.6 g/L of dibasic sodium phosphate dihydrate and 55.2 g/L of monobasic sodium phosphate monohydrate in water.

**Diluent:** Prepare a pH 11.0 diluent as follows. Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1000 mL.

**Mobile phase:** Transfer 150 mL of acetonitrile and 85 mL of the *Buffer* to a 1000-mL volumetric flask, and dilute with water to volume.

**Standard stock solution:** Prepare a solution containing 0.2 mg/mL of USP Omeprazole RS by dissolving a suitable amount first in alcohol, using 20% of the final volume, and then diluting with *Diluent* to volume.

**Standard solution:** 0.02 mg/mL of USP Omeprazole RS from the *Standard stock solution* in water

**Sample stock solution:** Transfer a portion of the Capsule content, equivalent to 20 mg of esomeprazole, to a 200-mL volumetric flask, add 120 mL of *Diluent*, and shake for 20 min to dissolve the pellets. Sonicate for a few min, if needed, to completely dissolve. Add 40 mL of alcohol, and sonicate for a few min. Cool, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 1- $\mu$ m pore size.

**Sample solution:** 0.01 mg/mL of esomeprazole from the *Sample stock solution* in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 302 nm

**Column:** 4.0-mm  $\times$  10-cm; 5- $\mu$ m packing L41

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]

#### Suitability requirements

**Resolution:** NLT 1.0 between the enantiomer peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the ratio of the retention times of the esomeprazole peak in the *Standard solution* and the *Sample solution*:

$$\text{Result} = (t_U/t_S)$$

$t_U$  = retention time of esomeprazole from the *Sample solution*

$t_S$  = retention time of esomeprazole from the *Standard solution*

**Acceptance criteria:** 0.98–1.02

### ASSAY

#### • PROCEDURE

**Buffer:** Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.

**Diluent:** Prepare as directed in *Identification test A*.

**Mobile phase:** Mix 350 mL of acetonitrile and 500 mL of the *Buffer*. Dilute with water to 1000 mL.

**Standard solution:** Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 40 mL of *Diluent*, and dilute with water to volume. This solution contains 0.04 mg/mL of USP Omeprazole RS.

**Sample stock solution:** Mix the contents of NLT 20 Capsules. Transfer a portion of the Capsule content, equivalent to 20 mg of esomeprazole, to a 100-mL volumetric flask, add 60 mL of *Diluent*, and shake for 20 min to dissolve the pellets. Sonicate for a few min, if needed, to completely dissolve. Add 20 mL of alcohol, and sonicate for a few min. Cool, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 1- $\mu$ m pore size.

**Sample solution:** 0.04 mg/mL of esomeprazole from the *Sample stock solution* in water. Store this solution protected from light.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 302 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of esomeprazole ( $C_{17}H_{19}N_3O_3S$ ) in the portion of the Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of esomeprazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium, if necessary, to a pH of  $6.8 \pm 0.05$ .

**Apparatus 2:** 100 rpm

**Time:** 30 min in a pH 6.8 phosphate buffer

**Standard solution:** Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute this solution with pH 6.8 phosphate buffer to obtain a solution containing ( $L/1000$ ) mg/mL, where  $L$  is the label claim, in mg/Capsule. Immediately add 2.0 mL of 0.25 M sodium hydroxide to 10.0 mL of this solution, and mix. [NOTE—Do not allow the solution to stand before adding the sodium hydroxide solution.]

**Sample solution:** After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.

**Buffer, Diluent, Mobile phase, System suitability, and Chromatographic system:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of esomeprazole ( $C_{17}H_{19}N_3O_3S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$V$  = volume of *Medium*, 1000 mL

**Tolerances:** NLT 75% ( $Q$ ) of the labeled amount of esomeprazole ( $C_{17}H_{19}N_3O_3S$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer:** Prepare a pH 7.6 phosphate buffer by mixing 5.2 mL of 1.0 M monobasic sodium phosphate buffer and 63 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.

**Solution A:** Mix 100 mL of acetonitrile and 100 mL of the *Buffer*. Dilute with water to 1000 mL.

**Solution B:** Mix 800 mL of acetonitrile and 10 mL of the Buffer. Dilute with water to 1000 mL.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	80	20
30	0	100
31	100	0
45	100	0

**Diluent:** Prepare as directed in Identification test A.

**System suitability stock solution:** 1 mg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS in methanol

**System suitability solution:** 1 µg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS from System suitability stock solution, in a mixture of Diluent and water (1:4)

**Sample solution:** Transfer a portion of the powdered pellets, from the Capsule content, equivalent to 80–90 mg of esomeprazole, to a 200-mL volumetric flask, add 20 mL of methanol, and shake for 30 s. Add 40 mL of Diluent, shake for 30 s by hand, and sonicate for a few min. Cool, and dilute with water to volume. Pass a portion of the solution through a filter of 0.45-µm pore size. [NOTE—The solution is stable for 3 h if stored protected from light.]

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 302 nm

**Column:** 4.6-mm × 10-cm; 3-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** System suitability solution

[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.5 between omeprazole related compound A and omeprazole

#### Analysis

**Sample:** Sample solution

Calculate the percentage of any individual impurity in the portion of the Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of all peak responses

**Acceptance criteria:** See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole sulfone <sup>a</sup>	0.93	0.5
Omeprazole	1.00	—
Any other individual impurity	—	0.2
Total impurities	—	2

<sup>a</sup> Omeprazole related compound A.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

#### USP REFERENCE STANDARDS <11>

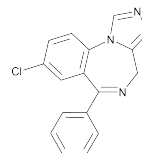
USP Omeprazole RS

USP Omeprazole Related Compound A RS

Omeprazole sulfone; 5-methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfonyl]-1H-benzimidazole.

C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S 361.42

## Estazolam



C<sub>16</sub>H<sub>11</sub>ClN<sub>4</sub> 294.74  
4H-[1,2,4]Triazolo[4,3-a][1,4]benzodiazepine, 8-chloro-6-phenyl-;  
8-Chloro-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine [29975-16-4].

#### DEFINITION

Estazolam contains NLT 98.0% and NMT 102.0% of C<sub>16</sub>H<sub>11</sub>ClN<sub>4</sub>, calculated on the dried basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION <197K>**

- B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer:** 2.8 g/L of potassium phosphate, monobasic in water. Adjust with 1 N NaOH to a pH of 6.5.

**Mobile phase:** Acetonitrile, methanol, and Buffer (10:35:55)

**Standard stock solution:** 0.5 mg/mL of USP Estazolam RS in Mobile phase

**Standard solution:** 0.02 mg/mL of USP Estazolam RS in water from Standard stock solution

**Sample stock solution:** 0.5 mg/mL of Estazolam in Mobile phase

**Sample solution:** 0.02 mg/mL of Estazolam in water from Sample stock solution

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L11

**Flow rate:** 1 mL/min

**Injection size:** 25 µL

**Run time:** 2.5 times the retention time of the estazolam peak

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of C<sub>16</sub>H<sub>11</sub>ClN<sub>4</sub> in the portion of Estazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

- $C_s$  = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Estazolam in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **HEAVY METALS**, *Method II* (231): NMT 20 ppm

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 1.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
 USP Estazolam RS  
 4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-  
 8-Chloro-6-phenyl-4*H*-s-triazolo[4,3-*a*]  
 [1,4]benzodiazepine.  
 $C_{16}H_{11}ClN_4$  294.74

**Estazolam Tablets****DEFINITION**

Estazolam Tablets contain an amount of Estazolam equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of estazolam ( $C_{16}H_{11}ClN_4$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 2.8 g/L of potassium phosphate, monobasic in water. Adjust to a pH of 6.5 with 1 N NaOH.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (10:35:55)

**Standard solution:** 0.02 mg/mL of USP Estazolam RS in *Mobile phase*

**Sample solution:** 0.02 mg/mL of Estazolam in *Mobile phase*, from NLT 20 finely powdered Tablets. [NOTE—Sonicate for 5 min. Pass a portion through a suitable filter with no glass prefilter.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L11

**Flow rate:** 1 mL/min

**Injection size:** 25 μL

**Run time:** 2.7 times the retention time of the estazolam peak

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{11}ClN_4$  in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of estazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** Water; 900 mL, deaerated

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer and Mobile phase:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.1 mg/mL of USP Estazolam RS in methanol

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of about (L/1000) mg/mL, where L is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size, discarding the first few mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Proceed as directed in the *Assay*, except for the injection size and run time.

**Injection size:** 100 μL

**Run time:** 1.6 times the retention time of estazolam

**System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of estazolam dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

$r_u$  = peak response of estazolam from the *Sample solution*

$r_s$  = peak response of estazolam from the *Standard solution*

$C_s$  = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

V = volume of *Medium* (mL), 900

**Tolerances:** NLT 80% (Q) of the labeled amount of estazolam is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****Organic Impurities****• PROCEDURE**

**Buffer, Mobile phase, Standard solution, and Sample solution:** Proceed as directed in the *Assay*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Proceed as directed in the *Assay*, except for the injection size and run time.

**Injection size:** 25 μL for the *Standard solution* and 50 μL for the *Sample solution*

**Run time:** 4 times the retention time of estazolam

**System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of the responses of all peaks from the *Sample solution*

**Acceptance criteria**

Any individual unspecified degradation product: NMT 0.5%

Total impurities: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

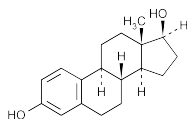
• **USP REFERENCE STANDARDS** (11)

USP Estazolam RS

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-

8-Chloro-6-phenyl-4*H*-s-triazolo[4,3-*a*][1,4]benzodiazepine.

C<sub>16</sub>H<sub>11</sub>ClN<sub>4</sub> 294.74

**Estradiol**

C<sub>18</sub>H<sub>24</sub>O<sub>2</sub> 272.38

Estra-1,3,5(10)-triene-3,17-diol, (17 $\beta$ )-.

Estra-1,3,5(10)-triene-3,17 $\beta$ -diol [50-28-2].

Hemihydrate 281.39 [35380-71-3].

» Estradiol contains not less than 97.0 percent and not more than 103.0 percent of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—The hemihydrate form is so labeled.

**USP Reference standards** (11)—

USP Estradiol RS

USP Estrone RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 50  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 280 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**Melting range**, *Class I* (741): between 173° and 179°.

[NOTE—Dry over silica gel for not less than 16 hours prior to testing.]

**Specific rotation** (781S): between +76° and +83°.

*Test solution:* 10 mg per mL, in dioxane.

**Water**, *Method I* (921): not more than 3.5%.

**Chromatographic purity**—[NOTE—Make all solutions fresh daily.]

*Mobile phase*—Prepare a filtered and degassed mixture of 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol (45:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluting solution*—Prepare a filtered and degassed mixture of *n*-butyl chloride and methanol (5:1).

*Test solution*—Transfer about 70 mg of Estradiol, accurately weighed, to a 10-mL volumetric flask, dissolve in *Diluting solution*, shake vigorously to aid dissolution, dilute with *Diluting solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between estradiol and any impurity is not less than 1.0; the column efficiency is not less than 800 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Estradiol taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the peak response for each impurity; and *r<sub>s</sub>* is the sum of the responses of all the peaks: not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Transfer about 300 mg of ethylparaben to a 500-mL volumetric flask, add methanol to volume, and mix.

*Standard preparation*—Dissolve accurately weighed quantities of USP Estradiol RS and USP Estrone RS in methanol to obtain a solution containing 0.40 mg and 0.24 mg, respectively, in each mL. Pipet 10 mL of this solution and 5 mL of the *Internal standard solution* into a 200-mL volumetric flask. Add 100 mL of methanol, dilute with water to volume, and mix to obtain a solution having a known concentration of about 20  $\mu$ g of USP Estradiol RS per mL.

*Assay preparation*—Transfer about 100 mg of Estradiol, accurately weighed, to a 250-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 5.0 mL of *Internal standard solution* and 100 mL of methanol, dilute with water to volume, and mix.

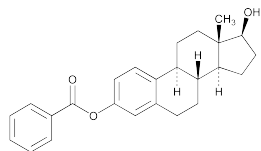
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 205-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for the internal standard, about 1.3 for estrone, and 1.0 for estradiol; the resolution, *R*, between the analyte and estrone is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub> in the portion of Estradiol taken by the formula:

$$5C(R_U / R_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Estradiol RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Estradiol Benzoate



$C_{25}H_{28}O_3$  376.49

Estra-1,3,5(10)-triene-3,17-diol, (17 $\beta$ )-, 3-benzoate.

Estradiol 3-benzoate [50-50-0].

» Estradiol Benzoate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{25}H_{28}O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is for veterinary use only. Label it to indicate whether it is coarse grade or fine grade.

**USP Reference standards** (11)—

USP Estradiol Benzoate RS

**Identification**—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between +57.0° and +63.0°.

*Test solution*: 10 mg per mL, previously dried, in dioxane.

**Loss on drying**—Dry it at 100° to 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%, a 250-mg specimen being used.

**Particle size** (786)—

*Suspension fluid*—To a mixture of glycerin and water (60:40, w/w) add a sufficient quantity of polysorbate 20 to obtain a solution having a concentration of 125  $\mu$ L of polysorbate 20 per 100 g of solution.

*Test suspension*—Saturate the *Suspension fluid* by adding about 100 mg of fine Estradiol Benzoate per 100 g of *Suspension fluid*, and sonicate for about 10 minutes. Filter the resulting suspension through a 0.45- $\mu$ m nylon filter. To the filtrate, add about 50 mg of Estradiol Benzoate per mL of the filtered, saturated *Suspension fluid*, and mix on a vortex mixer until dispersed (about 1 minute).

*Procedure*—Using a suitable multi-wavelength particle size analyzer,<sup>1</sup> determine the particle size distribution within the *Test suspension*, analyzing the results in the range from 5  $\mu$ m to 600  $\mu$ m. Not more than 50% of the particles are less than 30  $\mu$ m, and not less than 90% of the particles are less than 450  $\mu$ m. The mean diameter of fine grade Estradiol Benzoate is not more than 100  $\mu$ m, and the mean diameter of coarse grade Estradiol Benzoate is not less than 100  $\mu$ m and not more than 200  $\mu$ m.

**Limit of methanol and dichloromethane**—

*Internal standard solution*—Prepare a solution that contains 0.1% (v/v) ethyl acetate in pyridine. [NOTE—Inject 2  $\mu$ L of the pyridine into the *Chromatographic system* to confirm that it contains no peaks that would interfere with the analysis.]

*Standard solution*—Transfer 50  $\mu$ L of methylene chloride and 50  $\mu$ L of methanol to a 50-mL volumetric flask, and dilute with *Internal standard solution* to volume. Mix well.

<sup>1</sup>A suitable multi-wavelength particle size analyzer is model LS 13 320, obtained from Beckman Coulter, Inc., Fullerton, CA, or equivalent.

*Test solution*—Accurately weigh about 100 mg of Estradiol Benzoate into a low-actinic glass vial. Dissolve in 1.0 mL of *Internal standard solution*, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 3.2-mm  $\times$  1.8-m stainless steel column packed with support S3. The injection port temperature is maintained at about 165°, the detector temperature is maintained at about 165°, and the column temperature is maintained at 140° for 20 minutes, programmed thereafter to rise to 250° at a rate of 40° per minute, and then maintained at 250° for 15 minutes. Helium is used as the carrier gas, flowing at a rate of about 40 mL per minute. Chromatograph the *Internal standard solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the order of elution is methanol, methylene chloride, and ethyl acetate; no peaks are present within the *Internal standard solution* that would interfere with the integration of either the methanol or the methylene chloride peak; baseline resolution is achieved among the internal standard peak and residual solvent peaks; and the standard deviation for replicate injections of the *Standard solution* is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 2.0  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for methanol, methylene chloride, and ethyl acetate. Calculate the percentage (w/w) of each residual solvent in the portion of Estradiol Benzoate taken by the formula:

$$100C_i(D_i / C_u)(R_u / R_s)$$

in which  $C_i$  is the concentration, in  $\mu$ L per mL, of the solvent of interest in the *Standard solution*;  $D_i$  is the density, in g per mL, of the solvent of interest;  $C_u$  is the concentration, in mg per mL, of Estradiol Benzoate in the *Test solution*; and  $R_u$  and  $R_s$  are the peak response ratios of the solvent of interest to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: the sum of the percentages of methanol and methylene chloride is not more than 0.20%.

**Chromatographic purity**—

*Adsorbent*: a 0.25-mm layer of chromatographic silica gel mixture.

*Developing solvent system*—Use a mixture of toluene and ethyl acetate (70:30).

*Ammonium molybdate solution*—Dissolve 5 g of ammonium molybdate in 100 mL of 10% (v/v) sulfuric acid.

*Diluent*—Prepare a solution containing a mixture of methylene chloride and alcohol (2:1).

*Standard solution*—Dissolve an accurately weighed quantity of USP Estradiol Benzoate RS in *Diluent* to obtain a solution containing about 5 mg per mL.

*Test solution 1*—Dissolve an accurately weighed quantity of Estradiol Benzoate in *Diluent* to obtain a solution containing about 5 mg per mL.

*Test solution 2*—Transfer 200  $\mu$ L of *Test solution 1* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.

*Procedure* (see *Thin-Layer Chromatography* under *Chromatography* (621))—Apply to the thin-layer chromatographic plate 20- $\mu$ L aliquots of the *Standard solution* and *Test solution 1* and 20- $\mu$ L, 15- $\mu$ L, 10- $\mu$ L, 5- $\mu$ L, and 2- $\mu$ L aliquots of *Test solution 2*; the volumetric series of *Test solution 2* represents 2.0%, 1.5%, 1.0%, 0.5% and 0.2% of the concentration of Estradiol Benzoate within the *Test solution 1* spot. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate from the plate. Spray the plate thoroughly with the *Ammonium molybdate solution*, and dry. Heat the plate

in a drying oven for about 10 minutes at about 115°. Calculate the relative retardation factor,  $R_{rel}$ , (relative to estradiol benzoate) of all spots within the lanes for *Test solution 1* and *Test solution 2*. Possible estradiol benzoate impurities include, but are not limited to, estradiol [estra-1,3,5(10)-triene-3,17b-diol], 17 $\alpha$ -estradiol benzoate [estra-1,3,5(10)-triene-3,17 $\alpha$ -diol 3-benzoate], and estrone [estra-1,3,5(10)-triene-17-one, 3-hydroxy]; their relative retardation factors,  $R_{rel}$ , are about 0.84, 1.15, and 1.21, respectively. Determine the percentages of each impurity by comparing the intensity of the impurity spots within *Test solution 1* to those of the main spots obtained from the series of *Test solution 2*, ignoring any impurity peak less intense than the main spots found in the *Test solution 2* lane containing 0.2% of the amount of estradiol benzoate of *Test solution 1*. Not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability preparation**—Transfer an accurately weighed quantity of about 20.0 mg each of USP Estradiol Benzoate RS and estradiol 17-acetate into a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume, and mix.

**Standard preparation**—Transfer an accurately weighed quantity of about 20.0 mg of USP Estradiol Benzoate RS into a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately weighed quantity of about 20.0 mg of Estradiol Benzoate into a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector, a 4.6-mm  $\times$  4.5-cm guard column that contains packing L1, and a 4.6-mm  $\times$  25-cm analytical column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for estradiol 17-acetate and 1.0 for estradiol benzoate; the resolution,  $R$ , between estradiol 17-acetate and estradiol benzoate is not less than 6.0; the column efficiency is not less than 8000 theoretical plates for estradiol benzoate; the tailing factor for estradiol benzoate is not more than 2.0; and, using the *Standard preparation*, the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{25}H_{28}O_3$  in the portion of Estradiol Benzoate taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Estradiol Benzoate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Estradiol Vaginal Cream

### DEFINITION

Estradiol Vaginal Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ( $C_{18}H_{24}O_2$ ) in a suitable cream base.

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 0.5 mg/mL of USP Estradiol RS in chloroform

**Sample solution:** Transfer a portion of Vaginal Cream, equivalent to 1 mg of estradiol, to a 150-mL beaker. Add 25 mL of acetonitrile, and gently heat to boiling. Boil for 45 s, and cool to room temperature. Add 25 mL of water, and swirl. Filter with the aid of suction. Transfer the filtrate to a 125-mL separator, add 50 mL of chloroform, and shake. Allow the layers to separate, drain the chloroform layer into a flask, and evaporate in a rotary evaporator to dryness. Dissolve the residue in 2 mL of chloroform.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 50  $\mu$ L. Dry the applications with the aid of a stream of nitrogen.

**Developing solvent system:** Toluene and acetone (4:1)

**Spray reagent:** Sulfuric acid and methanol (1:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Spray the plate with a fine mist of *Spray reagent*, then heat the plate for 3–5 min at 90°. Observe the plate under visible light.

**Acceptance criteria:** The  $R_f$  value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (1:1)

**Internal standard solution:** 60  $\mu$ g/mL of dydrogesterone in acetonitrile. Use a freshly prepared solution.

**Standard solution:** 10  $\mu$ g/mL of USP Estradiol RS and 7.5  $\mu$ g/mL of USP Estrone RS, prepared as follows.

Transfer 10 mg of USP Estradiol RS and 7.5 mg of USP Estrone RS to a 1000-mL volumetric flask. Add 50.0 mL of *Internal standard solution* and 450 mL of acetonitrile, and mix. Dilute with water to volume.

**Sample solution:** Nominally 10  $\mu$ g/mL of estradiol, prepared as follows. Transfer a portion of Vaginal Cream, equivalent to 0.5 mg of estradiol, to a 150-mL beaker. Add 2.5 mL of *Internal standard solution*, 22.5 mL of acetonitrile, and a few boiling chips. Cover with a watch glass, and heat gently until the Vaginal Cream melts, swirling occasionally. Heat to boiling for about 45 s. Allow to cool to room temperature, add 25.0 mL of water, and mix. Pass first through paper and then through a microdisk filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for estradiol, estrone, and the internal standard are 1.0, 1.25, and 2.0, respectively.]



**Suitability requirements****Resolution:** NLT 1.9 between estradiol and estrone**Relative standard deviation:** NMT 3.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of estradiol ( $C_{18}H_{24}O_2$ ) in the portion of Vaginal Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 $R_U$  = peak response ratio of estradiol to the internal standard from the *Sample solution* $R_S$  = peak response ratio of estradiol to the internal standard from the *Standard solution* $C_S$  = concentration of USP Estradiol RS in the *Standard solution* ( $\mu\text{g/mL}$ ) $C_U$  = nominal concentration of estradiol in the *Sample solution* ( $\mu\text{g/mL}$ )**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **MINIMUM FILL** <755>: Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
- **PH** <791>: 3.5–6.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
- **USP REFERENCE STANDARDS** <11>  
USP Estradiol RS  
USP Estrone RS

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**Estradiol Vaginal Inserts**


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**DEFINITION**Estradiol Vaginal Inserts contain NLT 90% and NMT 107% of the labeled amount of estradiol ( $C_{18}H_{24}O_2$ ).**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>

[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]

**Standard solution:** 2.5 mg/mL of USP Estradiol RS in absolute alcohol**Sample solution:** Place a number of Inserts, equivalent to 0.38 or 0.95 mg of estradiol, into a vessel. Add 50 mL of isopropyl alcohol, and allow to disintegrate by stirring overnight. Centrifuge the suspension. Evaporate an aliquot of 40 mL of the supernatant to dryness, and dissolve the residue in 3 mL of isopropyl alcohol. Evaporate to dryness, reconstitute with 300  $\mu\text{L}$  of absolute alcohol to obtain a solution containing 1.0 or 2.5 mg/mL of estradiol, and centrifuge.**Adsorbent:** Use a suitable, high-performance thin-layer chromatographic plate.**Application volume:** NLT 5  $\mu\text{L}$  (equivalent to 10  $\mu\text{g}$  of estradiol)**Developing solvent system:** Chloroform and acetone (9:1)**Analysis:** Proceed as directed in the chapter, using the *Developing solvent system* described above. Develop the chromatogram over a path of a minimum of 8 cm, and allow the plate to air-dry. Remove the plate, mark thesolvent front, and allow solvent evaporation as described in the chapter. Heat at 100° for about 15 min. Allow the plate to cool, and then immerse it in a mixture of absolute alcohol and concentrated sulfuric acid (95:5). Remove it immediately, place the plate on absorbing paper, and allow it to air-dry. Heat the plate at 100° until the sulfuric acid has evaporated. Examine under UV light at  $\lambda = 365$  nm.**Acceptance criteria:** The principal spot obtained from the *Sample solution* has the same color and  $R_f$  value as that from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]

**Mobile phase:** Acetonitrile and water (11:9)**Diluent:** Absolute alcohol and water (1:1)**Estrone standard stock solution:** 0.1 mg/mL of USP Estrone RS in absolute alcohol**Estradiol standard stock solution:** 0.25 mg/mL of USP Estradiol RS in absolute alcohol**System suitability solution:** 0.6 and 2.0  $\mu\text{g/mL}$  of USP Estrone RS and USP Estradiol RS in *Diluent* from *Estrone standard stock solution* and *Estradiol standard stock solution*, respectively**Standard solution:** 1.0 or 2.5  $\mu\text{g/mL}$  of USP Estradiol RS in *Diluent* from *Estradiol standard stock solution***Sample solution:** 1.0 or 2.5  $\mu\text{g/mL}$  of estradiol prepared using 10 Inserts in *Diluent*. Stir the mixture overnight with a magnetic stirrer, shake thoroughly, and centrifuge if necessary.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 205 nm**Column:** 3.9-mm  $\times$  30-cm; 4- $\mu\text{m}$  packing L1**Flow rate:** 1 mL/min**Injection size:** 20  $\mu\text{L}$ **System suitability****Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 2.0 between estradiol and estrone**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of estradiol ( $C_{18}H_{24}O_2$ ) in the portion of Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Estradiol RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of estradiol in the *Sample solution* (mg/mL)**Acceptance criteria:** 90%–107%**PERFORMANCE TESTS**• **DISSOLUTION** <711>**Medium:** Phosphate buffer pH 4.75  $\pm$  0.05 (100 g of potassium dihydrogen phosphate in 10 L of water, adjusted with 1 N sodium hydroxide to a pH of 4.75  $\pm$  0.05); 500 mL**Apparatus 1:** 40 rpm**Time:** 3, 5, and 10 h**Mobile phase:** Methanol, acetonitrile, and water (27.5:27.5:45)

**Standard stock solution:** 0.1 mg/mL of USP Estradiol RS in absolute alcohol

**Standard solutions:** Quantitatively dilute with water the *Standard stock solution* to obtain solutions with final concentrations equal to approximately 20%, 60%, and 160% of the expected concentration of estradiol in the *Medium* for Inserts containing 0.025 mg, assuming complete dissolution, and approximately 20%, 50%, 150%, and 400% of the expected concentration of estradiol in the *Medium* for Inserts containing 0.01 mg of estradiol, assuming complete dissolution.

**Sample solutions:** Use the solution under test, unfiltered.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Fluorescence

**Excitation wavelength:** 230 nm

**Emission wavelength:** 310 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L1; or 4.6-mm × 7.5-cm; 5.0-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 200 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the amount of estradiol (C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>) dissolved:

$$\text{Result} = \{A_n \times V_n + [\sum_{i=1}^{n-1} (\Delta V_{(n-1)} \times A_{(n-1)})]\} / V$$

$A_n$  = percentage of estradiol, at the sample point  $n$  (e.g.,  $A_2$  at the second sampling point)

$V_n$  = volume of *Medium* in the vessel before the sample is taken (mL)

$\Delta V_{(n-1)}$  = volume of sample taken at the sampling point  $(n-1)$

$A_{(n-1)}$  = amount of estradiol (uncorrected) at the sample point  $(n-1)$

$V$  = volume of the medium, 500 mL

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved (%)
3	25–50
5	40–80
10	NLT 80

The percentage of the labeled amount of estradiol dissolved at the specified times conforms to *Acceptance Table 2* in <711>.

#### IMPURITIES

##### • ORGANIC IMPURITIES

[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]

**Solution A:** Acetonitrile

**Solution B:** Water

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	16	84

**Table 2** (Continued)

Time (min)	Solution A (%)	Solution B (%)
35	68	32

[NOTE—Before the next injection, run the system at the initial condition until equilibration is achieved.]

**System suitability solution:** 100 μg/mL of USP Estradiol RS, 0.5 μg/mL of USP Estradiol Related Compound B RS, and 0.5 μg/mL of USP Estradiol Related Compound C RS in absolute alcohol

**Sample solution:** Place a number of Inserts into a measured volume of absolute alcohol to obtain a solution having an estradiol concentration of 2.4 or 6.0 μg/mL. Stir for a minimum of 16 h, shake thoroughly, and centrifuge if necessary. Evaporate 10.0 mL of the supernatant to dryness. Dissolve the residue in 1.0 mL of water and add 7.0 mL of a mixture of toluene and acetone (5:2), mix on a whirl mixer, allow to stand for 1 h, and evaporate 5 mL of the organic phase to dryness. The residue is reconstituted in 450 μL of absolute alcohol to obtain a solution containing 38 or 95 μg/mL of estradiol. Centrifuge, and use the supernatant as the *Sample solution*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 25 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for estradiol related compound B and estradiol are about 0.96 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between estradiol related compound B and estradiol

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the Inserts taken:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of estradiol from the *Sample solution*

**Acceptance criteria:** See *Tables 3* and *4*.

**Table 3. For Inserts labeled to contain 0.025 mg of estradiol**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-ketoestradiol) <sup>a</sup>	0.71	2.4
Estradiol related compound B (6-dehydroestradiol) <sup>b</sup>	0.96	1.4
Estradiol	1.0	—

<sup>a</sup> 1,3,5(10)-Estratrien-3,17β-diol-6 one.

<sup>b</sup> 1,3,5(10),6-Estratetraen-3,17β-diol.

**Table 3. For Inserts labeled to contain 0.025 mg of estradiol** (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual impurity	—	0.8
Total impurities	—	4.4

<sup>a</sup> 1,3,5(10)-Estratrien-3,17 $\beta$ -diol-6 one.<sup>b</sup> 1,3,5(10),6-Estratetraen-3,17 $\beta$ -diol.**Table 4. For Inserts labeled to contain 0.01 mg of estradiol**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-ketoestradiol) <sup>a</sup>	0.71	1.5
Estradiol related compound B (6-dehydroestradiol) <sup>b</sup>	0.96	1.3
Estradiol	1.0	—
Any other individual impurity	—	1.3
Total impurities	—	4.0

<sup>a</sup> 1,3,5(10)-Estratrien-3,17 $\beta$ -diol-6-one.<sup>b</sup> 1,3,5(10),6-Estratetraen-3,17 $\beta$ -diol.**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Inserts meet the requirements of the tests for absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** (11)
  - USP Estradiol RS
  - USP Estradiol Related Compound B RS
  - 6-Dehydroestradiol.
  - USP Estradiol Related Compound C RS
  - 1,3,5(10)-Estratrien-3,17 $\beta$ -diol-6 one.
  - C<sub>18</sub>H<sub>22</sub>O<sub>3</sub>
  - USP Estrone RS

**Estradiol Pellets**

» Estradiol Pellets are sterile pellets composed of Estradiol in compressed form, without the presence of any binder, diluent, or excipient. They contain not less than 97.0 percent and not more than 103.0 percent of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>.

**Packaging and storage**—Preserve in tight containers, suitable for maintaining sterile contents, that hold 1 Pellet each.

**USP Reference standards** (11)—  
USP Estradiol RS

**Solubility in chloroform**—A solution of 25 mg of Pellets in 10 mL of chloroform is clear and practically free from insoluble residue.

**Weight variation**—Weigh 5 Pellets singly, and calculate the average weight. The average weight is between 95% and 105% of the labeled weight of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>, and each Pellet weighs between 90% and 110% of the labeled weight of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>.

**Other requirements**—Pellets meet the requirements under *Estradiol* and under *Sterility Tests* (71).

**Assay**—

**Standard preparation**—Prepare as directed in the Assay under *Estradiol Sterile Suspension*.

**Assay preparation**—Weigh and finely powder not less than 10 Pellets. Transfer a portion of the powder, equivalent to about 100 mg of estradiol, to a suitable container, dissolve in a sufficient quantity of a mixture of equal volumes of alcohol and chloroform to make 5.0 mL, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Estradiol Sterile Suspension*. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub> in the portion of Pellets taken by the formula:

$$5C(A_U / A_S)$$

in which all terms are as defined therein.

**Delete the following:**

**▲Estradiol Injectable Suspension****DEFINITION**

Estradiol Injectable Suspension is a sterile suspension of Estradiol in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197M)

**Sample:** Transfer a volume of well-mixed Injectable Suspension equivalent to 10 mg of estradiol to a flask, render it acid to bromophenol blue TS with dilute hydrochloric acid (1 in 12), and place in an ice bath for 15 min. Filter the acidified suspension with suction through a sintered-glass funnel. Wash the crystals of estradiol so isolated with five successive 5-mL portions of water, and dry the funnel and contents at 105° to constant weight.

**Acceptance criteria:** Meets the requirements.

• **B. MELTING RANGE OR TEMPERATURE, Class I** (741):

**Sample:** Proceed as directed in *Identification* test A. Dry over silica gel for NLT 16 h prior to testing.

**Acceptance criteria:** 173°–179°

**ASSAY**• **PROCEDURE**

[NOTE—The *Standard solution*, *Sample solution*, and the *Blank solution* should be prepared and analyzed concomitantly.]

**Standard stock solution:** 40  $\mu$ g/mL of USP Estradiol RS in methanol

**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* to a glass-stoppered, 16- × 150-mm test tubes, and evaporate with the aid of gentle heat and a current of air to dryness. Using a suitable syringe, add 1.0 mL of iron-phenol TS. Suspend the tube in a vigorously boiling water bath, and mix after heating for 5 min. Remove the tube after heating in the water bath for a total of 35 min, and immediately cool in an ice-water bath. Remove from the ice bath, add 10.0 mL of dilute

sulfuric acid (1 in 3) to obtain a homogeneous solution, and allow to reach room temperature.

**Sample stock solution:** Nominally 40 µg/mL of estradiol, prepared as follows. Transfer an aliquot of well-mixed Injectable Suspension equivalent to 1 mg of estradiol to a 100-mL beaker, and add water, if necessary, to obtain a volume of 5 mL. Add 6 g of purified siliceous earth, mix, and pack the mixture tightly into a 20- × 200-mm chromatographic tube containing in its base a pledget of fine glass wool. Dry-rinse the beaker with 1 g of purified siliceous earth, add the rinsing to the packed column, and wipe out the beaker with a pledget of glass wool used to top the column. Elute the column with 50 mL of ether that previously has been saturated with water, and collect the eluate in a glass-stoppered, 125-mL conical flask. Evaporate with the aid of gentle heat and a current of air to dryness, and add 25.0 mL of methanol to the residue.

**Sample solution:** Transfer 1.0 mL of the *Sample stock solution* to a glass-stoppered, 16- × 150-mm test tubes, and evaporate with the aid of gentle heat and a current of air to dryness. Using a suitable syringe, add 1.0 mL of iron-phenol TS. Suspend the tube in a vigorously boiling water bath, and mix after heating for 5 min. Remove the tube after heating in the water bath for a total of 35 min, and immediately cool in an ice-water bath. Remove from the ice bath, add 10.0 mL of dilute sulfuric acid (1 in 3) to obtain a homogeneous solution, and allow to reach room temperature.

**Blank solution:** Using a suitable syringe, add 1.0 mL of iron-phenol TS to a glass-stoppered, 16- × 150-mm test tubes, and evaporate with the aid of gentle heat and a current of air to dryness. Suspend the tube in a vigorously boiling water bath, and mix after heating for 5 min. Remove the tube after heating in the water bath for a total of 35 min, and immediately cool in an ice-water bath. Remove from the ice bath, add 10.0 mL of dilute sulfuric acid (1 in 3) to obtain a homogeneous solution, and allow to reach room temperature.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Analytical wavelength:** 520 nm

**Cell:** 1 cm

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*

Calculate the percentage of estradiol (C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>) in the portion of the Injectable Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Estradiol RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** <85>: NMT  $2.5 \times 10^2$  USP Endotoxin Units/mg of estradiol
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections* <1>.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** <11>
  - USP Endotoxin RS
  - USP Estradiol RS▲<sup>USP36</sup>

### Estradiol Transdermal System

#### DEFINITION

Estradiol Transdermal System contains NLT 85.0% and NMT 120.0% of the labeled amount of estradiol (C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Diluent:** Acetonitrile and water (1:1)

**Mobile phase:** Acetonitrile and water (55:45)

**Standard solution:** 0.1 mg/mL of USP Estradiol RS in *Diluent*

**Sample solutions:** Equivalent to 0.1 mg/mL of estradiol in *Diluent*, prepared as follows. Cut 10 Transdermal Systems into pieces, and keep the pieces from each system separate. Remove and discard the protective liners, if present, from the strips. Transfer the pieces of each system into separate stoppered flasks of suitable size, and add a measured volume of *Diluent* to each flask to provide the target estradiol concentration. Shake by mechanical means for about 3 h, and sonicate for 15 min.

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 25 µL

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Tailing factor:** 0.9–1.6

**Relative standard deviation:** NMT 2.5%

##### Analysis

**Samples:** *Standard solution* and *Sample solutions*

Calculate the percentage of estradiol (C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>) in each Transdermal System taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Estradiol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of estradiol in the *Sample solution* (mg/mL)

Use the individual assays to determine *Uniformity of Dosage Units*.

**Acceptance criteria:** 85.0%–120.0%

#### OTHER COMPONENTS

- **ALCOHOL CONTENT** (if present)

**Diluent:** Acetonitrile and water (1:1)

**Internal standard solution:** Prepare by diluting 4.0 mL of dehydrated methanol with water to 100 mL.

**Standard stock solution:** 5.0 mg/mL of ethanol in *Diluent*. Prepare by weighing by difference 1.6 mL of dehydrated alcohol into a tared 50-mL volumetric flask containing 15 mL of water, and dilute with *Diluent* to

volume. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

**Standard solution:** 2.5 mg/mL of ethanol. Prepare by pipeting 25.0 mL of the *Standard stock solution* into a 50-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.

**Sample solutions:** Prepare as directed for the *Sample solutions* in the *Assay*, with the following changes. Pipet 25.0 mL of each solution into individual 50-mL volumetric flasks. Add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  2-m glass; support S2

**Temperature**

**Column:** 100°

**Injection port:** 200°

**Detector:** 200°

**Carrier gas:** Helium

**Flow rate:** 30 mL/min

**Injection size:** 2  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the methanol and alcohol peaks are 0.4 and 1.0, respectively.]

#### Suitability requirements

**Relative standard deviation:** NMT 1.5% from the peak response ratio of alcohol to methanol

#### Analysis

**Samples:** *Standard solution* and *Sample solutions*

Calculate the percentage of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in each Transdermal System taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of alcohol to methanol from the *Sample solution*

$R_S$  = peak response ratio of alcohol to methanol from the *Standard solution*

$C_S$  = concentration of dehydrated alcohol in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alcohol in the *Sample solution* (mg/mL)

Average the percentage of alcohol found in the Transdermal Systems analyzed.

**Acceptance criteria:** 80%–120% of the labeled amount of C<sub>2</sub>H<sub>5</sub>OH

### PERFORMANCE TESTS

#### • DRUG RELEASE <724>

**Test 1:** For products labeled for dosing every 84 h

**Medium:** Water; 900 mL, deaerated

**Apparatus 5:** 50 rpm

**Times:** 24, 48, and 96 h

**Mobile phase:** Water and acetonitrile (3:2)

**Standard solution:** 9  $\mu$ g/mL of USP Estradiol RS in dehydrated alcohol. Dilute this solution with *Medium* to obtain solutions having concentrations of about 0.9, 0.45, and 0.045  $\mu$ g/mL.

**Sample solution:** At each sampling time interval, withdraw a 10-mL aliquot of the solution under test.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Fluorimetric, with excitation at 220 nm and emission at 270 nm

**Column:** 4.6-mm  $\times$  3-cm; packing L1

**Temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection size:** 50  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Tailing factor:** 0.9–2.5

**Relative standard deviation:** NMT 3.0%, using 0.45  $\mu$ g/mL of the *Standard solution*

**Analysis:** Plot the peak responses of the *Standard solutions* versus concentration, in  $\mu$ g/mL, of estradiol. From the graph determine the amount, in  $\mu$ g/mL, of estradiol released. Calculate the cumulative release rate as percentage of the labeled amount of estradiol: At 24 h:

$$\text{Result} = \{[900(A_1 - b)] / (1000 \times m \times L)\} \times 100$$

At 48 h:

$$\text{Result} = \{[890(A_2 - b) + 10(A_1 - b)] / (1000 \times m \times L)\} \times 100$$

At 96 h:

$$\text{Result} = \{[880(A_3 - b) + 10(A_2 - b) + 10(A_1 - b)] / (1000 \times m \times L)\} \times 100$$

$A_1$  = peak area of estradiol in the *Sample solution* at the first time interval

$A_n$  = peak area of estradiol in the *Sample solution* at the release interval  $n$

$m$  = slope of the calibration curve

$b$  = y-intercept of the calibration curve

$L$  = Transdermal System label claim (mg)

**Tolerances:** The percentage of the labeled amount of estradiol released at the times specified, conforms to *Acceptance Table 1*.

Time (h)	Amount Dissolved (release rate)
24	1.2%–6.0%
48	3.0%–11.4%
96	5.0%–16.3%

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.

**Medium:** 0.005 M phosphate buffer, pH 5.5, containing 0.3% sodium lauryl sulfate; 500 mL

**Apparatus 5:** 100 rpm. Use a 76-mm stainless steel disk assembly. Adhere the patch to the disk assembly using transfer tape. [NOTE—A suitable tape is available as 3M adhesive transfer tape 927, www.mmm.com.]

**Times:** 1, 4, 8, and 24 h

**Mobile phase:** Acetonitrile and water (1:1)

**Standard stock solution:** 800  $\mu$ g/mL of USP Estradiol RS in acetone

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a solution having a known concentration close to that expected in the solution under test, assuming 100% drug release.

**Sample solution:** At each sampling time interval, withdraw a known volume aliquot of the solution under test.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis: Calculate the amount of estradiol released at each sampling time:

$$M_i = (r_U/r_S) \times C_S \times V_i$$

$$m_1 = M_1$$

$$m_2 = M_2 + M_1(V_a/V_1)$$

$$m_3 = M_3 + M_2(V_a/V_2) + M_1(V_a/V_1)$$

$$m_4 = M_4 + M_3(V_a/V_3) + M_2(V_a/V_2) + M_1(V_a/V_1)$$

Calculate the percentage of the labeled amount of estradiol released at each sampling time:

$$\text{Result} = (m_i/L) \times 100$$

- $M_i$  = amount of estradiol released into the *Medium* at a given sampling time (mg)
- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of the *Standard solution* (mg/mL)
- $V_i$  = corrected volume of the *Medium* at a given sampling time (mL)
- $m_1, m_2, m_3, m_4$  = total amounts of estradiol released from the patch at given sampling times (mg)
- $M_1, M_2, M_3, M_4$  = amounts of estradiol released into the *Medium* at given sampling times (mg)
- $V_a$  = volume of the aliquot taken from the dissolution vessel at each sampling time (mL)
- $V_1, V_2, V_3$  = volumes of *Medium* at given sampling times (mL)
- $L$  = Transdermal System label claim (mg)

**Tolerances:** The percentage of the labeled amount of estradiol released at the times specified conforms to *Acceptance Table 1*.

Time (h)	Amount Dissolved (release rate)
1	15%–40%
4	45%–70%
8	70%–90%
24	NLT 80%

**Test 3:** If the product complies with this test, the labeling indicates that it meets *USP Drug Release Test 3*.

**Medium:** 1% (v/v) Polysorbate 40 in water; 900 mL

**Apparatus 5:** 50 rpm

**Times:** 4, 8, and 24 h

**Standard stock solution:** Known concentration (mg/mL) of USP Estradiol RS in methanol

**Standard solution:** Five different concentrations within the range of the expected release amounts of es-

tradiol, prepared as follows. Add 1.0 mL of Polysorbate 40 into a 100-mL volumetric flask, and then add the required amount of *Standard stock solution*. Mix well to dissolve the Polysorbate 40, and dilute with water to volume.

**Sample solution:** At each sampling time interval, withdraw a known volume aliquot of the solution under test.

**Mobile phase:** Acetonitrile and water (2:3)

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm, 5-µm packing L1 for 9-cm<sup>2</sup> systems; 4.6-mm × 12.5-cm, 5-µm packing L1 for 18-, 27-, or 36-cm<sup>2</sup> systems. In any case, a guard column containing packing L1 is used.

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Relative standard deviation: NMT 2.0%

Analysis: Calculate the cumulative release rate as a percentage of the labeled amount of estradiol:

$$\text{Result} = \{[900(A - b)] / (1000 \times m \times L)\} \times 100$$

$A$  = peak area of estradiol in the *Sample solution* at each time interval

$b$  = y-intercept of the calibration curve

$m$  = slope of the calibration curve

$L$  = Transdermal System label claim (mg)

**Tolerances:** The percentage of the labeled amount of estradiol released at the times specified conforms to the acceptance tables shown below.

L1 (6 units)

Time (h)	Amount Dissolved (individual values)
4	40%–71%
8	58%–94%
24	NLT 75%

L2 (12 units)

Time (h)	Amount Dissolved (average of 12)	Amount Dissolved (individual values)
4	40%–71%	34%–77%
8	58%–94%	50%–102%
24	NLT 75%	NLT 68%

L3 (24 units)

Time (h)	Amount Dissolved (average of 24)	Amount Dissolved (individual for 22 units of 24)	Amount Dissolved (individual for 24)
4	40%–71%	34%–77%	29%–82%
8	58%–94%	50%–102%	43%–109%
24	NLT 75%	NLT 68%	NLT 60%

- UNIFORMITY OF DOSAGE UNITS (905):** The results from the Transdermal Systems used in the *Assay* meet the requirements.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in hermetic, light-resistant, unit-dose pouches.
- **LABELING:** The label states the total amount of estradiol in the Transdermal System and the release rate, in mg/day, for the duration of application of one system.
- **USP REFERENCE STANDARDS** (11)  
USP Estradiol RS

**Estradiol Tablets**

» Estradiol Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{18}H_{24}O_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Estradiol RS

USP Estrone RS

**Identification**—Place a quantity of finely powdered Tablets, equivalent to about 4 mg of estradiol, in a screw-capped, 20-mL vial. Add 10 mL of chloroform, and sonicate for 2 minutes. Filter through medium-porosity filter paper. Apply 20  $\mu$ L each of this solution and a Standard solution of USP Estradiol RS in chloroform containing 0.4 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a lined chamber with a solvent system consisting of a mixture of toluene and acetone (4:1) until the solvent front has moved 10 cm beyond the starting line. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with a mixture of methanol and sulfuric acid (1:1), and heat at 100° for about 5 minutes: the principal spots obtained from the test solution and the Standard solution have the same color and  $R_F$  value.

**Dissolution** (711)—

*Medium:* 0.3% sodium lauryl sulfate in water; 500 mL.

*Apparatus 2:* 100 rpm.

*Time:* 60 minutes.

**Mobile phase**—Prepare a suitable degassed and filtered solution of water and acetonitrile (55:45).

**Standard solution**—Prepare a solution of USP Estradiol RS in methanol having an accurately known concentration of about 0.02 mg per mL. Dilute aliquots of this solution with *Medium* to obtain a final solution having a concentration approximately equal to the expected concentration of drug in the *Medium*, assuming 100% dissolution.

**Test solution**—Use a filtered portion of the solution under test from the dissolution vessel.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm  $\times$  7.5-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity of  $C_{18}H_{24}O_2$  dissolved by comparison of the peak areas obtained from the *Test solution* and the *Standard solution*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{24}O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Chromatographic purity**—

**Solution A**—Prepare a degassed mixture of water and acetonitrile (8:2).

**Solution B**—Prepare a degassed mixture of acetonitrile and water (8:2).

**Diluent**—Prepare a mixture of water and acetonitrile (6:4).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve accurately weighed quantities of USP Estradiol RS and USP Estrone RS in acetonitrile to obtain a solution having concentrations of 0.5 mg per mL and 0.3 mg per mL, respectively. Pipet 2 mL of this solution into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

**Test solution**—Transfer a number of Tablets, containing a combined amount of 4 to 8 mg of estradiol based on the label claim, to a suitable flask, add a volume of *Diluent* equivalent to about 5 mL per each mg of estradiol, swirl until the Tablets are completely disintegrated, then shake for 15 minutes, and allow the solids to settle. Pass a portion of this solution through a 0.45- $\mu$ m PVDF filter, discarding the first 2 mL of the filtrate. This solution contains about 0.2 mg of estradiol per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.0-mm  $\times$  12.5-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	100→90	0→10	linear gradient
5–15	90→50	10→50	linear gradient
15–25	50→0	50→100	linear gradient
25–35	0	100	isocratic
35–35.1	0→100	100→0	linear gradient
35.1–40	100	0	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*. Identify the components based on their relative retention times which are 1.0 for estradiol and about 1.1 for estrone: the resolution,  $R_s$ , between estradiol and estrone is not less than 3.0; the tailing factors for estradiol and estrone peaks are not more than 1.5; and the relative standard deviation for replicate injections is not more than 2% for each peak.

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 5% of total impurities is found. Disregard any peaks observed in the blank.

**Assay**—

**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay under *Estradiol*.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Transfer a portion of the powder, equivalent to about 8 mg of estradiol, to a 100-mL volumetric flask. Add 4 mL of water, and swirl. Add 10.0 mL of *Internal*

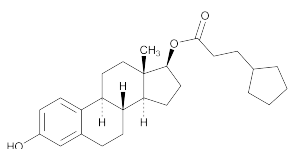
*standard solution* and about 60 mL of methanol. Shake by mechanical means for 15 minutes, dilute with methanol to volume, mix, and allow the solids to settle. Filter a portion, discarding the first 10 mL of the filtrate. Mix 5.0 mL of the subsequent filtrate with 5.0 mL of methanol and 10.0 mL of water.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Estradiol*. Calculate the quantity, in mg, of  $C_{18}H_{24}O_2$  in the portion of Tablets taken by the formula:

$$0.4C(R_U / R_S)$$

in which the terms are as defined therein.

## Estradiol Cypionate



$C_{26}H_{36}O_3$  396.56  
Estra-1,3,5(10)-triene-3,17-diol, (17 $\beta$ )-,  
17-cyclopentanepropionate;  
Estradiol 17-cyclopentanepropionate [313-06-4].

### DEFINITION

Estradiol Cypionate contains NLT 97.0% and NMT 103.0% of estradiol cypionate ( $C_{26}H_{36}O_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
*Sample solution:* 100  $\mu$ g/mL of estradiol cypionate in alcohol  
*Analytical wavelength:* 280 nm  
*Acceptance criteria:* Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

### ASSAY

- **PROCEDURE**  
*Mobile phase:* 0.8 g/L of ammonium nitrate. Dissolve in 300 mL of water, and combine with 700 mL of acetonitrile.  
*Internal standard solution:* 2.0 mg/mL of testosterone benzoate in tetrahydrofuran  
*Standard solution:* 1.0 mg/mL of USP Estradiol Cypionate RS in the *Internal standard solution*  
*Sample solution:* 1.0 mg/mL of Estradiol Cypionate in the *Internal standard solution*  
**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*).  
*Mode:* LC  
*Detector:* UV 280 nm  
*Column:* 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1  
*Flow rate:* 1 mL/min  
*Injection size:* 10  $\mu$ L  
**System suitability**  
*Sample:* *Standard solution*  
**Suitability requirements**  
*Resolution:* NLT 3.0 between the peaks for estradiol cypionate and the internal standard  
*Relative standard deviation:* NMT 1.5%  
**Analysis**  
*Samples:* *Standard solution* and *Sample solution*  
 Calculate the percentage of estradiol cypionate ( $C_{26}H_{36}O_3$ ) in the portion of Estradiol Cypionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = peak response ratio of estradiol cypionate to the internal standard from the *Sample solution*
- $R_S$  = peak response ratio of estradiol cypionate to the internal standard from the *Standard solution*
- $C_S$  = concentration of USP Estradiol Cypionate RS in the *Standard solution* (mg/mL)
- $C_U$  = concentration of the *Sample solution* (mg/mL)
- Acceptance criteria:** 97.0%–103.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 149°–153°
- **OPTICAL ROTATION, Specific Rotation** (781S)  
*Sample solution:* 20 mg/mL in dioxane  
*Acceptance criteria:* +39° to +44°
- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 1.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Estradiol Cypionate RS

## Estradiol Cypionate Injection

### DEFINITION

Estradiol Cypionate Injection is a sterile solution of Estradiol Cypionate in a suitable oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol cypionate ( $C_{26}H_{36}O_3$ ).

### IDENTIFICATION

- **A.**  
**Sulfanilic acid solution:** Mix 50 mg of sulfanilic acid with 2 mL of 3 N hydrochloric acid, warm the mixture, then cool it in ice water, and slowly add, with agitation, 0.3 mL of sodium nitrite solution (1 in 10).  
**Sample solution:** Transfer a volume of Injection, equivalent to 5 mg of estradiol cypionate, to a glass-stoppered, 50-mL test tube, and add 30 mL of alcohol.  
**Analysis:** Shake the *Sample solution* vigorously for 5 min, centrifuge until the two layers have separated, and transfer the alcohol layer, with the aid of a hypodermic syringe, to a 50-mL beaker. Evaporate on a steam bath to dryness, add 5 mL of potassium hydroxide solution (1 in 10), and heat on the steam bath for 15 min. Add the *Sulfanilic acid solution* to the saponified estradiol cypionate.  
**Acceptance criteria:** A red color is produced.

### ASSAY

- **PROCEDURE**  
*Mobile phase:* 0.8 g/L of ammonium nitrate. Dissolve in 300 mL of water, and combine with 700 mL of acetonitrile.  
*Internal standard solution:* 2.0 mg/mL of testosterone benzoate in tetrahydrofuran  
*Standard solution:* 0.1 mg/mL of USP Estradiol Cypionate RS in tetrahydrofuran, prepared as follows. Transfer 10 mg of USP Estradiol Cypionate RS to a 100-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume. Shake vigorously to dissolve.  
*Sample solution:* Using a “to contain” pipet, transfer an accurately measured volume, in mL, of Injection, equivalent to 10 mg of estradiol cypionate, to a 100-mL volumetric flask. Rinse the pipet with small portions of tetrahydrofuran, collecting the washings in the volumetric



flask. Add 10.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between the peaks for estradiol cypionate and the internal standard

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of estradiol cypionate ( $C_{26}H_{36}O_3$ ) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of estradiol cypionate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of estradiol cypionate to the internal standard from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **INJECTIONS** <1>: Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose, light-resistant containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** <11>  
USP Estradiol Cypionate RS

## Estradiol and Norethindrone Acetate Tablets

#### DEFINITION

Estradiol and Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ( $C_{18}H_{24}O_2$ ) and NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ( $C_{22}H_{28}O_3$ ).

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>

**Standard solution:** 0.5 mg/mL of USP Estradiol RS and 0.25 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol

**Sample solution:** Place 2 Tablets into a 10-mL vial, and add 0.2 mL of water. When the Tablets are partially disintegrated, add a few glass beads, and shake vigorously to disintegrate. Add 4.0 mL of dehydrated alcohol, and shake. [NOTE—Centrifuge until the supernatant is clear before application to the plate.]

**Adsorbent:** 0.25-mm chromatographic silica gel plate

**Application volume:** 2 µL

**Developing solvent system:** Chloroform and acetone (9:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, using the *Developing solvent system*. Apply the *Samples* and develop the plate. After removal of the plate, mark the solvent front, and allow the solvent to evaporate. Place the plate on a heating plate at 100° for 15 min. Allow the plate to cool, and then immerse it in a mixture of dehydrated alcohol and concentrated sulfuric acid (95:5). Place the plate on a piece of thick horizontal paper until it is almost dry. Heat the plate at 100° until it has fully developed. Examine under UV light at 365 nm.

**Acceptance criteria:** The color and  $R_F$  value of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

- **B.** The retention time of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and water (11:9)

**Diluent:** Dehydrated alcohol and water (1:1)

**Estrone standard stock solution:** 0.12 mg/mL of USP Estrone RS in dehydrated alcohol

**Estradiol standard stock solution:** 0.25 mg/mL of USP Estradiol RS in dehydrated alcohol

**Norethindrone acetate standard stock solution:** 0.15 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol

**Standard solution:** 20 µg/mL of USP Estradiol RS from the *Estradiol standard stock solution* and 10 µg/mL of USP Norethindrone Acetate RS from the *Norethindrone acetate standard stock solution* in *Diluent*

**System suitability solution:** Combine 800 µL of the *Estradiol standard stock solution*, 600 µL of the *Norethindrone acetate standard stock solution*, 200 µL of the *Estrone standard stock solution*, and 10.0 mL of *Diluent*.

**Sample solution:** Add 12 Tablets into a measured amount of *Diluent* to obtain a solution having an estradiol concentration of 20 µg/mL and a norethindrone acetate concentration of 10 µg/mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV Dual wavelength (254 nm/280 nm) or equivalent

[NOTE—The absorption of estradiol at 280 nm and norethindrone acetate at 254 nm can be included in a single run by altering the wavelength.]

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

[NOTE—Perform an investigational run to determine the retention times for estradiol and norethindrone acetate.]

**Injection size:** 50 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.8 between estradiol and estrone, *System suitability solution*

**Relative standard deviation:** NMT 3%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Measure the areas for the estradiol and norethindrone acetate peaks.]

Calculate the quantity, as a percentage, of  $C_{18}H_{24}O_2$  in each of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

- $r_s$  = peak area from the *Standard solution*  
 $C_s$  = concentration of USP Estradiol RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of estradiol in the *Sample solution* ( $\mu\text{g/mL}$ )

Calculate the quantity, as a percentage, of  $\text{C}_{22}\text{H}_{28}\text{O}_3$  in each of the Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak area from the *Sample solution*  
 $r_s$  = peak area from the *Standard solution*  
 $C_s$  = concentration of USP Norethindrone Acetate RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of norethindrone acetate in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of  $\text{C}_{18}\text{H}_{24}\text{O}_2$  and 90.0%–110.0% of the labeled amount of  $\text{C}_{22}\text{H}_{28}\text{O}_3$

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Medium:** 0.3% sodium lauryl sulfate; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min for Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate, and 50 min for Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate

**Mobile phase:** Acetonitrile and water (11:9)

**Standard stock solution A:** 20  $\mu\text{g/mL}$  of USP Estradiol RS in alcohol or in a mixture of alcohol and water

**Standard stock solution B:** 10  $\mu\text{g/mL}$  of USP Norethindrone Acetate RS in alcohol or in a mixture of alcohol and water

**Standard solution:** Dilute suitable quantities of *Standard stock solution A* and *Standard stock solution B* in *Medium* or a mixture of alcohol and water to obtain a final concentration of both analytes similar to the expected concentration of the *Sample solution*.

**Sample solution:** Pass a portion of the solution through a filter of 0.45- $\mu\text{m}$  pore size.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 241 nm for norethindrone acetate and 280 nm for estradiol

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 150  $\mu\text{L}$

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $\text{C}_{18}\text{H}_{24}\text{O}_2$  and of  $\text{C}_{22}\text{H}_{28}\text{O}_3$  dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of estradiol or norethindrone acetate from the *Sample solution*  
 $r_s$  = peak response of estradiol or norethindrone acetate from the *Standard solution*  
 $C_s$  = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of estradiol or norethindrone acetate in the *Sample solution* (mg/mL) (based on the label claim)

**Tolerances:** For Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate: NLT 75% (Q) of the labeled amounts of  $\text{C}_{18}\text{H}_{24}\text{O}_2$  and  $\text{C}_{22}\text{H}_{28}\text{O}_3$  is dissolved in 30 min. For Tablets labeled to contain

0.5 mg of estradiol and 0.1 mg of norethindrone acetate: NLT 75% (Q) of the labeled amounts of  $\text{C}_{18}\text{H}_{24}\text{O}_2$  and  $\text{C}_{22}\text{H}_{28}\text{O}_3$  is dissolved in 50 min.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

**Solution A:** Tetrahydrofuran and water (1:200)

**Solution B:** Acetonitrile, tetrahydrofuran, and water (160:1:40)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	65	35
35	20	80
49	20	80
50	80	20
60	80	20

**Diluent:** Dehydrated alcohol and water (1:1)

**System suitability solution:** 240  $\mu\text{g/mL}$  of USP Estradiol RS, 60  $\mu\text{g/mL}$  of USP Norethindrone Acetate RS, and 1  $\mu\text{g/mL}$  of USP Estrone RS in *Diluent*

**Estradiol standard stock solution:** 250  $\mu\text{g/mL}$  of USP Estradiol RS in alcohol

**Norethindrone acetate standard stock solution:** 150  $\mu\text{g/mL}$  of USP Norethindrone Acetate RS in alcohol

**Standard solution:** Combine 250  $\mu\text{L}$  of the *Estradiol standard stock solution* and 100  $\mu\text{L}$  of the *Norethindrone acetate standard stock solution*, and dilute with 50.0 mL of *Diluent*.

**Sample solution:** A quantity equivalent to 240  $\mu\text{g/mL}$  of estradiol and 120  $\mu\text{g/mL}$  of norethindrone acetate from NLT 20 finely ground Tablets in *Diluent*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 and 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 4- $\mu\text{m}$  packing L1

**Flow rate:** 0.8 mL/min

**Injection size:** 100  $\mu\text{L}$

### System suitability

**Sample:** *System suitability solution*

[NOTE— The relative retention times for estradiol, estrone, and norethindrone acetate are about 1.0, 1.4, and 3.0, respectively.]

### Suitability requirements

**Resolution:** NLT 1.3 between estrone and estradiol, measured at 254 nm

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any estradiol impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

- $r_u$  = peak response area at 235 nm for each impurity from the *Sample solution*  
 $r_s$  = peak response area at 235 nm from the *Standard solution*  
 $C_s$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = relative response factor (see *Impurity Table 1* or *Impurity Table 2*)

Calculate the percentage of any norethindrone acetate related impurities in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

- $r_U$  = peak response area at 254 nm for each impurity from the *Sample solution*  
 $r_S$  = peak response area at 254 nm from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = relative response factor (see *Impurity Table 1* or *Impurity Table 2*)

**Acceptance criteria:** The Tablets meet the requirements given in either *Impurity Table 1* or *Impurity Table 2*.

**Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6 $\alpha$ -Hydroxyestradiol	0.47	1.0	1.0
6 $\beta$ -Hydroxyestradiol	0.51	1.0	1.0
6-Ketoestradiol	0.62	1.0	1.0
6-Dehydroestradiol	0.95	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	0.5
Total estradiol related impurities	—	—	2.0
Norethindrone acetate related impurities			
6 $\beta$ -Hydroxynorethindrone acetate	0.58	1.0	1.0
Norethindrone	0.66	1.0	1.0
6-Ketonorethindrone acetate	0.79	0.56	1.0
6-Dehydronorethindrone acetate	0.97	0.45	1.0
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	0.5
Total norethindrone acetate related impurities	—	—	2.0

**Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6 $\beta$ -Hydroxy-estradiol	0.51	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	1.0
Total estradiol related impurities	—	—	2.5
Norethindrone acetate related impurities			
6 $\beta$ -Hydroxy-norethindrone acetate	0.58	1.0	1.5
Norethindrone	0.66	1.0	1.0
6-Keto-norethindrone acetate	0.79	0.56	2.5
6-Dehydro-norethindrone acetate	0.97	0.45	1.0

**Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate** (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	1.0
Total norethindrone acetate related impurities	—	—	4.0

## SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. The Tablets meet the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS** (11)
  - USP Estradiol RS
  - USP Estrone RS
  - USP Norethindrone Acetate RS

## Estradiol Valerate

$\text{C}_{23}\text{H}_{32}\text{O}_3$  356.50

Estra-1,3,5(10)-triene-3,17-diol(17 $\beta$ )-, 17-pentanoate.

Estradiol 17-valerate. [979-32-8].

» Estradiol Valerate contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{23}\text{H}_{32}\text{O}_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Estradiol Valerate RS

**Identification, Infrared Absorption** (197K).

**Melting range, Class Ia** (741): between 143° and 150°.

**Specific rotation** (781S): between +41° and +47°.

*Test solution:* 25 mg, uncorrected for moisture, per mL, in dioxane.

**Water, Method I** (921): not more than 0.1%.

**Limit of estradiol**—Apply 5  $\mu\text{L}$  of a solution of Estradiol Valerate in acetone, containing 5 mg per mL, and 5  $\mu\text{L}$  of a solution of estradiol in acetone, containing 50  $\mu\text{g}$  per mL, about 2.5 cm from the lower edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a solvent system consisting of a mixture of cyclohexane and ethyl acetate (7:3) in an unlined chamber until the solvent front has moved about 15 cm above the point of application. Remove the plate, dry at 90° for 30 minutes, and spray the plate lightly with a 3 in 10 solution of methanol in sulfuric acid, prepared by cautiously adding sulfuric acid to 30 mL of methanol in a 100-mL volumetric flask, in an ice bath, to volume. Heat the plate at 90° for 30 minutes: any spot in the chromatogram of Estradiol Valerate close to the origin and corresponding to the estradiol spot is not larger nor more intense than that produced by the standard. (The limit is 1.0% of estradiol.)

**Free acid**—Neutralize 25 mL of alcohol, in a conical flask, with 0.01 N sodium hydroxide VS to a faint blue color, using bromothymol blue TS. Accurately weigh 500 mg of Estradiol Valerate, and dissolve it in the neutralized alcohol. Titrate rapidly with 0.01 N sodium hydroxide VS to a faint blue color. Each mL of 0.01 N sodium hydroxide is equivalent to 1.021 mg of valeric acid. The free acid content, expressed as valeric acid, does not exceed 0.5%.

**Ordinary impurities** (466)—

*Test solution:* acetone.

*Standard solution:* acetone.

*Eluent:* a mixture of cyclohexane and ether (4:1).

*Visualization:* 5 followed by 1.

**Assay**—

*Mobile phase*—Dissolve 0.8 g of ammonium nitrate in 300 mL of water, add 700 mL of acetonitrile, and mix. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of testosterone benzoate in tetrahydrofuran having a concentration of about 2.0 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Estradiol Valerate RS in *Internal standard solution*, and dilute quantitatively with *Internal standard solution* to obtain a solution having a known concentration of about 1 mg of USP Estradiol Valerate RS per mL.

*Assay preparation*—Transfer about 25 mg of Estradiol Valerate, accurately weighed, to a 25-mL volumetric flask, add *Internal standard solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.2 for testosterone benzoate and 1.0 for estradiol valerate; the column efficiency determined from the analyte peak is not less than 1100 theoretical plates; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{23}H_{32}O_3$  in the portion of Estradiol Valerate taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Estradiol Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Estradiol Valerate Injection

### DEFINITION

Estradiol Valerate Injection is a sterile solution of Estradiol Valerate in a suitable vegetable oil. It contains NLT 90.0% and NMT 115.0% of the labeled amount of estradiol valerate ( $C_{23}H_{32}O_3$ ).

### IDENTIFICATION

#### • A.

**Phenol reagent** (Folin-Ciocalteu reagent): In a 1500-mL flask connected by a standard taper joint to a reflux

condenser, dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700 mL of water. Add 50 mL of phosphoric acid and 100 mL of hydrochloric acid, and reflux gently for 10 h. Cool, and add 150 g of lithium sulfate, 50 mL of water, and 4–6 drops of bromine. Boil the mixture without the condenser for 15 min to remove the excess bromine. Cool, transfer to a 1-L volumetric flask, dilute with water to volume, and filter. The filtrate is golden yellow in color, and has no greenish tint. Store the filtrate in a tight container in a refrigerator. Mix the filtrate with water (1:2) before use as the *Phenol reagent*.

**Analysis:** Transfer 0.5 mL of Injection to a separator containing 10 mL of solvent hexane and 10 mL of 80% methanol. Shake the contents for 2 min, and allow the phases to separate. Add 1 mL of *Phenol reagent* and 3 mL of sodium carbonate solution (1 in 5) to 1 mL of the bottom layer, and mix.

**Acceptance criteria:** A blue color develops.

### ASSAY

#### • PROCEDURE

*Mobile phase:* Dissolve 0.8 g of ammonium nitrate in 300 mL of water, add 700 mL of acetonitrile, and mix.

*Internal standard solution:* 8.0 mg/mL of testosterone benzoate in tetrahydrofuran

*Standard solution:* 0.8 mg/mL of USP Estradiol Valerate RS, prepared as follows. Transfer 20 mg of USP Estradiol Valerate RS to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume.

*Sample solution:* Nominally 0.8 mg/mL of estradiol valerate, prepared as follows. Using a “to contain” pipet, transfer an accurately measured volume of Injection, equivalent to 20 mg of estradiol valerate, to a 25-mL volumetric flask. Rinse the pipet with small portions of tetrahydrofuran, collecting the washings in the volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with tetrahydrofuran to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for estradiol valerate and testosterone benzoate are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between estradiol valerate and the internal standard

**Column efficiency:** NLT 1100 theoretical plates for estradiol valerate

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estradiol valerate ( $C_{23}H_{32}O_3$ ) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of estradiol valerate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of estradiol valerate to the internal standard from the *Standard solution*

- $C_s$  = concentration of USP Estradiol Valerate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

## IMPURITIES

### • LIMIT OF ESTRADIOL

**Standard solution:** A solution of estradiol in acetone containing 30.0% of the labeled concentration of the Injection. Dilute 1.0 mL of this solution with oil labeled as vehicle for Injection to 10.0 mL.

**Sample solution:** Injection

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Cyclohexane and ethyl acetate (7:3)

**Spray reagent:** A 3-in-10 solution of methanol in sulfuric acid, prepared as follows. To 30 mL of methanol in a 100-mL volumetric flask in an ice bath, cautiously add sulfuric acid to volume.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Apply the samples at spots 2.5 cm from the bottom edge of the plate. Allow the applications to be absorbed by the layer without air-drying. Develop the chromatogram in an unlined chamber until the solvent front has moved about 15 cm above the point of application. Remove the plate, dry at 90° for 30 min, and spray the plate lightly with *Spray reagent*. Heat the plate at 90° for 30 min.

**Acceptance criteria:** NMT 3.0%; any spot from the *Sample solution* close to the origin and corresponding to the estradiol spot is not larger or more intense than that from the *Standard solution*.

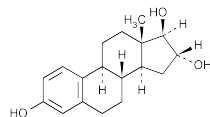
## SPECIFIC TESTS

- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* <1>

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I or Type III glass.
- **USP REFERENCE STANDARDS <11>**  
USP Estradiol Valerate RS

## Estriol



$C_{18}H_{24}O_3$  288.38

Estra-1,3,5(10)-triene-3,16,17-triol, (16 $\alpha$ ,17 $\beta$ )-.

Estriol [50-27-1].

» Estriol contains not less than 97.0 percent and not more than 102.0 percent of  $C_{18}H_{24}O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards <11>**—

USP Estriol RS

**Completeness of solution**—Dissolve 500 mg in 10 mL of pyridine: the solution is clear and free from undissolved solid.

### Identification—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 100  $\mu$ g per mL.

*Medium:* alcohol.

**Specific rotation** <781S>: between +54° and +62°.

*Test solution:* 4 mg per mL, in dioxane.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

### Chromatographic purity—

**Test preparation**—Prepare a solution of Estriol in a mixture of dioxane and water (9:1) to obtain a solution containing 20.0 mg per mL.

**Standard solution and Standard dilutions**—Prepare a solution of USP Estriol RS in a mixture of dioxane and water (9:1) to obtain a solution containing 20 mg per mL (*Standard solution*). Prepare a series of dilutions of the *Standard solution* in a mixture of dioxane and water (9:1) to obtain solutions containing 0.40, 0.20, 0.10, and 0.05 mg per mL (*Standard dilutions*).

**Chromatography chamber**—Line a suitable chamber (see *Chromatography* <621>) with absorbent paper, and pour into the chamber 200 mL of developing solvent, prepared by mixing, just prior to use, 90 mL of chloroform, 5 mL of methanol, 5 mL of acetone, and 5 mL of acetic acid. Equilibrate the chamber for 15 minutes before using.

**Procedure**—Apply 5- $\mu$ L volumes of the *Test preparation*, *Standard solution*, and each of the four *Standard dilutions* at equidistant points along a line 2.5 cm from one edge of a 20-  $\times$  20-cm thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in the *Chromatography chamber*, seal the chamber, and allow the chromatogram to develop until the solvent front has moved 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with a mixture of methanol and sulfuric acid (7:3), then heat the plate at 100° for 15 minutes. The lane of the *Test preparation* exhibits its principal spot at the same  $R_f$  value as the principal spot of the *Standard solution*. If spots other than the principal spot are observed in the lane of the *Test preparation*, estimate the concentration of each by comparison with the *Standard dilutions*. The spots from the 0.40-, 0.20-, 0.10-, and 0.05-mg-per-mL dilutions are equivalent to 2.0%, 1.0%, 0.5%, and 0.25% of impurities, respectively. The requirements of the test are met if the sum of impurities in the *Test preparation* is not greater than 2.0%.

**Assay**—Dissolve about 50 mg of Estriol, accurately weighed, in alcohol to make 100.0 mL, and mix. Dilute 10.0 mL of this solution with alcohol to 100.0 mL. Similarly, dissolve a suitable quantity of USP Estriol RS, accurately weighed, in alcohol to obtain a *Standard solution* having a known concentration of about 50  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 281 nm. Calculate the quantity, in mg, of  $C_{18}H_{24}O_3$  in the portion of Estriol taken by the formula:

$$C(A_u / A_s)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Estriol RS in the *Standard solution*, and  $A_u$  and  $A_s$  are the absorbances of the solution of Estriol and the *Standard solution*, respectively.

## Conjugated Estrogens

### DEFINITION

Conjugated Estrogens is a mixture of sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from estrone and equilin. It contains other conjugated estrogenic substances of the type excreted by pregnant mares. It is a dispersion of the estrogenic substances on a suitable powdered diluent.

Conjugated Estrogens contains NLT 52.5% and NMT 61.5% of sodium estrone sulfate and NLT 22.5% and NMT 30.5% of sodium equilin sulfate, and the total of sodium estrone sulfate and sodium equilin sulfate is NLT 79.5% and NMT 88.0% of the labeled content of Conjugated Estrogens. Conjugated Estrogens contains concomitant components as sodium sulfate conjugates NLT 13.5% and NMT 19.5% of 17 $\alpha$ -dihydroequilin, NLT 2.5% and NMT 9.5% of 17 $\alpha$ -estradiol, and NLT 0.5% and NMT 4.0% of 17 $\beta$ -dihydroequilin of the labeled content of Conjugated Estrogens.

### IDENTIFICATION

- **A.** The relative retention times of the 17 $\alpha$ -dihydroequilin peak, the estrone peak, and the equilin peak from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.
- **B.** The chromatogram of Conjugated Estrogens from the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17 $\alpha$ -estradiol and 17 $\beta$ -dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-O-methylestrone.

### ASSAY

#### • PROCEDURE

**Buffer:** Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of 5.2  $\pm$  0.1, if necessary.

**Internal standard solution:** 150  $\mu$ g/mL of 3-O-methylestrone in methanol

**Standard stock solution:** 160  $\mu$ g/mL, 70  $\mu$ g/mL, and 50  $\mu$ g/mL each of USP Estrone RS, USP Equilin RS, and USP 17 $\alpha$ -Dihydroequilin RS, respectively, in alcohol

**Standard solution:** Pipet 1.0 mL of the *Internal standard solution* and 1.0 mL of the *Standard stock solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue, add 15  $\mu$ L of dried pyridine and 65  $\mu$ L of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.

**System suitability solution:** Pipet 1.0 mL of a 2.0- $\mu$ g/mL solution of USP Estradiol RS (17 $\beta$ -estradiol) in alcohol, 1.0 mL of *Internal standard solution*, and 1.0 mL of *Standard stock solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture..."

**Sample solution:** Transfer a quantity of Conjugated Estrogens, equivalent to 2 mg of total conjugated estrogens, to a 50-mL centrifuge tube, fitted with a polytetrafluorinated screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0  $\pm$  0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by rapidly passing through a

filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture..."

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm  $\times$  15-m fused silica capillary column, bonded with a 0.25- $\mu$ m layer of phase G19

**Temperature**

**Column:** 208°

**Injector port:** 260°

**Detector:** 260°

**Carrier gas:** Hydrogen

**Flow rate:** 2 mL/min

**Injection mode:** Split

**Split flow rate:** 40–60 mL/min

**Injection size:** 1  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-O-methylestrone peak between 17 and 25 min.]

[NOTE—The relative retention times for 17 $\beta$ -estradiol, 17 $\alpha$ -dihydroequilin, estrone, equilin, and 3-O-methylestrone are 0.29, 0.30, 0.80, 0.87, and 1.00, respectively.]

### Suitability requirements

**Resolution:** NLT 1.2 between estrone and equilin, *System suitability solution*

**Tailing factor:** NMT 1.3 for the estrone peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the estrone peak ratios for NLT four injections of the *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of sodium estrone sulfate and sodium equilin sulfate in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

$R_U$  = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*

$R_S$  = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Standard solution*

$C_S$  = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of the *Sample solution* ( $\mu$ g/mL)

$F$  = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

**Acceptance criteria:** 52.5%–61.5% of sodium estrone sulfate and 22.5%–30.5% of sodium equilin sulfate

### OTHER COMPONENTS

- **CONTENT OF 17 $\alpha$ -DIHYDROEQUILIN, 17 $\beta$ -DIHYDROEQUILIN, AND 17 $\alpha$ -ESTRADIOL** (concomitant components)

**Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for 17 $\alpha$ -estradiol, 17 $\alpha$ -dihydroequilin, and 17 $\beta$ -dihydroequilin are about 0.82, 1.00, and 1.11, respectively.]

Identify the peaks for 17 $\alpha$ -estradiol, 17 $\alpha$ -dihydroequilin, and 17 $\beta$ -dihydroequilin from the *Sample solution*. Calculate the percentages of 17 $\alpha$ -estradiol, 17 $\alpha$ -dihydroequilin, and 17 $\beta$ -dihydroequilin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- $R_U$  = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*  
 $R_S$  = ratio of the peak response of 17 $\alpha$ -dihydroequilin to that of the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP 17 $\alpha$ -Dihydroequilin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381  
**Acceptance criteria:** NLT 13.5% and NMT 19.5% of 17 $\alpha$ -dihydroequilin, NLT 2.5% and NMT 9.5% of 17 $\alpha$ -estradiol, and NLT 0.5% and NMT 4.0% of 17 $\beta$ -dihydroequilin, as their sodium sulfate conjugates

## IMPURITIES

- LIMITS OF 17 $\alpha$ -DIHYDROEQUILININ, 17 $\beta$ -DIHYDROEQUILININ, AND EQUILENIN** (signal impurities)  
 Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 [NOTE—The relative retention times for dihydroequilenin, 17 $\beta$ -dihydroequilenin, 3-O-methylestrone, and equilenin are 0.56, 0.64, 1.0, and 1.3, respectively.]

Identify any peaks for dihydroequilenin, 17 $\beta$ -dihydroequilenin, 3-O-methylestrone, and equilenin from the *Sample solution*. Calculate the percentages of 17 $\alpha$ -dihydroequilenin, 17 $\beta$ -dihydroequilenin, and equilenin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- $R_U$  = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*  
 $R_S$  = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP Estrone RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

**Acceptance criteria:** NMT 3.25%, NMT 2.75%, and NMT 5.5% of the labeled content of Conjugated Estrogens for 17 $\alpha$ -dihydroequilenin, 17 $\beta$ -dihydroequilenin, and equilenin, respectively, as their sodium sulfate salts

- LIMITS OF 17 $\beta$ -ESTRADIOL AND  $\Delta^{8,9}$ -DEHYDROESTRONE**  
 Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 [NOTE—The relative retention times of 17 $\beta$ -estradiol, 3-O-methylestrone, and  $\Delta^{8,9}$ -dehydroestrone are about 0.29, 1.0, and 0.9, respectively.]

Identify any peaks for 17 $\beta$ -estradiol, 3-O-methylestrone, and  $\Delta^{8,9}$ -dehydroestrone from the *Sample solution*.

Calculate the percentages of 17 $\beta$ -estradiol and  $\Delta^{8,9}$ -dehydroestrone as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

- $R_U$  = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*  
 $R_S$  = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP Estrone RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

**Acceptance criteria:** NMT 2.25% and NMT 6.25% of the labeled content of Conjugated Estrogens for 17 $\beta$ -estradiol and  $\Delta^{8,9}$ -dehydroestrone, respectively, as their sodium sulfate salts

- LIMIT OF ESTRONE, EQUILIN, AND 17 $\alpha$ -DIHYDROEQUILIN** (free steroids)

Buffer, Internal standard solution, Standard stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

**Free steroids standard solution:** Dilute the *Standard stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture..."

**Sample solution:** Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the solution, instead of 3.0 mL, to the centrifuge tube.

**Blank solution:** Prepare a reagent blank in the same manner as the *Sample solution*.

**System suitability:** Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, from NLT two replicate injections.

### Analysis

**Samples:** *Free steroids standard solution* and *Sample solution*

Calculate the total percentage of estrone, equilin and 17 $\alpha$ -dihydroequilin (free steroids) in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = ratio of the sum of the estrone, equilin, and 17 $\alpha$ -dihydroequilin peak areas (corrected for any peaks found in the *Blank solution*) to the internal standard peak area from the *Sample solution*  
 $R_S$  = ratio of the estrone peak area to the internal standard peak area from the *Standard solution*  
 $C_S$  = concentration of USP Estrone RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
**Acceptance criteria:** NMT 1.3% of free steroids

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature, or under refrigeration.
- LABELING:** Label it to state the content of Conjugated Estrogens on a weight-to-weight basis.

• **USP REFERENCE STANDARDS** <11>

USP 17 $\alpha$ -Dihydroequilin RS  
Estra-1,3,5(10),7-tetraene-3,17 $\alpha$ -diol.  
 $C_{18}H_{22}O_2$  270.37  
USP Equilin RS  
USP Estradiol RS  
USP Estrone RS

## Conjugated Estrogens Tablets

### DEFINITION

Conjugated Estrogens Tablets contain NLT 73.0% and NMT 95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate in the Tablets is NLT 0.35 and NMT 0.65.

### IDENTIFICATION

- **A.** The relative retention times of the 17 $\alpha$ -dihydroequilin peak, the estrone peak, and the equilin peak from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.
- **B.** The chromatogram of the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17 $\alpha$ -estradiol and 17 $\beta$ -dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-O-methylestrone.

### ASSAY

• **PROCEDURE**

**Buffer:** Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of  $5.2 \pm 0.1$ , if necessary.

**Internal standard solution:** 150  $\mu$ g/mL of 3-O-methylestrone in methanol

**Standard stock solution:** 160, 70, and 50  $\mu$ g/mL each of USP Estrone RS, USP Equilin RS, and USP 17 $\alpha$ -Dihydroequilin RS, respectively, in alcohol

**Standard solution:** Pipet 1.0 mL of the *Internal standard solution* and 1.0 mL of the *Standard stock solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15  $\mu$ L of dried pyridine and 65  $\mu$ L of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.

**System suitability solution:** Pipet 1.0 mL of a 2.0- $\mu$ g/mL solution of USP Estradiol RS (17 $\beta$ -estradiol) in alcohol, 1.0 mL of *Internal standard solution*, and 1.0 mL of *Standard stock solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for the *Standard solution*, beginning with "Evaporate the mixture..."

**Sample solution:** If the Tablets are sugar coated, carefully remove the color and sugar coatings with water, leaving the shellac coating intact, and dry under nitrogen. Transfer an equivalent to 2 mg of total conjugated estrogens from NLT 20 finely powdered Tablets, to a 50-mL centrifuge tube fitted with a polytef-lined screw-cap and containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of  $5.0 \pm 0.5$ . Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the

lower layer is clear. Transfer as much of the organic phase as possible, and dry by rapidly passing through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed for the *Standard solution*, beginning with "Evaporate the mixture..."

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm  $\times$  15-m fused silica capillary; bonded with a 0.25- $\mu$ m layer of phase G19

**Temperatures**

**Column:** 208°

**Detector:** 260°

**Injection port:** 260°

**Carrier gas:** Hydrogen

**Flow rate:** 2 mL/min

**Injection mode:** Split

**Split flow rate:** 40–60 mL/min

**Injection volume:** 1  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-O-methylestrone peak at 17–25 min.]

[NOTE—The relative retention times for 17 $\beta$ -estradiol, 17 $\alpha$ -dihydroequilin, estrone, equilin, and 3-O-methylestrone are about 0.29, 0.30, 0.80, 0.87, and 1.00, respectively.]

### Suitability requirements

**Resolution:** NLT 1.2 between estrone and equilin, *System suitability solution*

**Tailing factor:** NMT 1.3 for the estrone peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the estrone peak ratios for NLT four injections of the *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

$R_U$  = peak response ratio of the appropriate analyte to that of the internal standard from the *Sample solution*

$R_S$  = peak response ratio of the appropriate analyte to that of the internal standard from the *Standard solution*

$C_S$  = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of estrone or equilin in the *Sample solution* ( $\mu$ g/mL)

$F$  = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

**Acceptance criteria:** 73.0%–95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.35 and NMT 0.65.

### PERFORMANCE TESTS

- **DISSOLUTION** <711>: Proceed as directed for *Extended-Release* articles.

**Test 1** (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.



**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 2, 5, and 8 h

**Buffer:** 0.025 M monobasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (1:3)

**Standard solution:** Transfer 10 Tablets to a 1000-mL volumetric flask, dilute with water to volume, and stir vigorously by mechanical means for at least 3 h. Pipet a filtered 100-mL aliquot of the solution into a 900-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Filter a portion of the solution under test. It is recommended that the filters selected be tested for binding affinity.

#### Chromatographic system

(see *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 3.0-cm; 3-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20–200 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]

#### Suitability requirements

**Resolution:** NLT 1.5 between equilin sulfate and estrone sulfate

**Relative standard deviation:** NMT 1.5% for the estrone sulfate peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estrone sodium sulfate dissolved:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = estrone sulfate peak response from the *Sample solution*

$r_S$  = estrone sulfate peak response from the *Standard solution*

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved (%)
2	19–49
5	66–96
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 2** (for products labeled as 0.9-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus, Times, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 1*.

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Amount Dissolved (%)
2	12–37
5	57–85
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 3** (for products labeled as 1.25- and 2.50-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 1*.

**Times:** 2, 5, 8, and 12 h

**Tolerances:** See *Table 3*.

**Table 3**

Time (h)	Amount Dissolved (%)
2	3–22
5	37–67
8	66–96
12	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 4** (for products labeled as 1.25-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** Acetate buffer, pH 4.5; 900 mL

**Apparatus 2:** 50 rpm, with sinkers

**Times:** 2, 4, 8, and 12 h

**Buffer:** 0.025 M monobasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (22:78)

**Standard solution:** Weigh 20 Tablets and determine the average tablet weight. Grind the Tablets to a uniform fine powder. Weigh a portion of the powdered Tablets equivalent to the average tablet weight, transfer to a 900-mL volumetric flask, and dilute with *Medium* to volume. Stir vigorously by mechanical means for at least 2 h or until the dissolution of the powder is complete. Pass a portion of the extract through a suitable filter of 10-μm pore size.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 10-μm pore size. It is recommended that the filters selected be tested for binding affinity.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.2-mm × 5.0-cm; 5-μm packing L1

**Flow rate:** 0.8 mL/min

**Injection volume:** 20–200 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]

#### Suitability requirements

**Resolution:** NLT 1.2 between equilin sulfate and estrone sulfate

**Relative standard deviation:** NMT 2.0% for the estrone sulfate peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estrone sulfate dissolved:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = estrone sulfate peak response from the *Sample solution*

$r_s$  = estrone sulfate peak response from the  
Standard solution

Tolerances: See Table 4.

Table 4

Time (h)	Amount Dissolved (%)
2	11–31
4	43–63
8	75–95
12	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 5** (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 4*.

**Times:** 1, 3, and 8 h

**Tolerances:** See Table 5.

Table 5

Time (h)	Amount Dissolved (%)
1	6–26
3	48–68
8	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 6** (for products labeled as 0.9-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 4*.

**Times:** 1, 3, and 8 h

**Tolerances:** See Table 6.

Table 6

Time (h)	Amount Dissolved (%)
1	3–23
3	41–61
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in <711>.

#### • UNIFORMITY OF DOSAGE UNITS

**Analysis:** Assay 10 individual Tablets as directed in the *Assay*, and calculate the average content of conjugated estrogens, as the average of the total contents of sodium estrone sulfate and sodium equilin sulfate, in the 10 Tablets.

**Acceptance criteria:** The requirements are met if the content of each of the Tablets is 85.0%–115.0% of the average content of conjugated estrogens. If the content of NMT 2 Tablets falls outside the range of 85.0%–115.0% of the average content but not outside the range of 75.0%–125.0%, assay an additional 20 Tablets. The requirements are met if the content of NMT 2 of the 30 Tablets falls outside the limits of 85.0%–115.0% of that average, and no unit is outside the range of 75.0%–125.0% of the average content.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The labeling indicates the Tablet strength and states with which in vitro *Dissolution Test* the product complies.
- **USP REFERENCE STANDARDS** <11>
  - USP 17 $\alpha$ -Dihydroequilin RS
  - Estra-1,3,5(10),7-tetraene-3,17 $\alpha$ -diol.
  - C<sub>18</sub>H<sub>22</sub>O<sub>2</sub> 270.37
  - USP Equilin RS
  - USP Estradiol RS
  - USP Estrone RS

### Esterified Estrogens

#### DEFINITION

Esterified Estrogens is a mixture of the sodium salts of the sulfate esters of the estrogenic substances, principally estrone. It is a dispersion of the estrogenic substances on a suitable powdered diluent. The content of total esterified estrogens is NLT 90.0% and NMT 110.0% of the labeled amount.

Esterified Estrogens contains NLT 75.0% and NMT 85.0% of sodium estrone sulfate, and NLT 6.0% and NMT 15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens.

#### IDENTIFICATION

- **A.** The *Sample solution* exhibits peaks for estrone and equilin at relative retention times corresponding to those from the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Internal standard solution:** 150  $\mu$ g/mL of 3-O-methylestrone in methanol

**Standard stock solution:** 160 and 70  $\mu$ g/mL each of USP Estrone RS and USP Equilin RS in alcohol

**Standard solution:** Pipet 1.0 mL of the *Standard stock solution* and 1.0 mL of *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15  $\mu$ L of dried pyridine and 65  $\mu$ L of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, and allow to stand for 15 min. Add 0.5 mL of toluene.

**System suitability stock solution:** 2  $\mu$ g/mL of USP Estradiol RS (17 $\beta$ -estradiol) in alcohol

**System suitability solution:** Pipet 1.0 mL of *System suitability stock solution*, 1.0 mL of *Standard stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture..."

**Buffer:** Mix 79 mL of sodium acetate TS with 21 mL of 1 N acetic acid, and dilute with water to 500 mL. If necessary, adjust the solution with 1 N acetic acid or sodium acetate TS to a pH of 5.2  $\pm$  0.1.

**Sample solution:** Transfer the equivalent to 2 mg of total esterified estrogens from Esterified Estrogens, to a 50-mL centrifuge tube, fitted with a polytetrafluoroethylene cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0  $\pm$  0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°.

Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by passing rapidly through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed under *Standard solution*, beginning with "Evaporate the mixture..."

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm × 15-m fused silica capillary column; with a 0.25-μm layer of phase G19

**Temperature**

**Column:** 208°

**Detector:** 260°

**Injector port:** 260°

**Carrier gas:** Hydrogen

**Flow rate:** 2 mL/min

**Injection mode:** Split

**Split flow rate:** 40–60 mL/min

**Injection size:** 1 μL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-O-methylestrone peak at between 17 and 25 min.]

[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-O-methylestrone are 0.29, 0.80, 0.87, and 1.00, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between estrone and equilin, *System suitability solution*

**Tailing factor:** NMT 1.3 for the estrone peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the estrone peak ratios for NLT 4 injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Separately calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Esterified Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

$R_U$  = ratio of the estrone or equilin peak area to the 3-O-methylestrone peak area from the *Sample solution*

$R_S$  = ratio of the estrone or equilin peak area to the 3-O-methylestrone peak area from the *Standard solution*

$C_S$  = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of the *Sample solution* (μg/mL)

$F$  = factor converting free estrogen to the conjugate ester sodium salt, 1.377 for estrone and 1.380 for equilin

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of total esterified estrogens; 75.0%–85.0% of sodium estrone sulfate; 6.0%–15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens

#### IMPURITIES

##### • FREE STEROIDS

**Internal standard solution, Buffer, Standard stock solution, Chromatographic system, and System suitability solution:** Proceed as directed in the *Assay*.

**Free steroids standard solution:** Dilute the *Standard stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture..."

**Sample solution:** Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the filtrate instead of 3.0 mL to the *Sample solution*.

**Blank solution:** Prepare a reagent blank in the same manner as the *Sample solution*.

**System suitability:** Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, on the basis of NLT two replicate injections.

#### Analysis

**Samples:** *Free steroids standard solution* and *Sample solution*

Calculate the total percentage of estrone and equilin (free steroids) in the portion of Esterified Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = ratio of the sum of the estrone and equilin peak areas (corrected for any peaks found in the *Blank solution*) to the 3-O-methylestrone peak area from the *Sample solution*

$R_S$  = ratio of the estrone peak area to the 3-O-methylestrone peak area from the *Free steroids standard solution*

$C_S$  = concentration of USP Estrone RS in the *Free steroids standard solution* (μg/mL)

$C_U$  = concentration of the *Sample solution* (μg/mL)

**Acceptance criteria:** NMT 3.0% of free steroids

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to state the content of Esterified Estrogens on a weight-to-weight basis.
- **USP REFERENCE STANDARDS** <11>
  - USP Equilin RS
  - USP Estradiol RS
  - USP Estrone RS

## Esterified Estrogens Tablets

#### DEFINITION

Esterified Estrogens Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of esterified estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.071 and NMT 0.20.

#### IDENTIFICATION

- **A.** The *Sample solution* exhibits peaks for estrone and equilin at relative retention times corresponding to those from the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Internal standard solution:** 150 μg/mL of 3-O-methylestrone in methanol

**Standard stock solution:** 160 and 70 µg/mL each of USP Estrone RS and USP Equilin RS in alcohol

**Standard solution:** Pipet 1.0 mL of the *Standard stock solution* and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, and allow to stand for 15 min. Add 0.5 mL of toluene.

**System suitability stock solution:** 2 µg/mL of USP Estradiol RS (17β-estradiol) in alcohol

**System suitability solution:** Pipet 1.0 mL of *System suitability stock solution*, 1.0 mL of *Standard stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture..."

**Buffer:** Mix 79 mL of sodium acetate TS with 21 mL of 1 N acetic acid, and dilute with water to 500 mL. If necessary, adjust the solution with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1.

**Sample solution:** Transfer an equivalent to 2 mg of total esterified estrogens from powdered Tablets (NLT 20), to a 50-mL centrifuge tube, fitted with a polytetrafluoroethylene-lined screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by passing rapidly through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed under *Standard solution*, beginning with "Evaporate the mixture..."

#### Chromatographic system

(See Chromatography <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm × 15-m fused silica capillary column; with a 0.25-µm layer of phase G19

**Temperature**

**Column:** 208°

**Detector:** 260°

**Injector port:** 260°

**Carrier gas:** Hydrogen

**Flow rate:** 2 mL/min

**Injection mode:** Split

**Split flow rate:** 40–60 mL/min

**Injection size:** 1 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-O-methylestrone peak between 17 and 25 min.]

[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-O-methylestrone are about 0.29, 0.80, 0.87, and 1.00, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between estrone and equilin, *System suitability solution*

**Tailing factor:** NMT 1.3 for the estrone peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the estrone peak ratios for NLT 4 injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Separately calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Esterified Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times (100)$$

$R_U$  = ratio of the estrone or equilin peak to the 3-O-methylestrone peak area from the *Sample solution*

$R_S$  = ratio of the estrone or equilin peak to the 3-O-methylestrone peak area from the *Standard solution*

$C_S$  = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of estrone or equilin in the *Sample solution* (µg/mL)

$F$  = factor converting free estrogen to the conjugate sodium salt, 1.377 for estrone and 1.380 for equilin

**Acceptance criteria:** 90.0%–115.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION <701>

**Simulated intestinal fluid:** Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, add 190 mL of 0.2 N sodium hydroxide and 400 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with 0.2 N sodium hydroxide to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.

**Analysis:** Place 1 Tablet in each of the six tubes of the basket, and immerse the basket in water at 25 ± 0.5° for 5 min to remove the coating. Add a disk to each tube, and operate the apparatus using simulated gastric fluid TS, maintained at 37 ± 2°, as the immersion fluid. After 30 min in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets.

**Acceptance criteria:** All the Tablets have disintegrated. If all the Tablets have not disintegrated completely, substitute *Simulated intestinal fluid*, maintained at 37 ± 2°, as the immersion fluid, and continue the test so that the total period of time, including previous exposure to water and simulated gastric fluid TS, does not exceed 90 min.

##### • UNIFORMITY OF DOSAGE UNITS (905)

**Analysis:** Test 10 individual Tablets as directed in the Assay, and calculate the average content of esterified estrogens, as the average of the total contents of sodium estrone sulfate and sodium equilin sulfate, in the 10 Tablets.

**Acceptance criteria:** The requirements are met if the content of each of the Tablets is 85.0%–115.0% of the average content of esterified estrogens. If the content of NMT 2 Tablets falls outside the range of 85.0%–115.0% of the average content but not outside the range of 75.0%–125.0%, test an additional 20 Tablets. The requirements are met if the content of NMT 2 of the 30 Tablets falls outside the limits of 85.0%–115.0% of the average, and no unit is outside the range of 75.0%–125.0% of the average content.

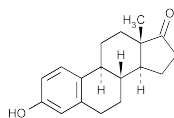
#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS <11>

USP Equilin RS  
USP Estradiol RS  
USP Estrone RS

## Estrone



$C_{18}H_{22}O_2$  270.37  
Estra-1,3,5(10)-trien-17-one, 3-hydroxy-.  
3-Hydroxyestra-1,3,5(10)-trien-17-one [53-16-7].

» Estrone contains not less than 97.0 percent and not more than 103.0 percent of  $C_{18}H_{22}O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Estrone RS

**Clarity of solution**—Add 100 mg to 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, heat on a steam bath until solution is complete, then cool, and transfer to a 100-mL color-comparison tube: the solution is clear.

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 50 µg per mL.

*Medium:* alcohol, heated on a steam bath and cooled to room temperature.

**Specific rotation** (781S): between +158° and +165°.

*Test solution:* 10 mg, previously dried, per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Limit of equilenin and equilin**—Dissolve 10 mg in sufficient alcohol to make 50 mL. Transfer 5 mL of the solution to a small beaker. Add 5 mL of a buffer solution prepared by dissolving 2 mL of glacial acetic acid and 13.3 g of anhydrous sodium acetate in water to make 100 mL, warm to about 50°, and add 1 mL of a freshly prepared 1 in 200 solution of 2,6-dibromoquinone-chlorimide in alcohol. Mix, and allow to stand for 30 minutes. Transfer the solution to a small separator, add 10 mL of chloroform and 20 mL of 1 N sodium hydroxide, and shake vigorously for 2 minutes. Separate the chloroform layer, and filter rapidly through a dry filter paper into a dry test tube, discarding the first 2 mL of the filtrate. Viewed transversely against a white background, the chloroform filtrate shows no more red color than that produced by similarly treating 5 mL of an alcohol solution containing 20 µg of equilenin.

### Ordinary impurities (466)—

*Test solution:* acetone.

*Standard solution:* acetone.

*Eluant:* a mixture of chloroform and acetone (9:1), in a nonequilibrated chamber.

*Visualization:* 5.

### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and 0.05 M monobasic potassium phosphate (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 20 mg of USP Estrone RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. If necessary, sonicate to aid solution. Transfer 5 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to

obtain a *Standard preparation* having a known concentration of about 40 µg of USP Estrone RS per mL.

*Assay preparation*—Transfer about 20 mg of Estrone, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. If necessary, sonicate to aid solution. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1500 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{22}O_2$  in the portion of Estrone taken by the formula:

$$0.5C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Estrone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Estrone Injectable Suspension

» Estrone Injectable Suspension is a sterile suspension of Estrone in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{18}H_{22}O_2$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Estrone RS

USP Progesterone RS

**Identification**—Transfer a volume of Injectable Suspension, equivalent to about 5 mg of estrone, to a glass-stoppered centrifuge tube, and add 2.5 mL of a mixture of ether and benzene (1:1). Shake for 2 minutes, and allow insoluble matter to settle, centrifuging, if necessary, to obtain a clear supernatant. Apply 5 µL each of this supernatant and a 1 in 500 solution of USP Estrone RS in a mixture of ether and benzene (1:1) to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of benzene and acetone (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol and sulfuric acid (3:1), and heat in an oven at 105° for 10 minutes: the  $R_f$  value and appearance (pale orange to amber by direct observation in daylight, and fluorescing pale yellow-green under long-wavelength UV light) of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

**Bacterial endotoxins** (85)—It contains not more than 88.0 USP Endotoxin Units per mg of estrone.

**Uniformity of dosage units** (905): meets the requirements.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay under Estrone*.

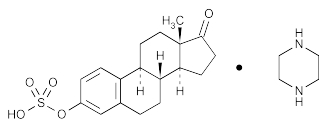
*Assay preparation*—Transfer an accurately measured volume of the well-mixed Injectable Suspension, equivalent to about 10 mg of estrone to a 50-mL volumetric flask. Add 30 mL of methanol and swirl for 5 minutes. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Estrone*. Calculate the quantity, in mg, of  $C_{18}H_{22}O_2$  in each mL of Injectable Suspension taken by the formula:

$$0.25(C/V)(r_U/r_S)$$

in which  $V$  is the volume, in mL, of the Injectable Suspension taken, and the other terms are as defined therein.

## Estropipate



$C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$  436.56

Estra-1,3,5(10)-trien-17-one, 3-(sulfooxy)-, compd. with piperazine (1:1).

Estrone hydrogen sulfate compound with piperazine (1:1) [7280-37-7].

» Estropipate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Estrone RS

USP Estropipate RS

**Identification, Infrared Absorption** (197K).

**Loss on drying** (731)—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Free estrone—**

*Stock impurity standard preparation*—Weigh accurately 25.0 mg of USP Estrone RS into a 100-mL volumetric flask, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

*Impurity standard preparation*—Weigh accurately 25.0 mg of USP Estropipate RS into a 25-mL volumetric flask, add 2.0 mL of *Stock impurity standard preparation*, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

*Standard preparation*—Weigh accurately 25.0 mg of USP Estropipate RS into a 25-mL volumetric flask, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

*Test preparation*—Using a portion of Estropipate, accurately weighed, prepare as directed under *Standard preparation*.

*Mobile phase*—Mix 650 mL of 0.025 M potassium dihydrogen phosphate with 350 mL of spectrophotometric-

grade acetonitrile. Filter the solution through a membrane filter having a porosity of 1  $\mu$ m or less, and degas at a pressure of less than 100 mm of mercury until no further bubbles appear. The concentration of acetonitrile may be varied to meet system suitability requirements and to provide a suitable elution time for all components.

*Chromatographic system*—Typically, a high-pressure liquid chromatograph, operated at room temperature, is fitted with a 30-cm  $\times$  3.9-mm stainless steel column that contains packing L1. The mobile phase is maintained at a pressure and flow rate (approximately 1.5 mL per minute) capable of giving the required resolution (see *System suitability test*) and a suitable elution time. An UV detector that monitors absorption at a wavelength of 213 nm is used with a recorder adjusted such that approximately 0.04 absorbance unit gives a full-scale reading.

*System suitability test*—Chromatograph two injections of the *Impurity standard preparation*, and determine that after the injection front the small peak (estrone) after the major peak does not differ in peak response between the duplicate injections by more than 4%. Also determine that the small peak after the major component has a retention time relative to the major component of approximately 5.5. (For a particular column, resolution may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.)

*Procedure*—Inject separately 5.0- $\mu$ L portions of the *Standard preparation*, the *Impurity standard preparation*, and the *Test preparation* into the high-pressure liquid chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the peak responses for the estrone peak relative to the estropipate peak obtained with the *Standard preparation*, the *Impurity standard preparation*, and the *Test preparation*. Calculate the percentage of free estrone taken by the formula:

$$2.5(C/W)(H_U/H_S)$$

in which  $H_U$  and  $H_S$  are the measured peak heights of the impurity (estrone) in the *Test preparation* and the *Impurity standard preparation* corrected for the peak height of estrone in the *Standard preparation*, respectively,  $W$  is the weight, in mg, of estropipate in the *Test preparation*, and  $C$  is the concentration, in  $\mu$ g per mL, of USP Estrone RS in the *Impurity standard preparation*. Not more than 2.0% is found.

**Assay—**

*Standard preparation*—Prepare as directed under *Free estrone*.

*Assay preparation*—Prepare as directed for *Test preparation* under *Free estrone*.

*Chromatographic system*—Use the same system as in test for *Free estrone*. Adjust the recorder so that approximately 0.4 absorbance unit gives a full-scale reading.

*System suitability test*—Chromatograph two injections of the *Standard preparation*, and determine that only one major peak is observed after the injection front. The peak responses between the duplicate injections for the major peak do not differ by more than 3%.

*Procedure*—Inject 5.0  $\mu$ L of the *Assay preparation* and the *Standard preparation* into the high-pressure liquid chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the peak heights for the respective estropipate peak (it is actually an estrone sulfate peak) obtained with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of  $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$  in the portion of Estropipate taken by the formula:

$$25C(H_U/H_S)$$

in which  $H_U$  and  $H_S$  are the peak heights obtained with the *Assay preparation* and the *Standard preparation*, respectively, and  $C$  is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation*.

## Estropipate Vaginal Cream

» Estropipate Vaginal Cream contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of estropipate ( $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ ), in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes.

**USP Reference standards** (11)—

USP Estropipate RS

**Identification**—

*Arsenomolybdate spray reagent*—Dissolve 25 g of ammonium molybdate in 450 mL of water, add 21 mL of sulfuric acid, and mix. Add 3 g of dibasic sodium arsenate heptahydrate dissolved in 25 mL of water, mix, and incubate at  $37 \pm 2^\circ$  for 24 to 48 hours. Store, protected from light.

*Procedure*—Transfer a portion of Vaginal Cream, equivalent to about 8 mg of estropipate, to a container. Add 20 mL of methanol, stir to obtain a homogeneous mixture, and pass through filter paper. Apply 30  $\mu$ L of the filtrate and 30  $\mu$ L of a Standard solution of USP Estropipate RS in methanol containing about 400  $\mu$ g per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (10:8) until the solvent front has moved about 14 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by lightly spraying with *Arsenomolybdate spray reagent* and by heating at  $105^\circ$  for about 20 minutes: the  $R_f$  values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.1 M monobasic potassium phosphate, methanol, and acetonitrile (215:59:59). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of water and methanol (1:1).

*Resolution solution*—Dissolve a suitable quantity of 4'-nitroacetophenone in methanol to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 5.0 mL of the solution so obtained to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 5.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with 0.05 M piperazine to volume, and mix. Transfer 5.0 mL of the solution so obtained to a 25-mL volumetric flask, add 5.0 mL of the *Resolution solution*, dilute with 0.05 M piperazine to volume, mix, and filter.

*Assay preparation*—Transfer an accurately weighed portion of Vaginal Cream, equivalent to about 1.5 mg of estropipate, to a 125-mL separator, add 50 mL of chloroform and 20 mL of 0.05 M piperazine, and extract for 3 minutes. Collect the organic extract in another separator, and extract the aqueous layer with an additional 20-mL portion of chloroform. Combine the organic phases, wash with a 20-mL portion of 0.05 M piperazine for 3 minutes, and collect the aqueous washings. Discard the organic layer. Rinse the separators with 0.05 M piperazine, and add the rinse to the aqueous washings. Transfer the aqueous extract and the aqueous washings to a 100-mL volumetric flask, dilute with 0.05 M piperazine to volume, and mix. Transfer 15.0 mL of

the solution so obtained to a 25-mL volumetric flask, dilute with 0.05 M piperazine to volume, mix, and filter.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 213-nm detector and 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. [NOTE—The injection of a diluted solution of 4'-nitroacetophenone can be used to better identify the peak locus.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for 4'-nitroacetophenone and 1.0 for estropipate; the resolution,  $R$ , between 4'-nitroacetophenone and estropipate is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 30 minutes for the *Standard preparation* and 90 minutes for the *Assay preparation*, and measure the responses for the major peaks. Calculate the quantity, in mg, of estropipate ( $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ ) in the portion of Vaginal Cream taken by the formula:

$$166.67C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Estropipate Tablets

» Estropipate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of estropipate ( $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Estropipate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: 0.05 M pH 6.8 phosphate buffer; 900 mL.

*Apparatus 2*: 75 rpm.

*Time*: 30 minutes.

Determine the amount of  $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$  dissolved by employing the following method.

*Diluent* and *Mobile phase*—Proceed as directed in the *Assay*.

*System suitability solution*—Transfer about 100 mg of 4'-nitroacetophenone, accurately weighed, to a 100-mL volumetric flask, and dissolve in methanol, sonicating if necessary. Dilute with methanol to volume, and mix. Pipet 2 mL of this solution into a 250-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution*—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, sonicating if necessary, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 2 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 4 mL of the solution so obtained into a second 100-mL volumetric flask, add 8.0 mL of the *System suitability solution*, dilute with water to volume, and mix.

**Test solution**—Transfer an accurately measured volume of a filtered portion of the solution under test, equivalent to about 20 µg of estropipate, to a 25-mL volumetric flask. Dilute with water to volume if the contents are less than the nominal volume of the flask, and mix.

**Chromatographic system** (see *Chromatography* <621>)—Prepare as directed in the Assay. To evaluate the system suitability requirements, use the *Standard solution*.

**Procedure**—Separately inject equal volumes (about 300 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of  $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

**Diluent, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system**—Prepare as directed in the Assay.

**Test preparation**—Transfer 1 Tablet to a 50-mL volumetric flask, add 20 mL of water, insert the stopper, and shake by mechanical means for about 30 minutes or until the Tablet disintegrates. Add 20 mL of methanol, insert the stopper, and shake by mechanical means for about 60 minutes. Dilute with *Diluent* to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 0.25 mg of estropipate, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass this solution through a solvent-resistant membrane filter having a 1-µm or finer porosity, discarding the first portion of the filtrate.

**Procedure**—Proceed as directed in the Assay, except to use the *Test preparation* instead of the *Assay preparation*.

**Assay**—

**Diluent**—Prepare a mixture of methanol and water (1:1).

**Mobile phase**—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and mix. Mix 600 mL of the resulting solution with 200 mL of methanol, add 200 mL of acetonitrile with stirring, and mix. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, sonicating if necessary, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1.0 mL of this solution into a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**System suitability solution**—Transfer about 100 mg of 4'-nitroacetophenone, accurately weighed, to a 100-mL volumetric flask, and dissolve in methanol, sonicating if necessary. Dilute with methanol to volume, and mix. Pipet 5 mL of this solution into a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pipet 5 mL of the solution so obtained into a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.

**Assay preparation**—Transfer 20 Tablets to a 1000-mL volumetric flask, add 200 mL of water, insert the stopper, and shake by mechanical means for about 30 minutes or until the Tablets disintegrate completely. Add 200 mL of methanol, insert the stopper, and shake by mechanical means for about 60 minutes. Dilute with *Diluent* to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 0.25 mg of estropipate, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass this solution through a solvent-resistant membrane filter having a 1-µm or finer porosity, discarding the first portion of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 213-nm detector and a 4.6-mm × 25-cm column that contains packing L1.

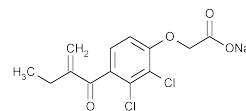
The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.85 for 4'-nitroacetophenone and 1.0 for estropipate; the resolution,  $R$ , between estropipate and 4'-nitroacetophenone is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of estropipate ( $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ ) in each Tablet taken by the formula:

$$(25C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation*;  $V$  is the volume, in mL, of the stock solution taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for estropipate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethacrynate Sodium for Injection



$C_{13}H_{11}Cl_2NaO_4$  325.12

Acetic acid, [2,3-dichloro-4-(2-methylene-1-oxobutyl)phenoxy]-, sodium salt.

Sodium [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetate [6500-81-8].

» Ethacrynate Sodium for Injection is a sterile, freeze-dried powder prepared by the neutralization of Ethacrynic Acid with the aid of Sodium Hydroxide. It contains an amount of ethacrynate sodium equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{13}H_{12}Cl_2O_4$ .

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.

**Labeling**—Label it to indicate that it was prepared by freeze-drying, having been filled into its container in the form of a true solution.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Ethacrynic Acid RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Identification, Ultraviolet Absorption** <197U>—

*Solution*: 50 µg per mL.

*Medium*: acidified methanol (prepared by adding 9 mL of hydrochloric acid to 100 mL of methanol).

**Bacterial endotoxins** <85>—It contains not more than 5.0 USP Endotoxin Units per mg of ethacrynate sodium.

**pH** <791>: between 5.0 and 7.0, in a solution containing the equivalent of about 50 mg of ethacrynic acid in 50 mL of Sterile Water for Injection.

**Other requirements**—It meets the requirements for *Sterility Tests* <71> and *Uniformity of Dosage Units* <905> under *Injections* <1>.



**Assay—**

*Triethylamine solution, Solvent mixture, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Ethacrynic Acid Tablets*.

*Assay preparation*—Select a number of containers of Ethacrynic Acid Sodium for Injection, the combined contents of which, on the basis of the labeled amount, are equivalent to about 500 mg of ethacrynic acid. Add about 5 mL of *Solvent mixture* to each container, mix to dissolve the contents, and combine the resulting solutions in a 200-mL volumetric flask. Rinse each container with two additional 5-mL portions of *Solvent mixture*, add the rinsings to the solution in the volumetric flask, dilute with *Solvent mixture* to volume, and mix. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Ethacrynic Acid Tablets*. Calculate the quantity, in mg, of  $C_{13}H_{12}Cl_2O_4$  in each container of Ethacrynic Acid Sodium for Injection taken by the formula:

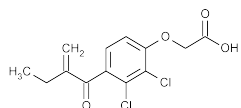
$$(1000C/N)(r_U / r_S)$$

in which *N* is the number of containers selected for the Assay preparation.

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**Ethacrynic Acid**


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$C_{13}H_{12}Cl_2O_4$  303.14

Acetic acid, [2,3-dichloro-4-(2-methylene-1-oxobutyl)-phenoxy]-, [2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid [58-54-8].

» Ethacrynic Acid contains not less than 97.0 percent and not more than 102.0 percent of  $C_{13}H_{12}Cl_2O_4$ , calculated on the dried basis.

**Caution**—Use care in handling Ethacrynic Acid, since it irritates the skin, eyes, and mucous membranes.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Ethacrynic Acid RS

**Identification—**

**A:** *Infrared Absorption* (197M).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 50 µg per mL.

*Medium:* methanol.

Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Add 2 mL of 1 N sodium hydroxide to about 25 mg of it, and heat for several minutes in a boiling water bath. Cool the solution, acidify with 0.25 mL of 18 N sulfuric acid, add 0.5 mL of chromotropic acid sodium salt solution (1 in 10), then add, cautiously, 2 mL of sulfuric acid TS: a deep violet color is produced.

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at 60° for 2 hours: it loses not more than 0.25% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Toluene extractives**—Accurately weigh about 1 g into a glass-stoppered, 100-mL cylinder. Add 50 mL of sodium sulfite solution (2 in 25), and agitate until the solid dissolves. Allow to stand for 20 minutes, add 5 mL of hydrochloric acid, and mix. Divide the solution between two centrifuge tubes, each of which contains 15 mL of toluene. Close each tube tightly, using a polyethylene stopper, and shake vigorously during 2 minutes, occasionally relieving the pressure from the sulfur dioxide by loosening the stoppers. Centrifuge the tubes, withdraw most of the upper layer by means of a syringe, avoiding withdrawal of any of the lower, aqueous phase, and transfer the toluene extracts to a tared evaporating dish. Repeat the extraction twice with additional 15-mL portions of toluene, and evaporate the combined extracts on a steam bath to dryness. Dry the residue at a pressure not exceeding 5 mm of mercury at 60° for 2 hours. Cool, and weigh: not more than 2.0% of extractives is found.

**Equivalent weight**—Dissolve about 400 mg, accurately weighed, in 100 mL of methanol, add 5 mL of water, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Calculate the equivalent weight on the dried basis: it is between 294 and 309.

**Heavy metals, Method II** (231): 0.001%.

**Assay**—Dissolve about 100 mg of Ethacrynic Acid, accurately weighed, in 20 mL of glacial acetic acid in an iodine flask. Pipet 20 mL of 0.1 N bromine VS into the flask, add 3 mL of hydrochloric acid, immediately insert the stopper, and seal the flask with a few mL of water in the stopper well. Swirl the flask, and allow to stand in the dark for 1 hour. Add 50 mL of water and 15 mL of potassium iodide TS, and immediately titrate with 0.1 N sodium thiosulfate VS, adding 2.0 mL of starch TS as the endpoint is approached. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N bromine is equivalent to 15.16 mg of  $C_{13}H_{12}Cl_2O_4$ .

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**Ethacrynic Acid Tablets**


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» Ethacrynic Acid Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ethacrynic acid ( $C_{13}H_{12}Cl_2O_4$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Ethacrynic Acid RS

**Identification—**

**A:** Weigh a portion of finely powdered Tablets, equivalent to about 50 mg of ethacrynic acid, and transfer to a separator containing 25 mL of 0.1 N hydrochloric acid. Extract with two 40-mL portions of methylene chloride, filter the extracts into a 100-mL volumetric flask, and dilute with methylene chloride to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, evaporate in a gentle current of air to dryness, and promptly dissolve the residue in a portion of a 9 in 1000 mixture of hydrochloric acid in anhydrous methanol, then dilute with the acidic methanol to volume: the UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of a solution of USP Ethacrynic Acid RS in the acidic methanol containing 50 µg per mL, concomitantly measured.

**B:** A portion of powdered Tablets, equivalent to 25 mg of ethacrynic acid, responds to *Identification* test C under *Ethacrynic Acid*.

**Dissolution** <711>—

**Medium:** 0.1 M phosphate buffer, prepared by mixing 13.6 g of monobasic potassium phosphate and 92.2 mL of 1 N sodium hydroxide with water to obtain 1000 mL of a solution having a pH of  $8.0 \pm 0.05$ ; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{13}H_{12}Cl_2O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 277 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Ethacrynic Acid RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{13}H_{12}Cl_2O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—Add 1 Tablet to a 100-mL volumetric flask containing 10 mL of water, and allow to stand for 15 minutes, shaking occasionally until the tablet is disintegrated. Add a 9 in 1000 mixture of hydrochloric acid in methanol to volume, and mix. Filter a portion of the mixture, and pipet a volume of the filtrate, equivalent to 5 mg of ethacrynic acid, into a 100-mL volumetric flask. Dilute with the acidic methanol to volume, and mix. Dissolve an accurately weighed quantity of USP Ethacrynic Acid RS in the acidic methanol, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using the acidic methanol as the blank. Calculate the quantity, in mg, of  $C_{13}H_{12}Cl_2O_4$  in the Tablet by the formula:

$$(T / D)C(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of ethacrynic acid in the Tablet; *D* is the concentration, in µg per mL, of ethacrynic acid in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution; *C* is the concentration, in µg per mL, of USP Ethacrynic Acid RS in the Standard solution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—

**Triethylamine solution**—Mix 10 mL of triethylamine and about 900 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of  $6.8 \pm 0.1$ , dilute with water to volume, mix, and filter.

**Solvent mixture**—Prepare a mixture of water and acetonitrile (3:2).

**Mobile phase**—Prepare a filtered and degassed mixture of *Triethylamine solution* and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ethacrynic Acid RS in *Solvent mixture*, and dilute quantitatively with *Solvent mixture*, to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of ethacrynic acid, to a 100-mL volumetric flask, add about 80 mL of *Solvent mixture*, and shake or sonicate to dissolve the ethacrynic acid. Dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from

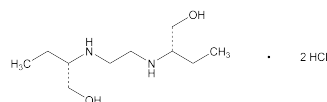
the analyte peak is not less than 1200 theoretical plates; the tailing factor for the analyte peak is not more than 2; the capacity factor, *k'*, is not less than 0.8; and the relative standard deviation for replicate injections is not more than 1.0 %.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ethacrynic acid ( $C_{13}H_{12}Cl_2O_4$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ethacrynic Acid RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethambutol Hydrochloride



$C_{10}H_{24}N_2O_2 \cdot 2HCl$  277.23  
1-Butanol, 2,2'-(1,2-ethanediyldiimino)bis-, dihydrochloride, [S-(R\*,R\*)]-;  
(+)-2,2'-(Ethylenediimino)-di-1-butanol dihydrochloride [1070-11-7].

**DEFINITION**

Ethambutol Hydrochloride contains NLT 98.0% and NMT 100.5% of  $C_{10}H_{24}N_2O_2 \cdot 2HCl$ , calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197K>
- B. IDENTIFICATION TESTS—GENERAL, Chloride** <191>: Meets the requirements  
**Sample:** 100 mg/mL in water

**ASSAY****PROCEDURE**

**Sample solution:** 200 mg of Ethambutol Hydrochloride in a mixture of 100 mL of glacial acetic acid and 5 mL of mercuric acetate TS. Add crystal violet TS.

**Analysis:** Titrate with 0.1 N perchloric acid VS (the color change at the endpoint is from blue to blue-green). Perform a blank determination, and make any necessary corrections (see *Titrimetry* <541>). Each mL of 0.1 N perchloric acid is equivalent to 13.86 mg of  $C_{10}H_{24}N_2O_2 \cdot 2HCl$ .

**Acceptance criteria:** 98.0%–100.5% on the dried basis

**IMPURITIES****Inorganic Impurities**

- HEAVY METALS, Method II** <231>: NMT 20 ppm

**Organic Impurities**

- PROCEDURE 1: ORDINARY IMPURITIES** <466>

**Sample solution:** Methanol

**Standard solution:** Methanol

**Eluant:** Methanol and ammonium hydroxide (18:1)

**Visualization:** 16

- PROCEDURE 2: LIMIT OF AMINOBUTANOL**

**Solution A:** 1.24 g of boric acid to a 100-mL volumetric flask. Dissolve in 90 mL of water, adjust with 5 N sodium hydroxide to a pH of 9.0, and dilute with water to volume.

**Fluorescamine solution:** 0.1 mg/mL of fluorescamine in acetone

**Standard solution:** 5.0 µg/mL of USP Aminobutanol RS

**Sample solution:** 0.5 mg/mL of Ethambutol Hydrochloride

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Fluorometry

**Analytical wavelength:** 485 nm, with the excitation wavelength at about 385 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Pipet a 10-mL portion of the *Sample solution* into a glass-stoppered, 100-mL conical flask, and add 10 mL of water and 20 mL of *Solution A*. To another 100-mL flask, add 10.0 mL of the *Sample solution*, 10.0 mL of the *Standard solution*, and 20 mL of *Solution A*. Place the flasks on a magnetic stirrer, and while the contents are being stirred rapidly, add 10 mL of *Fluorescamine solution* rapidly. Insert the stoppers in the flasks, invert, and shake briefly. After 1 min, accurately timed, determine the relative fluorescence intensities of both solutions in a suitable fluorometer.

**Acceptance criteria:** The fluorescence intensity of the solution from the *Sample solution* is NMT the difference between the intensities of the two solutions (NMT 1.0%).

**SPECIFIC TESTS**

• **LIMIT OF TOTAL STEREOISOMERS**

**Solution A:** 60 mg/mL of (*R*)-(+)-alpha-methylbenzyl isocyanate in acetonitrile

**Solution B:** Acetonitrile and water (1:1)

**Mobile phase:** Methanol and water (13:7)

**Standard solution 1:** Transfer 13 mg of USP Ethambutol Hydrochloride RS to a 10-mL volumetric flask. Add 2.0 mL acetonitrile and 260 µL of triethylamine, and mix for 1 min. Add 650 µL of *Solution A*, and mix for another min. Dilute with *Solution B* to volume.

**Standard solution 2:** Prepare as directed for *Standard solution 1* using USP Ethambutol Related Compound A RS and USP Ethambutol Related Compound B RS.

**System suitability solution:** Mix equal volumes of *Standard solution 1* and *Standard solution 2*.

**Sample solution:** Prepare as directed for *Standard solution 1* using Ethambutol Hydrochloride.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.7 mL/min

**Injection size:** 20 µL

**Run time:** 2.3 times the retention time of ethambutol

**System suitability**

**Samples:** *Standard solution 1* and *System suitability solution*

[NOTE—The relative retention times for ethambutol related compound B, ethambutol, and ethambutol related compound A are 0.85, 1.0, and 1.4, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between ethambutol and ethambutol related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution 1*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of stereoisomers in the portion of *Sample* taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of ethambutol related compound A or ethambutol related compound B from the *Sample solution*

$r_T$  = sum of all peak responses of ethambutol related compound A, ethambutol related compound B and ethambutol from the *Sample solution*

Calculate the percentage of total stereoisomers:

$$\text{Result} = \% \text{ ethambutol related compound A} + \% \text{ ethambutol related compound B}$$

**Acceptance criteria**

**Total impurities:** NMT 4.0%

• **OPTICAL ROTATION, Specific Rotation (781S):** +6.0° to +6.7°

**Sample solution:** 100 mg/mL in water

• **LOSS ON DRYING (731):** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Aminobutanol RS

$C_4H_{11}NO$  89.14

USP Ethambutol Hydrochloride RS

USP Ethambutol Related Compound A RS

(2*R*,2'*S*)-2,2'-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol.  
 $C_{10}H_{24}N_2O_2$  204.31

USP Ethambutol Related Compound B RS

(2*R*,2'*R*)-2,2'-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol.  
 $C_{10}H_{24}N_2O_2$  204.31

## Ethambutol Hydrochloride Tablets

**DEFINITION**

Ethambutol Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of ethambutol hydrochloride ( $C_{10}H_{24}N_2O_2 \cdot 2HCl$ ).

**IDENTIFICATION**

**Sample:** Triturate a quantity equivalent to 100 mg of ethambutol from powdered Tablets, with 3 mL of methanol in a glass mortar. Add 5 mL of methanol to obtain a suspension, then pass through a funnel lined with a suitable filter paper (Whatman No. 42 or equivalent) previously moistened with methanol, and collect the filtrate in a beaker containing 100 mL of acetone. Stir the mixture, and allow crystallization to proceed for 15 min. Decant the liquid, and gently dry the crystals with the aid of a current of air until the odor of methanol is no longer detectable: a portion of the crystals so obtained responds to the following tests.

• **A. INFRARED ABSORPTION (197K)**

• **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**

**Sample solution:** 100 mg/mL in water

**Acceptance criteria:** Meet the requirement

**ASSAY**

• **PROCEDURE**

**Buffer:** Mix 1.0 mL of triethylamine with 1 L of water, and adjust with phosphoric acid to pH 7.0.

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 0.30 mg/mL of USP Ethambutol Hydrochloride RS

**Sample solution:** Equivalent to 0.30 mg/mL of ethambutol hydrochloride from powdered Tablets (NLT 20). Filter the solution, discarding the first 10-mL portion.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 200 nm**Column:** 4.6-mm × 15-cm base-deactivated; 5-μm packing L10**Flow rate:** 1 mL/min**Injection volume:** 50 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of ethambutol hydrochloride (C<sub>10</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> · 2HCl) present in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of ethambutol hydrochloride from the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–105.0%**PERFORMANCE TESTS**• **DISSOLUTION** <711>**Medium:** Water; 900 mL**Apparatus 1:** 100 rpm**Time:** 45 min**Buffer:** 38.0 g/L of monobasic sodium phosphate and 2.0 g/L of anhydrous dibasic sodium phosphate**Bromocresol green solution:** Dissolve 200 mg of bromocresol green in 30 mL of water and 6.5 mL of 0.1 N sodium hydroxide. Dilute with *Buffer* to 500 mL, and add 0.1 N hydrochloric acid to adjust to a pH of 4.6 ± 0.1.**Standard solution:** 0.1 mg/mL of USP Ethambutol Hydrochloride RS**Sample solution:** Pass a portion of solution under test through a suitable filter.**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** Vis**Analytical wavelength:** 415 nm**Analysis****Samples:** *Standard solution*, *Sample solution*, and water (blank)

Into 3 separate, glass-stoppered, 50-mL centrifuge tubes, pipet 1 mL of water to provide the blank, 1 mL of *Standard solution*, and 1 mL of *Sample solution*. Add 5.0 mL of *Bromocresol green solution* to each tube, add 10.0 mL of chloroform to each, insert the stoppers, and shake the mixtures vigorously. Allow the mixtures to separate, discard the upper aqueous layers, and filter the 3 chloroform layers through separate pledgets of cotton.

Determine the percentage of the labeled amount of ethambutol hydrochloride (C<sub>10</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> · 2HCl) dissolved, using the blank to set the instrument.

**Tolerances:** NLT 75% (Q) of the labeled amount of C<sub>10</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> · 2HCl is dissolved.• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**IMPURITIES**• **LIMIT OF AMINOBUTANOL****Buffer:** 1.24 g of boric acid to a 100-mL volumetric flask. Dissolve in 90 mL of water, adjust with 5 N so-

dium hydroxide to a pH of 9.0, and dilute with water to volume.

**Fluorescamine solution:** 0.1 mg/mL of fluorescamine in acetone**Standard solution:** 5.0 μg/mL of USP Aminobutanol RS in water

**Sample solution:** Place a number of Tablets, equivalent to 400 mg of ethambutol hydrochloride, in a beaker, cover with acetone, and allow to stand for 15 min. Decant the acetone, dry the Tablets, and remove the coating. Grind the Tablet cores in a mortar to a fine powder, moisten with methanol, and triturate to a fine paste. Transfer the mixture with the aid of methanol to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pass the mixture through a dry, folded filter paper. Pipet 25 mL of the filtrate into a 200-mL volumetric flask, and dilute with water to volume. Allow to stand for 15 min, and pass through a dry, folded filter paper, discarding the first cloudy portions of the filtrate. The clear filtrate is the *Sample solution*.

**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** Fluorometry**Analytical wavelength:** Excitation wavelength at about 385 nm; emission wavelength at 485 nm**Cell:** 1 cm**Analysis****Samples:** *Standard solution* and *Sample solution*

Pipet a 10-mL portion of the *Sample solution* into a glass-stoppered, 100-mL conical flask, and add 10 mL of water and 20 mL of *Buffer*. To another 100-mL flask, add 10.0 mL of the *Sample solution*, 10.0 mL of the *Standard solution*, and 20 mL of *Buffer*. Place the flasks on a magnetic stirrer, and while the contents are being stirred rapidly, add 10 mL of *Fluorescamine solution* rapidly. Insert the stoppers in the flasks, invert, and shake briefly. After 1 min, accurately timed, determine the relative fluorescence intensities of both solutions.

**Acceptance criteria:** The fluorescence intensity of the solution from the *Sample solution* is NMT the difference between the intensities of the two solutions; NMT 1.0%.

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** <11>

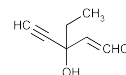
USP Aminobutanol RS

USP Ethambutol Hydrochloride RS

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**Ethchlorvynol**

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C<sub>7</sub>H<sub>9</sub>ClO 144.60

1-Penten-4-yn-3-ol, 1-chloro-3-ethyl-

1-Chloro-3-ethyl-1-penten-4-yn-3-ol [113-18-8].

» Ethchlorvynol contains not less than 98.0 percent and not more than 100.0 percent of *E*-ethchlorvynol (C<sub>7</sub>H<sub>9</sub>ClO), calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant glass or polyethylene containers, using polyethylene-lined closures.

**USP Reference standards** <11>—

USP Ethchlorvynol RS

**Identification—**

**A:** *Infrared Absorption* (197F).

**B:** Dissolve about 1 g in 20 mL of methanol. To 1 mL of the solution, add about 4 drops of 6 N ammonium hydroxide, mix, then add silver nitrate TS, a few drops at a time: a yellowish white precipitate is formed, and it at first redissolves, but becomes insoluble when an excess of silver nitrate TS has been added.

**C:** To 10 mL of the solution prepared in *Identification* test B add 5 mL of freshly prepared *m*-phenylenediamine hydrochloride-oxalic acid solution (prepared by dissolving 1 g of *m*-phenylenediamine hydrochloride and 1 g of oxalic acid in 35 mL of water and filtering, if necessary): a reddish orange color is produced in about 3 minutes.

**Refractive index** (831): between 1.476 and 1.480.

**Acidity**—Dissolve 5.0 mL of Ethchlorvynol in 50 mL of a mixture of equal volumes of water and methanol that has been neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide. Add 1 mL of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide to a pink endpoint: not more than 1.7 mL of 0.10 N sodium hydroxide is required for neutralization.

**Water, Method I** (921): not more than 0.2%.

**Chromatographic purity**—Calculate the percentage of each peak, other than the *E*-ethchlorvynol peak, observed in the chromatogram of the Ethchlorvynol obtained as directed in the Assay taken by the same formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of each secondary peak and  $r_t$  is the sum of all of the peaks observed in the chromatogram: not more than 0.2% of toluene, not more than 1.5% of *Z*-ethchlorvynol, and not more than 0.3% of any other impurity is found, and the total of all observed impurities is not more than 2.0%.

**Assay—**

**Resolution solution**—Add 2.5  $\mu$ L of toluene to 0.5 mL of USP Ethchlorvynol RS, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a thermal conductivity detector and a 1.8-m  $\times$  4-mm glass column (pretreated with 10% dimethyldichlorosilane in toluene) packed with 10% phase G16 on 60- to 80-mesh support S1AB. The column is maintained at about 160°, and the injector and the detector are maintained at about 200°. The carrier gas is dry helium, flowing at a rate of about 30 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times for toluene,  $\beta$ -chlorovinylethyl ketone (if present), *Z*-ethchlorvynol, and *E*-ethchlorvynol are about 0.1, 0.2, 0.8, and 1.0, respectively; the resolution,  $R$ , between the *Z*- and *E*-ethchlorvynol peaks is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Inject about 3  $\mu$ L of Ethchlorvynol into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of *E*-ethchlorvynol ( $C_7H_9ClO$ ) in the specimen of Ethchlorvynol taken by the formula:

$$100r_e / r_t$$

in which  $r_e$  is the response of the *E*-ethchlorvynol peak obtained in the chromatogram of the Ethchlorvynol and  $r_t$  is the sum of the responses of all of the peaks observed in the chromatogram.

**Ethchlorvynol Capsules**

» Ethchlorvynol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of *E*-ethchlorvynol ( $C_7H_9ClO$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Ethchlorvynol RS

**Identification—**

**A:** The retention time of the Ethchlorvynol peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Dissolve an amount of the contents of the Capsules, equivalent to about 1 g of ethchlorvynol, in 20 mL of methanol: the solution responds to *Identification* tests B and C under *Ethchlorvynol*.

**Dissolution** (711)—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements, chloroform being used as the solvent in the procedure for *Weight Variation*.

**Assay—**

**Methanol solution**—Add 600 mL of methanol to 1400 mL of water in a suitable container, and mix. Allow the solution to equilibrate to room temperature.

**Internal standard solution**—Dissolve a quantity of chlorobutanol in *Methanol solution* to obtain a solution having a known concentration of about 6 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ethchlorvynol RS in *Methanol solution* to obtain a solution having a known concentration of about 4 mg per mL. Transfer 5.0 mL of this solution to a suitable flask, add 5.0 mL of *Internal standard solution*, and mix to obtain a *Standard preparation* having a known concentration of about 2 mg of USP Ethchlorvynol RS per mL.

**Assay preparation**—Transfer not less than 20 Capsules, accurately counted, to a 500-mL volumetric flask. Add 250 mL of *Methanol solution*, heat at 70° to 80° for not more than 3 hours, and stir until the Capsules burst. Cool the solution to room temperature, add *Methanol solution* to volume, and mix. Quantitatively dilute a portion of this solution if necessary with *Methanol solution* to obtain a solution containing about 4 mg of *E*-ethchlorvynol per mL, and mix. Transfer 5.0 mL of this solution to a suitable flask, add 5.0 mL of *Internal standard solution*, and mix to obtain an *Assay preparation* containing about 2 mg of *E*-ethchlorvynol per mL.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 1.8-m  $\times$  4-mm glass column (pretreated with 10% dimethyldichlorosilane in toluene) packed with 10% phase G16 on 60- to 80-mesh support S1AB. The column is maintained at about 160°, and the injector and the detector are maintained at about 200°. The carrier gas is dry helium, flowing at a rate of about 30 mL per minute. Chromato-

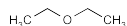
graph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the ethchlorvynol and chlorobutanol peaks is not less than 4.0, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the gas chromatograph, record the chromatographs, and measure the responses for the major peaks. The relative retention times are about 0.55 for chlorobutanol and 1.0 for *E*-ethchlorvynol. Calculate the quantity, in mg, of *E*-ethchlorvynol ( $C_7H_9ClO$ ) in each Capsule taken by the formula:

$$(CL / D)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ethchlorvynol RS in the *Standard preparation*,  $L$  is the labeled quantity, in mg, of ethchlorvynol in each Capsule,  $D$  is the concentration, in mg per mL, of ethchlorvynol in each mL of the *Assay preparation* based on the number of Capsules taken, the labeled quantity, in mg, of ethchlorvynol in each Capsule, and the extent of dilution, and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ether



$C_4H_{10}O$  74.12

Ethane, 1,1'-oxybis-

Ethyl ether [60-29-7].

» Ether contains not less than 96.0 percent and not more than 98.0 percent of  $C_4H_{10}O$ , the remainder consisting of alcohol and water.

**Caution**—Ether is highly volatile and flammable. Its vapor, when mixed with air and ignited, may explode.

**NOTE**—Ether to be used for anesthesia must be preserved in tight containers of not more than 3-kg capacity, and is not to be used for anesthesia if it has been removed from the original container longer than 24 hours. Ether to be used for anesthesia may, however, be shipped in larger containers for repackaging in containers as directed above, provided the ether at the time of repackaging meets the requirements of the tests of this Pharmacopeia.

**Packaging and storage**—Preserve in partly filled, tight, light-resistant containers, at a temperature not exceeding 30° remote from fire.

**Labeling**—Where Ether is intended for anesthetic use, the label so states.

**Specific gravity** (841): between 0.713 and 0.716 (indicating 96.0% to 98.0% of  $C_4H_{10}O$ ).

**Acidity**—To 10 mL of water in a glass-stoppered flask, add 0.10 mL of bromothymol blue TS and 0.010 N sodium hydroxide until a blue color persists after vigorous shaking. Add 25 mL of Ether, and shake briskly to mix the two layers.

If no blue color remains, titrate with 0.010 N sodium hydroxide until the blue color is restored and persists for several minutes: not more than 0.80 mL of 0.010 N sodium hydroxide is required (0.003% as  $CH_3COOH$ ). [NOTE—Exercise great care to avoid contamination from carbon dioxide when adding the Ether and titrating.]

**Water**, Method I (921): not more than 0.5%, except where labeled as intended for anesthetic use, it contains not more than 0.2%.

**Limit of nonvolatile residue**—Allow 50 mL to evaporate spontaneously from a tared dish, and dry at 105° for 1 hour: the weight of the residue does not exceed 1 mg (0.003%).

**Aldehyde**—Place 20 mL in a glass-stoppered cylinder, and add 7 mL of a mixture of 1 mL of alkaline mercuric-potassium iodide TS and 17 mL of a saturated solution of sodium chloride. Insert the stopper in the cylinder, shake vigorously for 10 seconds, then set aside for 1 minute: the water layer shows no turbidity.

### Limit of peroxide—

**Titanium tetrachloride solution**—Cool separately, in small beakers surrounded by crushed ice, 10 mL of 6 N hydrochloric acid and 10 mL of titanium tetrachloride. Add the titanium tetrachloride dropwise to the chilled acid. Allow the mixture to stand at ice-bath temperature until all of the yellow solid dissolves, dilute the solution with 6 N hydrochloric acid to 1000 mL, and mix.

**Peroxide standard solution**—Pipet 25 mL of hydrogen peroxide solution into a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 15 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL contains 0.011 mg of  $H_2O_2$ .

**Procedure**—To 50 mL of Ether, in a separator, add 5.0 mL of *Titanium tetrachloride solution*. Shake vigorously, allow the layers to separate, and drain the lower layer into a glass-stoppered, 25-mL graduated cylinder. Dilute with water to 10.0 mL, and mix. Any yellow color in the solution does not exceed that of a solution prepared by adding 5.0 mL of *Titanium tetrachloride solution* and 1.0 mL of *Peroxide standard solution* to a glass-stoppered, 25-mL graduated cylinder, diluting with water to 10.0 mL, and mixing, the colors being determined in 1-cm cells, with a suitable spectrophotometer, at a wavelength of 410 nm. The limit is 0.3 ppm.

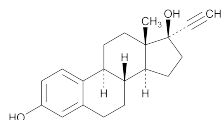
### Low-boiling hydrocarbons—

**Standard preparation**—Transfer about 50 mL of anhydrous ethyl ether, previously tested for absence of hydrocarbons, as directed under *Procedure*, to a 100-mL volumetric flask. Add 0.20 mL of pentane, dilute with the same anhydrous ethyl ether to volume, and mix.

**Apparatus**—Under typical conditions, a gas chromatograph (see *Chromatography* (621)) is equipped with a flame-ionization detector, and contains a 3.7-m  $\times$  2-mm (ID) stainless steel column packed with 30% phase G22 on 30- to 60-mesh support S1C. The injector port, column, and detector are maintained at temperatures of about 230°, 80°, and 250°, respectively, nitrogen being used as the carrier gas at a flow rate of about 30 mL per minute.

**Procedure**—Inject 1  $\mu$ L of *Standard preparation* and Ether, respectively, into the apparatus, and measure the total area under the hydrocarbon peaks (under typical conditions the retention times are about 2.3 minutes for isopentane, 2.7 minutes for pentane, and 4.3 minutes for 2-methylpentane) in the Ether. The total area of the hydrocarbon peaks does not exceed that of the peak for pentane in the *Standard preparation* (0.2%).

## Ethinyl Estradiol



$C_{20}H_{24}O_2$  296.40  
 19-Norpregna-1,3,5(10)-trien-20-yne-3, 17-diol, (17 $\alpha$ )-;  
 19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,17-diol  
 [57-63-6].

### DEFINITION

Ethinyl Estradiol contains NLT 97.0% and NMT 102.0% of  $C_{20}H_{24}O_2$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
*Sample solution:* 50  $\mu$ g/mL in alcohol  
*Wavelength:* 281 nm  
*Acceptance criteria:* Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

### ASSAY

- **PROCEDURE**  
*Mobile phase:* Acetonitrile and water (1:1)  
*Internal standard solution:* 0.5 mg/mL of ethylparaben in *Mobile phase*  
*Standard solution:* 0.2 mg/mL of USP Ethinyl Estradiol RS in *Mobile phase*, prepared as follows. Transfer 10 mg of USP Ethinyl Estradiol RS to a 50-mL volumetric flask, and add 10 mL of *Mobile phase* and 5.0 mL of *Internal standard solution*. Dilute with *Mobile phase* to volume.  
*Sample stock solution:* 1.0 mg/mL of Ethinyl Estradiol in *Mobile phase*  
*Sample solution:* 0.2 mg/mL of Ethinyl Estradiol, prepared as follows. Combine 10.0 mL of the *Sample stock solution* and 5.0 mL of *Internal standard solution* in a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.  
**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*.)  
*Mode:* LC  
*Detector:* UV 280 nm  
*Column:* 4.6-mm  $\times$  15-cm; packing L1  
*Flow rate:* 1 mL/min  
*Injection size:* 25  $\mu$ L  
**System suitability**  
*Sample:* *Standard solution*  
 [NOTE—The relative retention times for ethylparaben and ethinyl estradiol are about 0.6 and 1.0, respectively.]  
**Suitability requirements**  
*Resolution:* NLT 4.5 between the ethylparaben and ethinyl estradiol peaks  
*Relative standard deviation:* NMT 2.0%  
**Analysis**  
*Samples:* *Standard solution* and *Sample solution*  
 Calculate the percentage of ethinyl estradiol ( $C_{20}H_{24}O_2$ ) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio from the *Sample solution*  
 $R_S$  = peak response ratio from the *Standard solution*

$C_S$  = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Ethinyl Estradiol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

- **COMPLETENESS OF SOLUTION:** Dissolve 100 mg in 5 mL of alcohol; the solution is clear and free from undissolved solid.

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 180°–186°. It may exist also in a polymorphic modification, melting at 142°–146°.
- **OPTICAL ROTATION, Specific Rotation** (781S)  
*Sample solution:* 50 mg/mL, using sonication if necessary, in colorless pyridine from a freshly opened container  
*Acceptance criteria:* –28.0° to –29.5°
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, nonmetallic, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Ethinyl Estradiol RS

## Ethinyl Estradiol Tablets

### DEFINITION

Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of  $C_{20}H_{24}O_2$ .

### IDENTIFICATION

- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)  
*Standard solution:* 0.03 mg/mL of USP Ethinyl Estradiol RS in methanol  
*Sample solution:* Transfer 25 Tablets to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if needed, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to 10 mL.  
*Spray reagent:* Methanol and sulfuric acid (1:1)  
*Application volume:* 30  $\mu$ L  
*Developing solvent system:* Chloroform and alcohol (24:1)  
**Analysis**  
*Samples:* *Standard solution* and *Sample solution*  
 Proceed as directed under *Chromatography* (621), *Thin-Layer Chromatography*. Spray the plate with *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.  
**Acceptance criteria:** Meet the requirements

### ASSAY

- **PROCEDURE**  
*Mobile phase:* Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)  
*Diluent:* Acetonitrile and water (1:1)  
*Standard stock solution:* 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol  
*Standard solution:* 0.12  $\mu$ g/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*  
*Sample solution:* 0.12  $\mu$ g/mL of ethinyl estradiol from NLT 20 Tablets in *Diluent*. [NOTE—Shake for about 30 min before makeup to volume. Centrifuge a portion of the solution, and makeup with *Diluent*.]

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** Spectrofluorometric, with an excitation wavelength of 285 nm and an emission wavelength of 310 nm**Column:** 4.6-mm × 15-cm; packing L11**Guard column:** 4.6-mm × 12.5-mm; packing L11**Flow rate:** 2 mL/min**Injection size:** 200 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for ethinyl estradiol**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>20</sub>H<sub>24</sub>O<sub>2</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of ethinyl estradiol from the *Sample solution* $r_S$  = peak response of ethinyl estradiol from the *Standard solution* $C_S$  = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (µg/mL) $C_U$  = nominal concentration of the *Sample solution* (µg/mL)**Acceptance criteria:** 90.0%–115.0%**PERFORMANCE TESTS**

- **DISSOLUTION** [NOTE—Care must be taken not to expose any of the solutions to plastic or rubber. Fluorescent material will leach into the solutions and interfere with the quantitation of ethinyl estradiol. Also, adsorption may occur.]

**Test 1****Medium:** 0.3% sodium lauryl sulfate in water; 500 mL, degassed**Apparatus 2:** 100 rpm**Time:** 30 min**pH 6.0 phosphate buffer:** Transfer 2.7 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.0, and dilute with water to volume.**Mobile phase:** pH 6.0 phosphate buffer and acetonitrile (1:1)**Standard stock solution:** 0.25 mg/mL of USP Ethinyl Estradiol RS in methanol. This solution is stable for 14 days.**Standard solution:** Dilute the *Standard stock solution* with *Medium* to a final concentration of 0.06 µg/mL. Add 1 or 2 drops of methanol to dissipate the bubbles, if necessary. This solution is stable for 24 h.**Sample solution:** Centrifuge the solution under test for 10 min at 2000 rpm. Use the supernatant.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** Fluorescence, excitation at 285 nm, emission at 310 nm**Column:** 4.6-mm × 15-cm, 5-µm packing L11**Guard column:** 4.6-mm × 1.25-cm, 5-µm packing L11**Flow rate:** 2.0 mL/min**Injection size:** 200 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 3.0%**Analysis:** Calculate the percentage of ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $L$  = label claim (mg/Tablet) $V$  = volume of *Medium*, 500 mL**Tolerances:** NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** 5 ppm of polysorbate 80 in water; 500 mL, deaerated with helium**Apparatus 2:** 75 rpm**Time:** 45 min**Standard stock solution:** Transfer 10 mg of USP Ethinyl Estradiol RS and 50 mg of USP Norgestrel RS to a 500-mL volumetric flask. Add 250 mL of acetonitrile, and sonicate until dissolved. Cool to room temperature, and dilute with water to volume. The final concentration is about 20 µg/mL of ethinyl estradiol and 100 µg/mL of norgestrel. This solution is stable for 15 days.**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.02 µg/mL of ethinyl estradiol. This solution is stable for 6 days.**Sample solution:** Centrifuge the solution under test at about 3000 rpm for 20 min. Use the supernatant. This solution is stable for 12 h.**Mobile phase:** Water, acetonitrile, and methanol (55:40:5)**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 200 nm**Column:** 4.6-mm × 10-cm, 3-µm packing L1**Column temperature:** 30°**Flow rate:** 1.2 mL/min**Injection size:** 200 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 6.0 between the ethinyl estradiol and norgestrel peaks**Relative standard deviation:** NMT 3.0%, for ethinyl estradiol**Analysis:** Calculate the percentage of ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $L$  = label claim (mg/Tablet) $V$  = volume of *Medium*, 500 mL**Tolerances:** NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.



- **UNIFORMITY OF DOSAGE UNITS:** Meet the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE**

**Solution A:** Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)

**Solution B:** Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (4:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	2
20	100	0	2
20.1	100	0	2.5
25.0	0	100	2.5
25.1	0	100	3
30.0	0	100	3
30.1	0	100	2
32.0	100	0	2
35.0	100	0	2

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol

**Standard solution:** 0.12 µg/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*

**Sample solution A:** Transfer 20 Tablets into a 200-mL volumetric flask. Add 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the dissolution sample, and use the clear supernatant.

**Sample solution B:** 0.6 µg/mL of ethinyl estradiol from *Sample solution A* in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detectors:** UV detector at 210 nm; and a spectrofluorometric detector with an excitation wavelength of 285 nm and an emission wavelength of 310 nm

**Column:** 4.6-mm × 15-cm; packing L11

**Guard column:** 4.6-mm × 12.5-mm; packing L11

**Flow rate:** See the gradient table above.

**Injection size:** 200 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for ethinyl estradiol

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Sample solution A* and *Sample solution B*

[NOTE— Measure the peak heights for the major peaks obtained within 20 min. Use the response from *Sample solution A* for estrone and all other impurities. Use *Sample solution B* for 17β-ethinyl estradiol.]

Calculate the percentage of 17β-ethinyl estradiol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = height of any peak at the relative retention time of 1.16, using the spectrofluorometric detector

$r_S$  = peak height of ethinyl estradiol, using the spectrofluorometric detector

Calculate the percentage of estrone in the portion of Tablets taken:

$$\text{Result} = [(r_U/r_S) \times 100] - E$$

$r_U$  = height of any peak at the relative retention time of 1.2, using the UV detector at 210 nm

$r_S$  = peak height of ethinyl estradiol, using the UV detector at 210 nm

$E$  = percentage of 17β-ethinyl estradiol

Calculate the percentage of any other impurity:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = height of any peak other than those mentioned above

$r_S$  = peak height of ethinyl estradiol, using the UV detector at 210 nm

**Acceptance criteria**

17β-Ethinyl estradiol: NMT 0.5%

Estrone: NMT 0.5%

Any other impurity: NMT 0.5%

Total impurities: NMT 2.0 %

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- **USP REFERENCE STANDARDS** <11>

USP Ethinyl Estradiol RS

USP Norgestrel RS

**Ethiodized Oil Injection**

» Ethiodized Oil Injection is an iodine addition product of the ethyl ester of the fatty acids of poppyseed oil, containing not less than 35.2 percent and not more than 38.9 percent of organically combined iodine. It is sterile.

**Packaging and storage**—Preserve in well-filled, light-resistant, single-dose or multiple-dose containers.

**Identification**—Place 1 drop in a test tube, and heat directly in a flame: the violet color of iodine vapors is observed.

**Specific gravity** <841>: between 1.280 and 1.293, at 15°.

**Viscosity—Capillary Viscometer Methods** <911> and **Rotational Rheometer Methods** <912>: between 50 centipoises and 100 centipoises, at 15°.

**Sterility** <71>: meets the requirements.

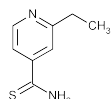
**Acidity**—Dissolve 1.0 mL in 10 mL of chloroform in a glass-stoppered cylinder, add phenolphthalein TS and 0.30 mL of sodium hydroxide solution (1 in 250), insert the stopper, and shake vigorously: a red color is produced.

**Free iodine**—Dissolve 1.0 mL in 5 mL of chloroform, add 20 mL of potassium iodide solution (1 in 20), agitate vigorously, and add 2 drops of starch TS: no blue color is produced.

**Assay**—[Caution—Observe rigorously the precautions set forth for *Procedure under Oxygen Flask Combustion* <471>.] Weigh accurately about 30 mg (1 drop) of Injection in a tared cellulose acetate capsule, and proceed as directed for *Procedure under Oxygen Flask Combustion* <471>, beginning with "Place the specimen," and using a thick-walled, 500-mL combustion flask. Use 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of freshly prepared sodium bisulfite solution (1 in 100) as the absorbing liquid. Pipet 1 mL of bromine-sodium acetate TS into the cup of the flask, loosen the stopper, and allow the solution to be sucked into the flask. Wash down the cup and the ground joint with water, insert the

stopper in the flask, shake it vigorously, then add 5 drops of formic acid, and again shake the flask. Remove the stopper, and rinse the stopper and the specimen holder with water, collecting the rinsings in the flask. Bubble nitrogen through the solution to displace all of the oxygen from the solution and the flask. Add 0.50 g of potassium iodide and 3 mL of 2 N sulfuric acid, allow the mixture to stand for 2 minutes, add 3 mL of starch TS, and titrate the liberated iodine with 0.05 N sodium thiosulfate VS. Each mL of 0.05 N sodium thiosulfate is equivalent to 1.058 mg of iodine (I).

## Ethionamide



$C_8H_{10}N_2S$  166.24

4-Pyridinecarbothioamide, 2-ethyl-

2-Ethylthioisonicotinamide [536-33-4].

» Ethionamide contains not less than 98.0 percent and not more than 102.0 percent of  $C_8H_{10}N_2S$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ethionamide RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—Test solution versus standard solution, prepared as directed in the Assay.

**Melting range:** (741): between 158° and 164°.

**pH** (791): between 6.0 and 7.0, in a 1 in 100 slurry in water.

**Water, Method I** (921): not more than 2.0%.

**Residue on ignition** (281): not more than 0.2%.

**Selenium** (291): 0.003%, a 200-mg test specimen being used.

**Assay**—Transfer about 100 mg of Ethionamide, accurately weighed, to a 250-mL volumetric flask, dissolve in about 100 mL of methanol, dilute with methanol to volume, and mix. Transfer a 5-mL aliquot to a 200-mL volumetric flask, dilute with methanol to volume, and mix (test solution). Dissolve a suitable quantity of USP Ethionamide RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 290 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of  $C_8H_{10}N_2S$  in the portion of Ethionamide taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Ethionamide RS in the Standard solution, and  $A_U$  and  $A_S$  are the absorbances of the test solution and the Standard solution, respectively.

## Ethionamide Tablets

» Ethionamide Tablets contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of  $C_8H_{10}N_2S$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ethionamide RS

**Identification**—

**A:** The solution of Tablets employed for measurement of absorbance in the Assay exhibits an absorbance maximum at  $290 \pm 2$  nm.

**B:** Digest a quantity of powdered Tablets, equivalent to about 1 g of ethionamide, with 50 mL of methanol, and filter through a medium-porosity, sintered-glass funnel. Evaporate the filtrate on a steam bath to dryness: the residue so obtained melts between 155° and 164°.

**Dissolution** (711)—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_8H_{10}N_2S$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ethionamide RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_8H_{10}N_2S$  is dissolved in 45 minutes.

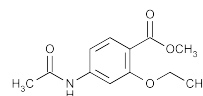
**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of ethionamide, to a medium-porosity, sintered-glass funnel that is fitted into a 250-mL suction flask. Extract the specimen by stirring it with successive 10-mL portions of methanol, using a total of about 100 mL of solvent, drawing off each portion of liquid with gentle suction before adding the next portion. Transfer the combined methanol extracts to a 250-mL volumetric flask, dilute with methanol to volume, and mix. Proceed as directed in the Assay under *Ethionamide*, beginning with "Transfer a 5-mL aliquot." Calculate the quantity, in mg, of  $C_8H_{10}N_2S$  in the portion of Tablets taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Ethionamide RS in the Standard solution, calculated on the anhydrous basis, and  $A_U$  and  $A_S$  are the absorbances of the solution of Ethionamide Tablets and the Standard solution, respectively.

## Ethopabate



$C_{12}H_{15}NO_4$  237.25

Benzoic acid, 4-(acetamido)-2-ethoxy-, methyl ester.

Methyl 4-acetamido-2-ethoxybenzoate [59-06-3].

» Ethopabate contains not less than 96.0 percent and not more than 101.0 percent of  $C_{12}H_{15}NO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Ethopabate RS

USP Ethopabate Related Compound A RS

Methyl-4-acetamido-2-hydroxybenzoate.

$C_{10}H_{11}NO_4$  209.20

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 10 µg per mL.

*Medium:* methanol.

**Loss on drying** (731)—Dry 1.0 g of it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Melting range** (741): between 146° and 151°.

**Residue on ignition** (281): not more than 0.5%.

**Chromatographic purity**—Examine the chromatogram of the *Assay preparation*, as obtained in the *Assay*, for peaks that elute at the following retention times in relation to ethopabate: 0.33, *p*-aminosalicylic acid (ethopabate related compound B); 0.64, methyl 2-ethoxy-4-aminobenzoate (ethopabate related compound C); 0.68, methyl 2-hydroxy-4-aminobenzoate (ethopabate related compound D); 0.9, methyl 4-acetamido-2-hydroxybenzoate (ethopabate related compound A); and 1.6, ethyl 4-acetamido-2-ethoxybenzoate (ethopabate related compound E). Calculate the percentage of diazotizable substances, represented by peaks for ethopabate related compounds B, C, and D, if present, by the formula:

$$(0.72r_B + 0.68r_C + 0.74r_D) / 0.01r_U$$

in which 0.72, 0.68, and 0.74 are the response factors of ethopabate related compounds B, C, and D, respectively, relative to that of ethopabate,  $r_B$ ,  $r_C$ , and  $r_D$  are the responses of the peaks observed for ethopabate related compounds B, C, and D, respectively, and  $r_U$  is the ethopabate peak response obtained from the *Assay preparation*: not more than 0.5% of diazotizable substances is found. Calculate the percentage of any other impurities by the formula:

$$100 - A_E - A_S$$

in which  $A_E$  is the percentage of total peak area represented by the main ethopabate peak in the chromatogram obtained from the *Assay preparation*, and  $A_S$  is the percentage of peak area represented by the sum of the peaks for ethopabate related compounds B, C, and D: not more than 2.0% of other impurities is found. [NOTE—Exclude from the total peak area the responses of any minor peaks that are 0.01% or less than that of the main ethopabate peak.]

**Assay**—

*Mobile phase*—Dissolve 3 g of sodium 1-hexanesulfonate in 1 L of water, and adjust with phosphoric acid to a pH of 2.5. Prepare a filtered and degassed mixture of this solution, methanol, and acetonitrile (450:150:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Prepare a mixture of methanol and water (50:50).

*Standard preparation*—Prepare a solution of USP Ethopabate RS in *Diluent* having a known concentration of about 0.4 mg per mL. If necessary, filter this solution through a filter having a porosity of 0.5 µm or finer, and use the fil-

trate as the *Standard preparation*. Use this solution on the day prepared.

*Assay preparation*—Transfer about 40 mg of Ethopabate, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Diluent*, and dissolve with the aid of sonication. Mix and dilute to volume with *Mobile phase*. If necessary, filter this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Assay preparation*. Use this solution on the day prepared.

*Resolution solution*—Prepare a solution in *Diluent* containing about 0.4 mg of USP Ethopabate RS and 0.1 mg of USP Ethopabate Related Compound A RS per mL.

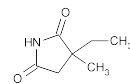
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 268-nm detector and a 3.9-mm × 30-cm column that contains packing L11 and is maintained at about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.9 for methyl 4-acetamido-2-hydroxybenzoate (ethopabate related compound A) and 1.0 for ethopabate, the column efficiency is not less than 4000 theoretical plates, the resolution, *R*, between the ethopabate related compound A peak and the ethopabate peak is not less than 1.2, and the tailing factor is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of  $C_{12}H_{15}NO_4$  in the portion of Ethopabate taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ethopabate RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the ethopabate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethosuximide



$C_7H_{11}NO_2$  141.17

2,5-Pyrrolidinedione, 3-ethyl-3-methyl-, (±)-.

(±)-2-Ethyl-2-methylsuccinimide. [77-67-8].

» Ethosuximide contains not less than 98.0 percent and not more than 101.0 percent of  $C_7H_{11}NO_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ethosuximide RS

**Identification**—

**A:** Infrared Absorption (197S)—

*Solution:* 1 in 15.

*Medium:* chloroform.

**Melting range** (741): between 47° and 52°.

**Water, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.5%.

**Limit of cyanide**—Dissolve 1 g in 10 mL of alcohol, and add 3 drops of ferrous sulfate TS, 1 mL of 1 N sodium hy-

dioxide, and a few drops of ferric chloride TS. Warm gently, and acidify with 2 N sulfuric acid: no blue precipitate or blue color is formed within 15 minutes.

**Limit of 2-ethyl-2-methylsuccinic acid and other impurities—**

*pH 3.0 Phosphate buffer, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve accurately weighed quantities of USP Ethosuximide RS and 2-ethyl-2-methylsuccinic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg of each per mL.

*Test solution*—Transfer about 1 g of Ethosuximide, accurately weighed, into a 10-mL volumetric flask, dissolve in *Mobile phase*, sonicating if necessary, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks with an area greater than 0.1% of the total area, except the ethosuximide peak. Calculate the percentage of 2-ethyl-2-methylsuccinic acid in the portion of Ethosuximide taken by the formula:

$$(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of 2-ethyl-2-methylsuccinic acid in the *Standard solution*; W is the weight, in g, of Ethosuximide taken to prepare the *Test solution*; and  $r_i$  and  $r_s$  are the peak responses of 2-ethyl-2-methylsuccinic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found. Calculate the percentage of any other impurity in the portion of Ethosuximide taken by the formula:

$$(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Ethosuximide RS in the *Standard solution*; W is the weight, in g, of Ethosuximide taken to prepare the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution* other than 2-ethyl-2-methylsuccinic acid; and  $r_s$  is the peak response for ethosuximide obtained from the *Standard solution*: not more than 0.1% of any other impurity is found; and not more than 0.5% of total impurities is found.

**Assay—**

*pH 3.0 Phosphate buffer*—Add 4.1 mL of phosphoric acid to 1000 mL of water, and adjust with sodium hydroxide TS to a pH of 3.0.

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 3.0 Phosphate buffer* and acetonitrile (90:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*System suitability solution*—Dissolve suitable quantities of 2-ethyl-2-methylsuccinic acid and USP Ethosuximide RS in an appropriate volume of *Mobile phase* to obtain a solution containing about 2 mg per mL and 10 mg per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ethosuximide RS in *Mobile phase*, and dilute with *Mobile phase* to obtain a solution having a known concentration of about 10.0 mg per mL.

*Assay preparation*—Transfer about 100 mg of Ethosuximide to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm  $\times$  30.0-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between 2-ethyl-

2-methylsuccinic acid and ethosuximide is not less than 6.6; the column efficiency determined from ethosuximide is not less than 2900 theoretical plates; the tailing factor for the ethosuximide peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 0.4%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_7H_{11}NO_2$  in the portion of Ethosuximide taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ethosuximide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethosuximide Capsules

» Ethosuximide Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_7H_{11}NO_2$ , present in the form of a solution of Ethosuximide in Polyethylene Glycol 400 or other suitable solvent.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Ethosuximide RS

**Identification**—Place a portion of the Capsule contents, equivalent to about 300 mg of ethosuximide, in a separator containing 50 mL of ether. Shake with three 10-mL portions of water, discarding the aqueous extracts. Add about 5 g of anhydrous sodium sulfate, swirl for 3 minutes, and filter through a small pledget of cotton that previously has been washed with ether, into a small flask. Evaporate the ether solution at room temperature in a current of air to dryness, and dissolve the residue in 5 mL of chloroform: the IR absorption spectrum of the solution, in the region between 3000  $cm^{-1}$  and 1650  $cm^{-1}$ , determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of a 1 in 15 solution of USP Ethosuximide RS in chloroform.

**Dissolution, Procedure for a Pooled Sample** <711>—

*Medium*: pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 1*: 50 rpm.

*Time*: 30 minutes.

*Mobile phase*—Prepare a degassed and filtered mixture of water and acetonitrile (80:20).

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Inject an accurately measured volume (about 50  $\mu$ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the amount of  $C_7H_{11}NO_2$  dissolved by comparison with a *Standard solution*, having a known concentration of USP Ethosuximide RS in the same *Medium*, similarly chromatographed.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_7H_{11}NO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of 2-ethyl-2-methylsuccinic acid—**

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of 2-ethyl-2-methylsuccinic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.026 mg per mL.

*Test solution*—Transfer 20 Capsules into a 2-liter volumetric flask, dissolve in 1800 mL of *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of 2-ethyl-2-methylsuccinic acid in the portion of Capsules taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of 2-ethyl-2-methylsuccinic acid in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of ethosuximide in the *Test solution*, based on the number of Capsules taken, the labeled quantity, in mg, of ethosuximide in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses for 2-ethyl-2-methylsuccinic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% is found.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (875:125:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve suitable quantities of USP Ethosuximide RS and 2-ethyl-2-methylsuccinic acid in *Mobile phase* to obtain a solution containing about 0.062 mg per mL and 0.064 mg per mL, respectively.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ethosuximide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.062 mg per mL.

*Assay preparation*—Transfer 20 Capsules, accurately weighed, into a 2-liter volumetric flask, dissolve in 1800 mL of *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL into a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between ethosuximide and 2-ethyl-2-methylsuccinic acid is not less than 3.5; the tailing factor is not more than 1.5; and the relative standard deviations for replicate injections determined from ethosuximide and 2-ethyl-2-methylsuccinic acid are not more than 2.0% and 5.0%, respectively.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ethosuximide ( $C_7H_{11}NO_2$ ) in the portion of each Capsule taken by the formula:

$$4000C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ethosuximide RS in the *Standard preparation*; and  $r_U$  and  $r_S$

are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethosuximide Oral Solution

» Ethosuximide Oral Solution contains not less than 90.0 percent and not more than 105.0 percent of the labeled amount of ethosuximide ( $C_7H_{11}NO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ethosuximide RS

**Identification—**

**A: Infrared Absorption** (197S)—

*Spectral range:* 3000  $cm^{-1}$  to 1650  $cm^{-1}$ .

*Test solution*—Transfer a portion of Oral Solution, equivalent to 150 mg of ethosuximide, to a 125-mL separatory funnel, add 50 mL of ether, and shake well. Allow the layers to separate, and retain the ether layer. Wash the ether layer with three 10-mL portions of water. Transfer the ether layer to a suitable beaker, add 5 g of anhydrous sodium sulfate, and swirl. Filter the mixture into a 50-mL volumetric flask through a small pledget of cotton that has been previously washed with ether. Evaporate to dryness. Dissolve the residue in 5 mL of chloroform.

*Standard solution*—Dissolve 150 mg of USP Ethosuximide RS in 5.0 mL of chloroform.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**pH** (791): between 4.5 and 5.8.

**Limit of 2-ethyl-2-methylsuccinic acid—**

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay under *Ethosuximide Capsules*.

*Standard solution*—Dissolve an accurately weighed quantity of 2-ethyl-2-methylsuccinic acid in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.05 mg per mL.

*Test solution*—Using a “to contain” pipet, transfer a volume of Oral Solution, equivalent to 250 mg of ethosuximide, to a 100-mL volumetric flask. Rinse the pipet several times with *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of 2-ethyl-2-methylsuccinic acid, in relation to the quantity of ethosuximide, in the volume of Oral Solution taken by the formula:

$$40C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of 2-ethyl-2-methylsuccinic acid in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses for 2-ethyl-2-methylsuccinic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 2.0% is found.

**Assay—**

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Ethosuximide Capsules*.

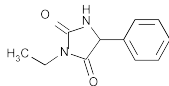
**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 250 mg of ethosuximide, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ethosuximide ( $C_7H_{11}NO_2$ ) in the volume of Oral Solution taken by the formula:

$$4000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ethosuximide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the ethosuximide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethotoin



$C_{11}H_{12}N_2O_2$  204.23  
2,4-Imidazolidinedione, 3-ethyl-5-phenyl-, ( $\pm$ )-.  
( $\pm$ )-3-Ethyl-5-phenylhydantoin [86-35-1].

» Ethotoin contains not less than 97.5 percent and not more than 102.0 percent of  $C_{11}H_{12}N_2O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ethotoin RS

USP 5-Phenylhydantoin RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 1 mg per mL.

*Medium:* alcohol.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it in vacuum at 60° for 4 hours; it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride**—Transfer 1.0 g of Ethotoin to a suitable separator, and dissolve in 50 mL of ether. Extract with three 15-mL portions of water, collect the combined extracts in a beaker, heat on a steam bath to expel any traces of ether, and allow to cool to room temperature. Transfer the solution to a 50-mL color-comparison tube, add 2 N nitric acid until the solution is acidic, add 1 mL of 2 N nitric acid in excess, mix, add 1 mL of silver nitrate TS, dilute with water to 50 mL, and allow to stand for 5 minutes, protected from direct sunlight. The turbidity produced does not exceed that of a solution prepared by mixing 2 mL of freshly prepared 0.002 N

hydrochloric acid, 1 mL of 2 N nitric acid, 1 mL of silver nitrate TS, and 46 mL of water (0.014%).

**Heavy metals, Method II** (231): 0.002%.

**Related compounds**—

**Buffer solution**—Dissolve about 1 g of monobasic sodium phosphate in 1 L of water. Adjust the solution with 1.5 M phosphoric acid to a pH of  $3.5 \pm 0.1$ .

**Diluent**—Prepare a mixture of *Buffer solution* and methanol (65:35).

**Solution A**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (80:20).

**Solution B**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (60:40).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Ethotoin RS, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 2.5  $\mu$ g of ethotoin per mL.

**Test solution**—Transfer about 50 mg of Ethotoin, accurately weighed, to a 200-mL volumetric flask, dissolve in about 100 mL of *Diluent* with sonication, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The column temperature is maintained at 40°. The flow rate is about 0.8 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–10	100	0	isocratic
10–30	100→0	0→100	linear gradient
30–40	0	100	isocratic
40–42	0→100	100→0	linear gradient
42–55	100	0	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0 for ethotoin; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—Discard any peak due to the *Diluent*.] Calculate the percentage of any impurity in the portion of Ethotoin taken by the formula:

$$(20,000/F)(C/W)(r_i / r_S)$$

in which C is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard solution*; F is the response factor for the impurity as shown in the table below; W is the weight, in mg, on the anhydrous basis, of the portion of Ethotoin taken;  $r_i$  is the peak area for any impurity in the *Test solution*; and  $r_S$  is the peak area for ethotoin in the *Standard solution*. The impurities meet the requirements given in the table below.

Compound name	RRT <sup>1</sup>	RRF <sup>2</sup>	Limit (%)
5-Phenylhydantoin	about 0.4	1.0	1.5
3-Methyl-5-phenylhydantoin	about 0.6	1.0	0.9
Ethotoin	1.0	—	—
Ethotoin/5-Phenylhydantoin dimer	about 1.9	1.0	0.3
Ethotoin dimer	about 2.5	0.58	0.4
Unknown impurities	—	1.0	0.1 Individual 1.0 Total Un- known
Total	—	—	2.0

<sup>1</sup>RRT—Relative retention time.<sup>2</sup>RRF—Relative response factor.**Assay—**

**Mobile phase**—Dissolve 0.65 g of monobasic potassium phosphate in 600 mL of water, adjust with phosphoric acid solution (1 in 10) to a pH of  $3.5 \pm 0.1$ , and dilute with water to 650 mL. Add 350 mL of methanol, mix, filter through a membrane filter of 0.5  $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**5-Phenylhydantoin stock solution**—Dissolve, with the aid of sonication if necessary, an accurately weighed quantity of USP 5-Phenylhydantoin RS in *Mobile phase* to obtain a solution having a known concentration of about 0.37 mg per mL.

**Standard preparation**—Dissolve, with the aid of sonication if necessary, an accurately weighed quantity of USP Ethotoin RS in *Mobile phase* to obtain a solution having a known concentration of about 0.25 mg of ethotoin per mL.

**System suitability solution**—Transfer 25 mg of USP Ethotoin RS, accurately weighed, to a 100-mL volumetric flask, add about 1.0 mL of *5-Phenylhydantoin stock solution*, add *Mobile phase* to volume, and sonicate to dissolve.

**Assay preparation**—Transfer about 50 mg of Ethotoin, accurately weighed, to a 200-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and sonicate to dissolve.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the 5-phenylhydantoin and ethotoin peaks is not less than 6.0. The relative retention times are about 0.4 for 5-phenylhydantoin and 1.0 for ethotoin. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{11}H_{12}N_2O_2$  in the portion of Ethotoin taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Ethotoin Tablets**

» Ethotoin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{11}H_{12}N_2O_2$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Ethotoin RS

USP Ethylparaben RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Dissolution** <711>—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 100 rpm.

**Time:** 60 minutes.

**Standard solution**—Transfer about 100 mg of USP Ethotoin RS, accurately weighed, to a 25-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add *Dissolution Medium* to volume, and mix.

**Procedure**—Determine the amount of  $C_{11}H_{12}N_2O_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 257 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with the *Standard solution*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{11}H_{12}N_2O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay—**

**Diluent**—Prepare a mixture of water, acetonitrile, and phosphoric acid (750:250:1).

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Prepare a solution of USP Ethylparaben RS in *Diluent* having a concentration of 0.02 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ethotoin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL. Immediately transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a suitable container, and mix well.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of ethotoin, to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, shake vigorously for 60 minutes, dilute with *Mobile phase* to volume, mix, and immediately filter. Without delay, transfer

5 mL of the filtrate and 5 mL of the *Internal standard solution* to a suitable container, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—For the purpose of identification, the relative retention times are about 0.5 for ethotoin and 1.0 for ethylparaben.] Calculate the quantity, in mg, of ethotoin ( $C_{11}H_{12}N_2O_2$ ) in the portion of Tablets taken by the formula:

$$200C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ethyl Chloride



$C_2H_5Cl$  64.51  
Ethane, chloro-  
Chloroethane [75-00-3].

» Ethyl Chloride contains not less than 99.5 percent and not more than 100.5 percent of  $C_2H_5Cl$ . [Caution—Ethyl Chloride is highly flammable. Do not use where it may be ignited.]

**Packaging and storage**—Preserve in tight containers, preferably hermetically sealed, and remote from fire.

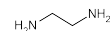
**Reaction**—Shake 10 mL with 10 mL of water, both having been previously cooled to 0°, and allow the supernatant layer of ethyl chloride to volatilize spontaneously: the remaining liquid is neutral to litmus.

**Limit of nonvolatile residue and odor**—Allow 5 mL to evaporate spontaneously from a tared, shallow dish: no foreign odor is perceptible while the last portions evaporate, and the weight of the residue is negligible.

**Chloride**—Add a few drops of silver nitrate TS to 10 mL of alcohol, cool to 0°, and add to the clear liquid about 500 µL of Ethyl Chloride cooled to the same temperature: no turbidity is produced immediately.

**Assay**—Introduce about 1.5 mL of cold Ethyl Chloride into a tared glass-stoppered pressure bottle containing 25.0 mL of 1 N alcoholic potassium hydroxide VS, rapidly replace the stopper, and weigh accurately. Tie down the stopper, insert the bottle in a wire basket, and immerse in a water bath at room temperature. [Caution—Before raising the bath temperature, take adequate precautions to cover the bottle or erect a suitable safety shield to prevent injury in case the bottle should burst.] Heat the water bath to boiling, maintain at this temperature for 30 minutes, and then cool gradually to room temperature before handling the bottle. Remove the stopper, add phenolphthalein TS, and titrate the excess alkali with 1 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* <541>). Each mL of 1 N alcoholic potassium hydroxide is equivalent to 64.51 mg of  $C_2H_5Cl$ .

## Ethylenediamine



$C_2H_8N_2$  60.10  
1,2-Ethanediamine.  
Ethylenediamine [107-15-3].

» Ethylenediamine contains not less than 98.0 percent and not more than 100.5 percent, by weight, of  $C_2H_8N_2$ .

[Caution—Use care in handling Ethylenediamine because of its caustic nature and the irritating properties of its vapor.]

NOTE—Ethylenediamine is strongly alkaline and may readily absorb carbon dioxide from the air to form a nonvolatile carbonate. Protect Ethylenediamine against undue exposure to the atmosphere.

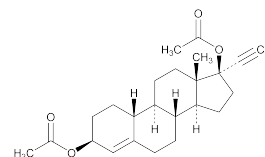
**Packaging and storage**—Preserve in well-filled, tight, glass containers.

**Identification**—To 2 mL of cupric sulfate solution (1 in 100) add 3 drops of a solution of Ethylenediamine (1 in 6), and shake: a purplish blue color is produced.

**Heavy metals, Method I** <231>—Evaporate 5.0 mL on a steam bath to dryness, add to the residue 1 mL of hydrochloric acid and 0.5 mL of nitric acid, and evaporate again to dryness. Dissolve the residue in 20 mL of warm water, cool, dilute with water to 100 mL, mix, and use 20 mL of this solution for the test: the limit is 0.002%.

**Assay**—Weigh accurately about 1 mL of Ethylenediamine in a tared, glass-stoppered flask containing about 25 mL of water. Dilute with water to about 75 mL, add a mixed indicator of bromocresol green TS and methyl red TS (5 in 6), mix, and titrate with 1 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 1 N hydrochloric acid is equivalent to 30.05 mg of  $C_2H_8N_2$ .

## Ethynodiol Diacetate



$C_{24}H_{32}O_4$  384.51  
19-Norpregn-4-en-20-yne-3,17-diol, diacetate, (3β,17α)-;  
19-Nor-17α-pregn-4-en-20-yne-3β,17-diol diacetate  
[297-76-7].

### DEFINITION

Ethynodiol Diacetate contains NLT 97.0% and NMT 102.0% of ethynodiol diacetate ( $C_{24}H_{32}O_4$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>

### ASSAY

- **PROCEDURE**

**Mobile phase:** Acetonitrile and water (41:59)

**Standard stock solution:** 2.5 mg/mL of USP Ethynodiol Diacetate RS, prepared as follows. Transfer a sufficient



amount of USP Ethynodiol Diacetate RS into a suitable volumetric flask and dissolve, by sonication, in a volume of acetonitrile equivalent to 50% of the flask volume. Dilute with water to volume.

**Standard solution:** 0.25 mg/mL of USP Ethynodiol Diacetate RS in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** 2.5 mg/mL of Ethynodiol Diacetate, prepared as follows. Transfer a sufficient amount of Ethynodiol Diacetate into a suitable volumetric flask and dissolve, by sonication, in a volume of acetonitrile equivalent to 50% of the flask volume. Dilute with water to volume.

**Sample solution:** 0.25 mg/mL of Ethynodiol Diacetate in *Mobile phase* from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 15-cm; packing L11

**Column temperature:** 40°

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for ethynodiol diacetate is NLT 18 min.]

#### Suitability requirements

**Tailing factor:** 0.75–2.0

**Relative standard deviation:** NMT 0.7%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of ethynodiol diacetate (C<sub>24</sub>H<sub>32</sub>O<sub>4</sub>) in the portion of Ethynodiol Diacetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ethynodiol Diacetate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ethynodiol Diacetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0%

#### IMPURITIES

##### • PROCEDURE 1

**Mobile phase, Sample stock solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the area percentages of the individual impurities in the portion of Ethynodiol Diacetate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area of each individual peak between the solvent front and the ethynodiol diacetate peak

$r_T$  = sum of the areas of all peaks appearing after the solvent front

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
α-Ethynodiol diacetate	0.87	1.5
Ethynodiol diacetate	1.0	—
Any other individual impurity	—	0.5
Total impurities	—	2.0

##### • PROCEDURE 2: LIMIT OF CONJUGATED DIENE

**Sample solution:** 0.5 mg/mL in methanol

**Blank:** Methanol

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** about 236 nm

**Cell:** 1 cm

**Acceptance criteria:** Absorbance is NMT 0.500.

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 10 mg/mL in chloroform

**Acceptance criteria:** −70° to −76°

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** <11>  
USP Ethynodiol Diacetate RS

## Ethynodiol Diacetate and Ethinyl Estradiol Tablets

#### DEFINITION

Ethynodiol Diacetate and Ethinyl Estradiol Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of ethynodiol diacetate (C<sub>24</sub>H<sub>32</sub>O<sub>4</sub>), and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>).

#### IDENTIFICATION

• The retention times of the ethynodiol diacetate and ethinyl estradiol peaks from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Methanol, acetonitrile, and water (3:7:10)

**Standard solution:** Dissolve, with the aid of sonication if necessary, quantities of USP Ethynodiol Diacetate RS and USP Ethinyl Estradiol RS in *Mobile phase*. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations, in mg/mL, of the Reference Standards, corresponding to about 1/25 of the labeled amounts of ethynodiol diacetate and ethinyl estradiol in the Tablets.

**Sample solution:** Place 10 Tablets in a 250-mL volumetric flask. Add a portion of *Mobile phase*, and sonicate until the Tablets are completely disintegrated. Cool to room temperature, dilute with *Mobile phase* to volume, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L10

**Flow rate:** 2 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates for ethynodiol diacetate

**Tailing factor:** NMT 1.5 for ethynodiol diacetate

**Relative standard deviation:** NMT 2.0% for each peak due to ethynodiol diacetate and ethinyl estradiol

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{24}H_{32}O_4$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of ethynodiol diacetate from the *Sample solution*  
 $r_S$  = peak response of ethynodiol diacetate from the *Standard solution*  
 $C_S$  = concentration of USP Ethynodiol Diacetate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of ethynodiol diacetate in the *Sample solution*

Calculate the percentage of  $C_{20}H_{24}O_2$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of ethinyl estradiol from the *Sample solution*  
 $r_S$  = peak response of ethinyl estradiol from the *Standard solution*  
 $C_S$  = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of ethinyl estradiol in the *Sample solution*

**Acceptance criteria:** 93.0%–107.0% of the labeled amount of  $C_{24}H_{32}O_4$  and 90.0%–110.0% of the labeled amount of  $C_{20}H_{24}O_2$

**PERFORMANCE TESTS**

- **DISINTEGRATION** <701>: 15 min, the use of disks being omitted
- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements for *Content Uniformity* with respect to ethynodiol diacetate and to ethinyl estradiol

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** <11>  
USP Ethinyl Estradiol RS  
USP Ethynodiol Diacetate RS

## Ethynodiol Diacetate and Mestranol Tablets

**DEFINITION**

Ethynodiol Diacetate and Mestranol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of ethynodiol diacetate ( $C_{24}H_{32}O_4$ ) and mestranol ( $C_{21}H_{26}O_2$ ).

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution A:** 5 mg/mL of USP Ethynodiol Diacetate RS in chloroform

**Standard solution B:** 0.5 mg/mL of USP Mestranol RS in chloroform

**Sample solution:** Transfer an amount equivalent to 10 mg of ethynodiol diacetate from powdered Tablets to a stoppered, 15-mL centrifuge tube. Add 10 mL of acetonitrile, insert the stopper in the tube, and mix by shaking and inversion for 2 min. Centrifuge at 1200 rpm for 10 min, and decant the supernatant through filter paper into a suitable container. Evaporate a 5-mL aliquot of the filtrate on a steam bath with the aid of a stream of nitrogen, and dissolve the residue in 1 mL of chloroform.

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent:** Cyclohexane and ethyl acetate (70:30)

**Spray reagent:** A 1-in-2 solution of sulfuric acid in water

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Remove the plate when the solvent has moved about 15 cm above the initial spots. Spray the plate with *Spray reagent*, and heat it at 80° for 10 min: the ethynodiol diacetate spots appear yellowish tan and the mestranol spots appear pink when viewed under white light.

**Acceptance criteria:** The ethynodiol diacetate and mestranol spots from the *Sample solution* have the same relative positions on the plate as the spots from the respective *Standard solutions*. The  $R_f$  values for ethynodiol diacetate and mestranol are 0.8 and 0.6, respectively.

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol and water (4:1)

**Mobile phase:** Methanol, tetrahydrofuran, and water (63:7:30)

**Standard solution:** 0.2 mg/mL of USP Ethynodiol Diacetate RS and 0.02 mg/mL of USP Mestranol RS in *Diluent*

**Sample solution:** Transfer 10 Tablets to a glass-stoppered flask, and pipet a sufficient volume of *Diluent* into the flask to obtain a solution having a final nominal concentration of 0.2 mg/mL of ethynodiol diacetate and 0.02 mg/mL of mestranol. Agitate vigorously until the Tablets are completely disintegrated. Shake vigorously by mechanical means for 30 min. Allow the solids to settle for 10 min, and pass the solution through a filter of 0.5- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 204 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L13

**Flow rate:** 1.2 mL/min

**Injection volume:** 25  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for mestranol and ethynodiol diacetate are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4.0 between mestranol and ethynodiol diacetate

**Relative standard deviation:** NMT 2.0% for both mestranol and ethynodiol diacetate

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethynodiol diacetate ( $C_{24}H_{32}O_4$ ) and mestranol ( $C_{21}H_{26}O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of the corresponding analyte from the *Sample solution*  
 $r_S$  = peak response of the corresponding analyte from the *Standard solution*  
 $C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of ethynodiol diacetate ( $C_{24}H_{32}O_4$ ) and mestranol ( $C_{21}H_{26}O_2$ )

**PERFORMANCE TESTS**• **DISINTEGRATION** <701>

**Time:** 15 min, omitting the use of disks

**Acceptance criteria:** Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** <905>

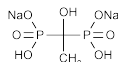
**Procedure for content uniformity:** Proceed as directed in the *Assay*, except reduce by half the concentrations in the *Standard solution* and the *Sample solution* and inject twice the volume into the chromatograph.

**Acceptance criteria:** Meet the requirements for *Content Uniformity* with respect to ethynodiol diacetate and to mestranol

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** <11>

USP Ethynodiol Diacetate RS

USP Mestranol RS

**Etidronate Disodium**

$C_2H_6Na_2O_7P_2$  249.99

Phosphonic acid, (1-hydroxyethylidene)bis-, disodium salt.  
Disodium dihydrogen (1-hydroxyethylidene)diphosphonate  
[7414-83-7].

» Etidronate Disodium contains not less than 97.0 percent and not more than 101.0 percent of  $C_2H_6Na_2O_7P_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Etidronate Disodium RS

USP Etidronate Disodium Related Compound A RS

Sodium phosphite dibasic pentahydrate.

$Na_2HPO_3 \cdot 5H_2O$  216.04 [CAS-13708-85-5].

USP Etidronic Acid Monohydrate RS

$C_2H_8O_7P_2 \cdot H_2O$  224.04

**Identification**—

**A: Infrared Absorption** <197>—The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of it, separately prepared from a test specimen recrystallized from water and dried at 105° for 1 hour, exhibit maxima in the regions of 4000 to 1350  $cm^{-1}$  and 1350 to 450  $cm^{-1}$ , respectively, only at the same wavelengths as those of similar preparations of USP Etidronate Disodium RS.

**B:** A solution (1 in 100) meets the requirements of the flame test for *Sodium* <191>.

**pH** <791>: between 4.2 and 5.2, in a solution (1 in 100).

**Water, Method Ic** <921>: not more than 5.0%, determined on a preparation containing 100 mg of finely powdered etidronate disodium in 10 mL of a mixture of acetic acid and formamide (1:1).

**Limit of phosphite**—

**Solution A**—Prepare an aqueous solution containing 0.65 mg per mL of anhydrous sodium carbonate and 0.40 mg per mL of sodium bicarbonate.

**Solution B**—Prepare an aqueous solution containing 4.68 mg per mL of anhydrous sodium carbonate and 2.89 mg per mL of sodium bicarbonate.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Dissolve suitable quantities of USP Etidronate Disodium Related Compound A RS and dibasic sodium phosphate in *Solution A* to obtain a solution having a known concentration of 0.016 mg of dibasic sodium phosphite on the anhydrous basis and 0.015 mg of dibasic sodium phosphate in each mL. [NOTE—Etidronate disodium related compound A is dibasic sodium phosphite pentahydrate.]

**Suppressor regenerant solution**—Use 12.5 mM sulfuric acid. [NOTE—This solution is needed only if the chemical suppression option is used.]

**Test solution**—Transfer approximately 50 mg of Etidronate Disodium, accurately weighed, to a suitable flask. Dissolve in 10.0 mL of *Solution A*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a conductivity detector, a 4-mm  $\times$  25-cm column and a 4-mm  $\times$  50-mm guard column both containing packing L61, and either a 4-mm anion self-regenerating suppressor or a suitable chemical suppressor. The flow rate is about 1.0 mL per minute for the *Mobile phase*. When a chemical suppressor is used, the flow rate is 3 to 5 mL per minute for the *Suppressor regenerant solution*. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6.0	100	0	isocratic
6.0–6.1	100→0	0→100	linear gradient
6.1–8.0	0	100	isocratic
8.0–8.1	0→100	100→0	linear gradient
8.1–15	100	0	isocratic

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the elution order is phosphite, followed by phosphate; the resolution,  $R$ , between phosphite and phosphate is not less than 2.5; and the relative standard deviation for replicate injections is not more than 10% for each peak.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the phosphite peaks. Calculate the percentage of phosphite, determined as monobasic sodium phosphite, in the portion of Etidronate Disodium taken by the formula:

$$100(103.98/125.96)(C/W)(r_U / r_S)$$

in which 103.98 and 125.96 are the molecular weights of monobasic sodium phosphite and dibasic sodium phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Etidronate Disodium Related Compound A RS on the anhydrous basis in the *Standard solution*;  $W$  is the weight, in mg, of Etidronate Disodium taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the phosphite peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% of phosphite, determined as monobasic sodium phosphite, is found.

**Heavy metals, Method II** <231>—Use 0.5 g of Etidronate Disodium for the *Test Preparation* and 2.5 mL of *Standard Lead Solution* for the *Standard Preparation*. Transfer the *Test Preparation* and the *Standard Preparation* to separate quartz crucibles, add 0.5 g of magnesium oxide to each crucible, and mix. Evaporate the *Standard Preparation* to dryness at 110° for 1 hour, and ignite each crucible over a flame to a light gray color. Ignite at 800° for 1 hour, cool, and dissolve the residues by the dropwise addition of hydrochloric acid, and add 3 mL of water. Adjust with ammonia TS to a pH of 8.5, then adjust with acetic acid to a pH of 4. Make a final pH adjustment to  $3.4 \pm 0.05$ , using dilute hydrochloric acid. Filter into 50-mL color comparison tubes, and dilute with water to 40 mL. The limit is 0.005%.

**Assay—**

**Mobile phase**—Prepare a 35 mM to 40 mM ammonium nitrate solution in water, and adjust with dilute ammonium hydroxide to a pH of 7.0.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Etidronic Acid Monohydrate RS in a mixture of 1 mL of 1 N sodium hydroxide solution and 150 mL of *Mobile phase*, to obtain a solution having a known concentration between 0.73 and 0.75 mg of etidronic acid monohydrate per mL.

**Assay preparation**—Transfer between 42.0 and 43.0 mg of Etidronate Disodium, accurately weighed, to a 50-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.6- × 150-mm column that contains packing L23. The column and the detector temperatures are maintained at 32°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_2H_6Na_2O_7P_2$  in the portion of Etidronate Disodium taken by the formula:

$$100(250.00/224.05)(C_s / C_u)(r_u / r_s)$$

in which 250.00 and 224.05 are the molecular weights of etidronate disodium and etidronic acid monohydrate, respectively;  $C_s$  is the concentration, in mg per mL, of USP Etidronic Acid Monohydrate RS in the *Standard preparation*;  $C_u$  is the concentration, in mg per mL, of Etidronate Disodium in the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## **Etidronate Disodium Tablets**

» Etidronate Disodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etidronate disodium ( $C_2H_6Na_2O_7P_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Etidronate Disodium RS

USP Etidronic Acid Monohydrate RS

$C_2H_6O_7P_2 \cdot H_2O$  224.04

**Identification, Infrared Absorption** (197)—The spectra of trifluorovinyl chloride polymer and mineral oil dispersions, separately prepared from finely powdered Tablets recrystallized from water and dried at 105° for 1 hour, exhibit maxima in the regions of 4000 to 1350  $cm^{-1}$  and 1350 to 450  $cm^{-1}$ , respectively, only at the same wavelengths as those of similar preparations of USP Etidronate Disodium RS.

**Dissolution** (711)—

**Medium:** water; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_2H_6Na_2O_7P_2$  dissolved by employing the following method.

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay under Etidronate Disodium*.

**Standard solution**—

FOR PRODUCTS LABELED TO CONTAIN 200 MG OF ETIDRONATE DISODIUM—Transfer approximately 20 mg of USP Etidronic Acid Monohydrate RS to a 100-mL volumetric flask, dissolve in 50 mL of water, add 2.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix.

FOR PRODUCTS LABELED TO CONTAIN 400 MG OF ETIDRONATE DISODIUM—Transfer approximately 20 mg of USP Etidronic Acid Monohydrate RS to a 50-mL volumetric flask, dissolve in 25 mL of water, add 2.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix.

**Test solution**—Transfer a portion of the solution under test to an HPLC vial.

**Procedure**—Separately inject equal volumes (about 100 µL for products labeled to contain 200 mg of Etidronate Disodium, and about 50 µL for products labeled to contain 400 mg of Etidronate Disodium) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities of  $C_2H_6Na_2O_7P_2$  dissolved, employing the procedure set forth in the *Assay*, making any necessary volumetric adjustments.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_2H_6Na_2O_7P_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay under Etidronate Disodium*.

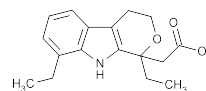
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 160 to 170 mg of Etidronate Disodium, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume. Agitate the solution for at least 5 minutes, and pass through a 0.45-µm nylon filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Etidronate Disodium*. Calculate the quantity, in mg, of etidronate disodium ( $C_2H_6Na_2O_7P_2$ ) in the portion of Tablets taken by the formula:

$$C_s V_u (250.00/224.05)(r_u / r_s)$$

in which 250.00 and 224.05 are the molecular weights of etidronate disodium and etidronic acid monohydrate, respectively;  $C_s$  is the concentration, in mg per mL, of USP Etidronic Acid Monohydrate RS in the *Standard preparation*;  $V_u$  is the volume, in mL, of the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## **Etodolac**



$C_{17}H_{21}NO_3$  287.35

Pyran[3,4-*b*]indole-1-acetic acid, 1,8-diethyl-1,3,4,9-tetrahydro-(±)-.

(±)-1,8-Diethyl-1,3,4,9-tetrahydropyrano [3,4-*b*]indole-1-acetic acid [41340-25-4].

» Etodolac contains not less than 98.0 percent and not more than 102.0 percent of  $C_{17}H_{21}NO_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.

$C_{16}H_{19}NO_3$  273.33

**Identification**, *Infrared Absorption* (197K).

**Water**, *Method I* (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Limit of chloride**—Dissolve 1.0 g of Etodolac in 60 mL of methanol, dilute with 10 mL of water, add 20 mL of 2 N nitric acid, and titrate with 0.01 N silver nitrate determining the endpoint potentiometrically (see *Titrimetry* (541)): the limit is not more than 0.3 mg per g.

**Heavy metals**, *Method II* (231): 0.001%.

**Limit of alcohol and methanol**—

*Internal standard stock solution*—Dissolve suitable quantities of isopropyl alcohol in dimethylformamide to obtain a solution containing about 2.5  $\mu$ L per mL.

*Internal standard solution*—Pipet 5.0 mL of *Internal standard stock solution* into a 100-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. This solution contains 0.125  $\mu$ L of isopropyl alcohol.

*Standard stock solution*—Transfer 5.0 mL of methanol and 5.0 mL of alcohol to a 200-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. Pipet 5.0 mL of this solution into a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

*Standard solution*—Pipet 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard stock solution* into a 100-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. This solution contains 0.101 mg of methanol and 0.099 mg of alcohol per mL.

*Test solution*—Transfer about 0.5 g of Etodolac, accurately weighed, to a 10-mL flask. Pipet 5.0 mL of *Internal standard solution* into the flask. Insert a stopper into the flask, and sonicate to dissolve.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm  $\times$  25-m fused silica capillary column coated with a 5- $\mu$ m film of phase G36. The carrier gas is helium, with a split flow rate of 50 mL per minute. The injection port temperature is maintained at 200°, and the detector is maintained at 300°. The column temperature is maintained at 45° for 5 minutes, then programmed to increase at the rate of 30° per minute to 280° and to maintain this temperature for 27 minutes. Chromatograph the *Standard solution* and the *Test solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for methanol, 0.8 for alcohol, and 1.0 for isopropyl alcohol; and the resolution, *R*, between peaks is not less than 1.

*Procedure*—Separately inject equal volumes (about 1  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Identify by their retention times any peaks present in the chromatogram obtained from the *Test solution* that correspond to those in the chromatogram obtained from the *Standard solution*. Calculate the percentages of  $C_2H_5OH$  and  $CH_3OH$  in the portion of Etodolac taken by the formula:

$$500(C/W)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of  $C_2H_5OH$  or  $CH_3OH$  in the *Standard solution*; *W* is the weight, in mg, of Etodolac taken to prepare the *Test solution*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak area ratios of the relevant analyte to the internal standard obtained from the *Test solution* and the *Stan-*

*dard solution*, respectively: not more than 0.1% of each is found.

**Chromatographic purity**—

*Solution A*—Mix 0.6 mL of phosphoric acid with 100 mL of water.

*Solution B*—Mix 0.6 mL of phosphoric acid with 100 mL of acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in acetonitrile to obtain a solution containing about 0.01 mg per mL and 0.2 mg per mL, respectively.

*Test solution*—Transfer about 25 mg of Etodolac, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0-5	60	40	isocratic
5-35	60→20	40→80	linear gradient
	60	40	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 3; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%.

*Procedure*—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Etodolac taken by the formula:

$$100(r_i/r_s)$$

in which *r<sub>i</sub>* is the peak response for each impurity; and *r<sub>s</sub>* is the sum of the responses of all of the peaks: not more than 0.5% of any individual impurity is found; and the total of all impurities is not more than 2.0%.

**Assay**—Dissolve about 230 mg of Etodolac in 60 mL of methanol. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 28.74 mg of  $C_{17}H_{21}NO_3$ .

## Etodolac Capsules

» Etodolac Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac ( $C_{17}H_{21}NO_3$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid. $C_{16}H_{19}NO_3$  273.33

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*: pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the amount of  $C_{17}H_{21}NO_3$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{21}NO_3$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500:500:0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Etodolac RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Prepare this solution fresh daily.

*System suitability solution*—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in *Mobile phase* to obtain a solution containing about 0.01 mg of etodolac related compound A and 0.2 mg of etodolac per mL.

*Assay preparation*—Weigh not fewer than 20 Capsules, and transfer the contents as completely as possible to a suitable container. Remove any residual powder from the empty capsules with the aid of a current of air, and weigh the capsule shells, determining the weight of the contents by difference. Mix the contents of the Capsules, and transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of etodolac, to a 500-mL volumetric flask, add 300 mL of *Mobile phase*, shake for 15 minutes, sonicate for 5 minutes, cool, dilute with *Mobile phase* to volume, and mix. Allow to settle for 10 minutes. Pipet 10.0 mL of the solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a 0.45- $\mu$ m or finer porosity, prior to use.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*; the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of etodolac ( $C_{17}H_{21}NO_3$ ) in the portion of Capsule contents taken by the formula:

$$5000C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the etodolac peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Etodolac Tablets**


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» Etodolac Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac ( $C_{17}H_{21}NO_3$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid. $C_{16}H_{19}NO_3$  273.33

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905): meet the requirements.

**Dissolution** (711)—

*Medium*: pH 6.8 Phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

*Apparatus 1*: 100 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the amount of  $C_{17}H_{21}NO_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 274 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{21}NO_3$  is dissolved in 30 minutes.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500:500:0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in *Mobile phase* to obtain a solution containing about 0.01 mg of etodolac related compound A and 0.2 mg of etodolac per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Etodolac RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Prepare this solution fresh daily.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of etodolac, to a 500-mL volumetric flask, add 300 mL of *Mobile phase*, shake for 15 minutes, sonicate for 5 minutes, cool, dilute with *Mobile phase* to volume, and mix. Allow to settle for 10 minutes. Pipet 10.0 mL of the solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a porosity of 0.45  $\mu$ m or less.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of etodolac (C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub>) in the portion of Tablets taken by the formula:

$$5000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the etodolac peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Etodolac Extended-Release Tablets

» Etodolac Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac (C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub>).

**Packaging and storage**—Preserve in well-closed containers. Store at controlled room temperature, protected from light.

**Labeling**—When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.

### USP Reference standards <11>—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.

C<sub>16</sub>H<sub>19</sub>NO<sub>3</sub> 273.33

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution <711>—

TEST 1—

*Medium*: 0.05 M phosphate buffer, pH 7.4; 1000 mL.

*Apparatus 2*: 75 rpm, with USP sinker.

*Times*: 3, 6, 10, and 16 hours.

**Procedure**—Determine the amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 279 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Etodolac RS in the same *Medium*. Use *Medium* as the blank.

**Tolerances**—The percentages of the labeled amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 15% and 40%
6	between 35% and 70%

Time (hours)	Amount dissolved
10	between 60% and 95%
16	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Medium*: 0.05 M phosphate buffer, pH 7.5; 1000 mL.

*Apparatus 2*: 100 rpm.

*Times*: 2, 4, 8, and 14 hours.

**Procedure**—Determine the amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved by comparing the difference between the absorbances of the filtered portions of the solution under test determined at 278 nm and 245 nm with the difference between the absorbances of a *Standard solution* having a known concentration of USP Etodolac RS in the same *Medium* determined at the same wavelengths. Use *Medium* as the blank, and use a 0.05-cm silica cell.

**Tolerances**—The percentages of the labeled amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 35%
4	between 30% and 55%
8	between 60% and 90%
14	not less than 85%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

*Medium*: 0.05 M phosphate buffer, pH 6.8; 1000 mL.

*Apparatus 2*: 75 rpm.

*Times*: 2, 4, 8, and 14 hours.

**Procedure**—Determine the amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on portions of the solution under test passed through a 10-µm polyethylene filter, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Etodolac RS in the same *Medium*. Use *Medium* as the blank, and use a 0.05-cm silica cell.

**Tolerances**—The percentages of the labeled amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 30% and 50%
8	between 55% and 75%
14	not less than 80%

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

*Medium*: 0.05 M phosphate buffer, pH 6.8; 900 mL.

*Apparatus 2*: 75 rpm, with a wire helix sinker.

*Times*: 2, 4, 8, and 18 hours.

**Test solution**—Pass a portion of the solution under test through a suitable 70-µm filter.

**Standard solution**—From a solution containing about 4 mg per mL of USP Etodolac RS in *Mobile phase*, make dilutions with *Medium* to obtain a solution with a final concentration of about *L*/1500 mg per mL, where *L* is the tablet label claim, in mg.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, and concentrated phosphoric acid (500:500:0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.5 mL per minute. The column is maintained at 25°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of etodolac dissolved at the times specified.

**Tolerances**—The percentages of the labeled amount of C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 20% and 45%
8	between 40% and 65%
18	not less than 80%

**Uniformity of dosage units** <905>: meet the requirements.

#### Chromatographic purity—

**Diluent, Mobile phase, and System suitability solution**—Proceed as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system**—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Inject a volume (about 10 μL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which *r<sub>i</sub>* is the peak area for each impurity, and *r<sub>s</sub>* is the sum of the areas of all the peaks: not more than 0.2% of any individual impurity is found; and not more than 0.75% of total impurities is found.

#### Assay—

**Diluent**—Use acetonitrile.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500:500:0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve accurately weighed quantities of USP Etodolac RS and USP Etodolac Related Compound A RS in *Diluent*, and quantitatively dilute with *Diluent* to obtain a solution having known concentrations of about 0.48 mg per mL and 0.05 mg per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Etodolac RS in *Diluent*, and quantitatively dilute with *Diluent* to obtain a solution having a known concentration of about 0.6 mg per mL.

**Assay preparation**—[NOTE—Do not finely powder Tablets.] Weigh and powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to

about 600 mg of etodolac, to a 200-mL volumetric flask. Add about 100 mL of *Diluent*, mix, and shake for 40 minutes by mechanical means. Dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45-μm porosity, discarding the first 3 mL of the filtrate. Transfer 2.0 mL of the filtrate to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

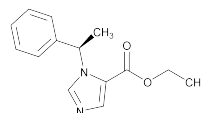
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 274-nm detector, a 4.0-mm × 4.0-cm guard column that contains 5-μm packing L7, and a 4.0-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2.5; and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of etodolac (C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub>) in the portion of Tablets taken by the formula:

$$1000C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Etomidate



C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> 244.29  
1*H*-Imidazole-5-carboxylic acid, 1-(1-phenylethyl)-, ethyl ester, (+)-;  
(+)-Ethyl 1-(α-methylbenzyl)imidazole-5-carboxylate  
[33125-97-2].

#### DEFINITION

Etomidate contains NLT 98.0% and NMT 102.0% of C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>, calculated on the dried basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 0.7 g/L of monobasic sodium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (2:3)

**Standard solution:** 0.16 mg/mL of USP Etomidate RS in acetonitrile

**Sample solution:** 0.16 mg/mL of Etomidate in acetonitrile



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; packing L1**Flow rate:** 2.3 mL/min**Injection size:** 20 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> in the portion of Etomidate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Etomidate RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Etomidate in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS, Method II** <231>: NMT 20 ppm

**Organic Impurities****• PROCEDURE****Solution A:** Dissolve 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid in 1 L of water.**Solution B:** Acetonitrile**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	30	70
21	95	5
30	95	5

**Diluent:** Weigh 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid into a 1000-mL volumetric flask. Add 500 mL of water, and shake to dissolve. Add 110 mL of acetonitrile and 50 mL of methanol, and dilute with water to volume.

**Standard solution:** 4 µg/mL of USP Metomidate Hydrochloride RS in *Diluent***Sensitivity solution:** 0.8 µg/mL of USP Metomidate Hydrochloride RS in *Diluent*, from the *Standard solution***System suitability solution:** 0.02 mg/mL of USP Etomidate RS and 0.02 mg/mL of USP Metomidate Hydrochloride RS in *Diluent***Sample solution:** 0.8 mg/mL of Etomidate in *Diluent***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; packing L1**Flow rate:** 2.0 mL/min**Injection size:** 50 µL**System suitability****Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution***Suitability requirements****Resolution:** NLT 2.0 between etomidate and metomidate, *System suitability solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Relative standard deviation:** NMT 3.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Etomidate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of metomidate from the *Standard solution* $C_S$  = concentration of metomidate hydrochloride in the *Standard solution* (mg/mL) $C_U$  = concentration of Etomidate in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of metomidate free base, 230.26 $M_{r2}$  = molecular weight of metomidate hydrochloride, 266.72**Acceptance criteria****Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any impurity peaks less than 0.05%.]**Total impurities:** NMT 1.0%**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Etomidate acid <sup>a</sup>	0.34	0.1
Metomidate <sup>b</sup>	0.90	0.1
Etomidate	1.0	—
Any unspecified impurity	—	0.1

<sup>a</sup> 1-(1-Phenylethyl)-1*H*-imidazole-5-carboxylic acid.<sup>b</sup> Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.**SPECIFIC TESTS**

- **LOSS ON DRYING, Method 1c** <731>: Dry 1 g of sample over phosphorus pentoxide for 16 h: it loses NMT 0.5% of its weight.
- **OPTICAL ROTATION, Specific Rotation** <781S>: 67.0° to 70.0° (t = 20°), calculated on the dried basis  
**Sample solution:** 10 mg/mL in dehydrated alcohol

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well closed, light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Etomidate RS  
USP Metomidate Hydrochloride RS  
Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate hydrochloride.  
C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub> · HCl 266.72

**Etomidate Injection****DEFINITION**

Etomidate Injection is a sterile, nonpyrogenic solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of etomidate (C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>). It may contain suitable buffers and preservatives.

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION** <197U>  
**Sample solution:** 10 µg/mL of etomidate in isopropyl alcohol  
**Medium:** Isopropyl alcohol
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** 0.7 g/L of monobasic sodium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (2:3)

**Standard solution:** 0.16 mg/mL of USP Etomidate RS in acetonitrile

**Sample solution:** 0.16 mg/mL of etomidate, from the Injection, in acetonitrile

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.3 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Etomidate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of etomidate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****Organic Impurities****• PROCEDURE 1: RELATED COMPOUNDS**

**Solution A:** Dissolve 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid in 1 L of water.

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	30	70
21	95	5
30	95	5

**Diluent:** Weigh 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid into a 1000-mL volumetric flask. Add 500 mL of water, and shake to dissolve. Add 110 mL of acetonitrile and 50 mL of methanol, and dilute with water to volume.

**Standard stock solution:** 0.1 mg/mL of USP Metomidate Hydrochloride RS in methanol

**Standard solution:** 4 µg/mL of USP Metomidate Hydrochloride RS in *Diluent*, from the *Standard stock solution*

**Sensitivity solution:** 0.8 µg/mL of USP Metomidate Hydrochloride RS in *Diluent*, from the *Standard solution*

**System suitability solution:** 0.02 mg/mL of USP Etomidate RS and 0.02 mg/mL of USP Metomidate Hydrochloride RS in *Diluent*

**Sample solution:** 0.8 mg/mL of etomidate, from the Injection, in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.0 mL/min

**Injection size:** 50 µL

**System suitability**

**Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between etomidate and metomidate, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of metomidate from the *Standard solution*

$C_S$  = concentration of USP Metomidate Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of etomidate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of metomidate free base, 230.26

$M_{r2}$  = molecular weight of metomidate hydrochloride, 266.72

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any impurity peaks less than 0.05%.]

**Total impurities:** NMT 1.6%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Etomidate acid <sup>a</sup>	0.34	1.4
Propylene glycol ester <sup>b</sup>	0.77	—
Metomidate <sup>c</sup>	0.90	0.1
Etomidate	1.0	—
Any unspecified impurity	—	0.1

<sup>a</sup> 1-(1-Phenylethyl)-1*H*-imidazole-5-carboxylic acid.

<sup>b</sup> This is quantitated in *Procedure 2: Total Propylene Glycol Ester*.

<sup>c</sup> Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.

- PROCEDURE 2: TOTAL PROPYLENE GLYCOL ESTER:** [NOTE—Perform this test if propylene glycol is used in the formulation.]

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 25 µg/mL of USP Etomidate RS in acetonitrile

**Sensitivity solution:** 1.6 µg/mL of USP Etomidate RS in acetonitrile

**Sample solution:** 1.6 mg/mL of etomidate, from the Injection, in acetonitrile

**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Tailing factor:** NMT 2.0, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Relative standard deviation:** NMT 3.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of etomidate from the *Standard solution* $C_S$  = concentration of USP Etomidate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of etomidate in the *Sample solution* (mg/mL) $F$  = relative response factor for each individual impurity (see *Impurity Table 2*)**Acceptance criteria****Individual impurities:** See *Impurity Table 2*. [NOTE—Disregard any impurity peaks less than 0.05%.]**Total impurities:** NMT 4.0%. [NOTE—Total impurities include all impurities from *Impurity Table 1* and *Impurity Table 2*.]**Impurity Table 2**

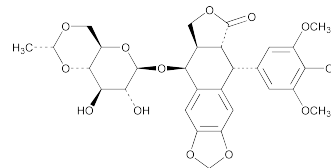
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total propylene glycol ester <sup>a</sup>	0.40	0.9	2.8
Etomidate	1.0	1.0	—
Any unspecified impurity	—	1.0	0.1

<sup>a</sup> 2-Hydroxypropyl ester and 2-hydroxy-1-methylethyl ester.**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 8.35 USP Endotoxin Units/mg of etomidate.
- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH** (791): Between 4.0 and 7.0
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* (1).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Endotoxin RS
  - USP Etomidate RS
  - USP Metomidate Hydrochloride RS
  - Methyl 1-(1-phenylethyl)-1H-imidazole-5-carboxylate hydrochloride.
  - $C_{13}H_{14}N_2O_2 \cdot HCl$  266.72

**Etoposide** $C_{29}H_{32}O_{13}$  588.56

Furo[3',4':6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one-, 9-[(4,6-O-ethylidene-β-D-glucopyranosyl)oxy]5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl), [5R-[5α,5aβ,8α,9β(R\*)]]-, 4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-D-glucopyranoside] [33419-42-0].

» Etoposide contains not less than 95.0 percent and not more than 105.0 percent of  $C_{29}H_{32}O_{13}$ , calculated on the anhydrous basis.**Caution**—Etoposide is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.**Packaging and storage**—Preserve in tight, light-resistant containers.**USP Reference standards** (11)—

USP Etoposide RS

USP Etoposide Resolution Mixture RS

**Identification**—**A:** *Infrared Absorption* (197K).**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Specific rotation** (781S): between  $-110^\circ$  and  $-118^\circ$  ( $t = 20^\circ$ ).**Test solution:** 5 mg per mL, in a mixture of chloroform and methanol (9:1).**Water**, *Method I* (921): not more than 6.0%.**Residue on ignition** (281): not more than 0.1%.**Heavy metals**, *Method II* (231): 0.002%.**Related compounds**—**Buffer solution**—Prepare as directed in the *Assay*.**Solution A**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (80:20).**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (60:40).**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).**Diluting solution**—Prepare a filtered mixture of 0.02 M sodium acetate, previously adjusted with acetic acid to a pH of 4.0, and acetonitrile (70:30).**Standard solution**—Dissolve an accurately weighed quantity of USP Etoposide RS in *Diluting solution* to obtain a *Standard stock solution* having a known concentration of about 2.0 mg per mL. Dilute this *Standard stock solution* quantitatively and stepwise with *Diluting solution* to obtain a *solution* having a known concentration of about 10 μg per mL.**System suitability solution**—Transfer about 20 mg of *n*-propylparaben, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with *Diluting solution* to volume. Transfer 5 mL of this solution and 5 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluting solution* to volume. Transfer 5.0 mL of this solu-

tion to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

**Test solution**—Transfer about 100 mg of Etoposide, accurately weighed, to a 50-mL volumetric flask, and dissolve in and dilute with *Diluting solution* to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains packing L11 having a diameter of less than 5 μm. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution* using *Solution A*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.20 for lignan P, 1.0 for etoposide, and 1.43 for picroetoposide; and the resolution, *R*, between propylparaben and etoposide is not less than 1.1. The chromatograph is programmed for *Procedure* as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–15	100	0	isocratic
15–30	100→40	0→60	linear gradient
30–40	40	60	isocratic
40–42	40→0	60→100	linear gradient
42–45	0	100	isocratic
45–47	0→100	100→0	linear gradient
47–50	100	0	re-equilibration

**Procedure**—Separately inject equal volumes (about 25 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least 40 minutes, and measure the peak responses. Calculate the percentages of lignan P and picroetoposide in the portion of Etoposide taken by the formula:

$$5000(C/W)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Etoposide RS in the *Standard solution*; *W* is the weight, in mg, of Etoposide taken to prepare the *Test solution*; *r<sub>i</sub>* is the peak response for each related compound obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response for etoposide obtained from the *Standard solution*: not more than 0.5% of lignan P and 1.0% of picroetoposide is found. Calculate the quantity of any other impurity observed in the chromatogram of the *Test solution* by the same formula: not more than 2.0% of all related compounds and other impurities is found.

#### Assay—

**Buffer solution**—Dissolve 5.44 g of sodium acetate in 2000 mL of water, adjust with glacial acetic acid to a pH of 4.0, and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (74:26). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Etoposide RS in acetonitrile to obtain a Standard stock solution having a known concentration of about 2.0 mg per mL. Transfer 5.0 mL of this Standard stock solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability solution**—Dissolve an accurately weighed quantity of USP Etoposide Resolution Mixture RS in *Mobile phase* to obtain a solution having a known concentration of 0.3 mg per mL.

**Assay preparation**—Transfer about 100 mg of Etoposide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the etoposide and α-etoposide peaks is not less than 1.35. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and allow the *Assay preparation* to elute for not less than 1.5 times the retention time of etoposide. Record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of C<sub>29</sub>H<sub>32</sub>O<sub>13</sub> in the portion of Etoposide taken by the formula:

$$500C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Etoposide RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the etoposide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Etoposide Capsules

» Etoposide Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etoposide (C<sub>29</sub>H<sub>32</sub>O<sub>13</sub>).

**Caution**—Etoposide is potentially cytotoxic. Great care should be taken to prevent inhaling particles of Etoposide and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers in a cold place. Do not freeze.

#### USP Reference standards <11>—

USP Etoposide RS

USP Etoposide Resolution Mixture RS

#### Identification—

**A: Infrared Absorption** <197K>—

**Test specimen**—Transfer a suitable quantity of the contents of Capsules, equivalent to about 100 mg of etoposide, to a separator containing 100 mL of water. Extract twice with 20-mL portions of chloroform, separate and combine the organic layer, dry over anhydrous sodium sulfate, and filter. Transfer the dried filtrate to a second separator, extract with 30 mL of water, and allow the layers to separate. Drain the chloroform layer through a bed of anhydrous sodium sulfate contained in a filter funnel into a round bottom flask, and evaporate the chloroform at a temperature of 30 ± 5° using a rotary evaporator. Dissolve the oily residue obtained in 5 mL of water, shake gently, and allow to stand for 30 minutes. Filter, collecting the precipitate formed on a glass filter funnel, wash the precipitate with three 20-mL portions of water, and allow the precipitate to dry on the filter for about 90 minutes in a vacuum oven at 40°. Prepare a dispersion of the precipitate in KBr at a ratio 1 in 100.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution <711>—

**Medium:** pH 4.5 acetate buffer; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of C<sub>29</sub>H<sub>32</sub>O<sub>13</sub> dissolved by employing the following method.

**Buffer solution**—Dissolve 5.44 g of sodium acetate in 2000 mL of water, adjust with acetic acid to a pH of 4.0, and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (74:26). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve, with sonication, an accurately weighed quantity of USP Etoposide RS in a volume of methanol equivalent to 2% of the final solution volume. Dilute with *Dissolution Medium* to obtain a solution having a known concentration of about 55 µg per mL, and mix.

**Test solution**—At 30 minutes withdraw a 10-mL aliquot from the dissolution vessel.

**Chromatographic system**—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the filtered portions of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Determine the amount of C<sub>29</sub>H<sub>32</sub>O<sub>13</sub> dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of C<sub>29</sub>H<sub>32</sub>O<sub>13</sub> is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—Proceed as directed in the test for *Related compounds* under *Etoposide*: not more than 2.0% of picroetoposide is found; and not more than 3.0% of total impurities is found.

#### Assay—

*Buffer solution, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Etoposide*.

**Assay preparation**—Transfer an accurately counted number of Capsules, equivalent to about 500 mg of etoposide, to a 500-mL volumetric flask, add about 400 mL of *Mobile phase*, and stir using a magnetic bar for about 15 minutes, followed by sonication for about 1 hour with occasional shaking. Cool, dilute with *Mobile phase* to volume, stir for an additional 5 minutes, and filter. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Etoposide*. Calculate the quantity, in mg, of etoposide (C<sub>29</sub>H<sub>32</sub>O<sub>13</sub>) per Capsule by the formula:

$$2500(C/N)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Etoposide RS in the *Standard preparation*; N is the number of Capsules taken; and  $r_U$  and  $r_S$  are the etoposide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Etoposide Injection

» Etoposide Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etoposide (C<sub>29</sub>H<sub>32</sub>O<sub>13</sub>) in a sterile solution in a nonaqueous medium intended for dilution with a suitable parenteral vehicle prior to intravenous infusion.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers of Type I glass.

**Labeling**—Label it to indicate that it must be diluted with suitable parenteral vehicle prior to intravenous infusion.

#### USP Reference standards (11)—

USP Etoposide RS

USP Etoposide Resolution Mixture RS

USP Endotoxin RS

#### Identification—

**A: Diluting solution**—Prepare a mixture of chloroform and methanol (9:1).

**Spray reagent**—Add 10 mL of sulfuric acid with cooling and stirring to 70 mL of dehydrated alcohol in a 100-mL volumetric flask. Dilute with dehydrated alcohol to volume, and mix.

**Standard solution**—Dissolve USP Etoposide RS in *Diluting solution* to obtain a solution having a known concentration of about 0.8 mg per mL.

**Test solution**—Transfer a volume of Injection, equivalent to about 20 mg of etoposide, to a 25-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

**Procedure**—Apply separately 10 µL each of the *Standard solution* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the plate in a suitable chromatographic chamber containing a developing solvent consisting of a mixture of chloroform, acetone, alcohol, and water (80:25:2.5:0.5). Allow the chromatogram to develop until the solvent front has moved about 17 cm from the origin. Remove the plate, and allow it to air-dry in a fume hood for 5 minutes. Replace the plate in the tank and develop again to a distance of about 17 cm from the origin. Remove the plate and air-dry it in a fume hood for about 20 minutes. Spray the plate with the *Spray reagent* and heat in a forced-air oven at 120° for about 15 minutes: the principal spot from the *Test solution* corresponds in appearance and  $R_f$  value to that from the *Standard solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** (791): between 3.0 and 4.0 in a solution prepared by diluting 5.0 mL of it with 45 mL of water.

**Bacterial endotoxins** (85)—Use a test solution, prepared by diluting the Injection with Sterile Water for Injection to obtain a concentration of 0.31 mg etoposide activity per mL: it contains not more than 2.0 USP Endotoxin Units per mg of etoposide.

**Alcohol content, Method II** (611) (if present): between 90.0% and 110.0% of the labeled amount of C<sub>2</sub>H<sub>5</sub>OH, *n*-propyl alcohol being used as the internal standard.

#### Benzyl alcohol content (if present)—

*Buffer solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Etoposide*.

**Standard preparation**—Transfer 0.75 mL of freshly distilled benzyl alcohol, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test preparation**—Use the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the benzyl alcohol peaks. Calculate the

quantity, in mg per mL, of benzyl alcohol in the volume of Injection taken by the formula:

$$500(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of benzyl alcohol in the *Standard preparation*; V is the volume of Injection taken; and  $r_U$  and  $r_S$  are the peak responses of benzyl alcohol obtained from the *Test preparation* and the *Standard preparation*, respectively: between 90.0% and 110.0% of the labeled amount is found.

**Related compounds**—Proceed as directed in the *Related compounds* test under *Etoposide*. Not more than 3.0% total impurities is found.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

*Buffer solution*, *Mobile phase*, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Etoposide*.

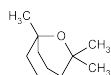
*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of etoposide, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into another 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Etoposide*. Calculate the quantity, in mg, of etoposide ( $C_{29}H_{32}O_{13}$ ) in each mL of the Injection taken by the formula:

$$500(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Etoposide RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the responses of the etoposide peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Eucalyptol



$C_{10}H_{18}O$  154.25  
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane;  
1,8-Epoxy-*p*-menthane [470-82-6].

#### DEFINITION

Eucalyptol is obtained from oil of eucalyptus and from other sources. It contains NLT 98.0% and NMT 100.0% of  $C_{10}H_{18}O$ .

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197F)
- **B.** Add 1 mL of phosphoric acid to 1 mL of Eucalyptol contained in a test tube maintained in an ice bath. A solid white crystalline mass is formed, from which eucalyptol separates upon addition of warm water.

#### ASSAY

##### • PROCEDURE

**System suitability solution:** 0.2 mg/mL of limonene and 0.9 mg/mL of USP Eucalyptol RS in methanol

**Sample solution:** 0.9 mg/mL of Eucalyptol in methanol

**Blank:** Methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 60-m fused-silica capillary column coated with phase G16

**Temperature**

**Injector port:** 250°

**Detector:** 250°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	6	200	—

**Split flow rate:** 50 mL/min

**Carrier gas:** Helium

**Column head pressure:** 30 psi

**Injection size:** 1 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between limonene and eucalyptol

**Column efficiency:** NLT 150,000 theoretical plates, eucalyptol

**Analysis**

**Samples:** *Sample solution* and *Blank*

[NOTE—Identify any peaks from the *Sample solution* that correspond to those in the *Blank* by their retention times.]

Calculate the percentage of eucalyptol ( $C_{10}H_{18}O$ ) in the portion of Eucalyptol taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak response of eucalyptol from the *Sample solution*

$r_T$  = sum of all peak responses from the *Sample solution*, other than the responses corresponding to those of the *Blank*

**Acceptance criteria:** 98.0%–100.0%

#### IMPURITIES

##### • LIMIT OF PHENOLS

**Analysis 1:** Shake 5 mL with 5 mL of sodium hydroxide TS.

**Acceptance criteria 1:** The volume of Eucalyptol is not diminished.

**Analysis 2:** Shake 1 mL with 20 mL of water and allow the liquids to separate. To 10 mL of the water layer add 1 drop of ferric chloride TS.

**Acceptance criteria 2:** The mixture develops no violet color.

#### SPECIFIC TESTS

- **SPECIFIC GRAVITY** (841): 0.921–0.924
- **CONGEALING TEMPERATURE** (651): NLT 0°
- **OPTICAL ROTATION**, *Angular Rotation* (781A):  $-0.5^\circ$  to  $+0.5^\circ$
- **DISTILLING RANGE**, *Method I* (721): 174°–177°
- **REFRACTIVE INDEX** (831): 1.455–1.460 at 20°

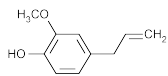
#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Eucalyptol RS

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**Eugenol**

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 $C_{10}H_{12}O_2$ 

164.20

Phenol, 2-methoxy-4-(2-propenyl)-;  
4-Allyl-2-methoxyphenol [97-53-0].

**DEFINITION**

Eugenol is obtained from clove oil and from other sources.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197F)

**IMPURITIES**

- **HEAVY METALS**, *Method II* (231): NMT 40 ppm
- **HYDROCARBONS**

**Analysis:** Dissolve 1 mL in 20 mL of 0.5 N sodium hydroxide in a 50-mL stoppered tube, add 18 mL of water, and mix.

**Acceptance criteria:** A clear mixture results immediately, but it may become turbid when exposed to air.

- **LIMIT OF PHENOL**

**Analysis:** Shake 1 mL with 20 mL of water. Filter, and add 1 drop of ferric chloride TS to 5 mL of the clear filtrate.

**Acceptance criteria:** The mixture exhibits a transient grayish-green color but not a blue or violet color.

**SPECIFIC TESTS**

- **SPECIFIC GRAVITY** (841): 1.064–1.070
- **DISTILLING RANGE**, *Method II* (721): NLT 95% distills from 250°–255°
- **REFRACTIVE INDEX** (831): 1.540–1.542 at 20°
- **SOLUBILITY IN 70% ALCOHOL:** One volume dissolves in 2 volumes of 70% alcohol.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Eugenol RS

## Factor IX Complex

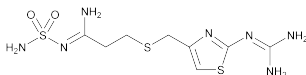
» Factor IX Complex conforms to the regulations of the federal Food and Drug Administration concerning biologics (see *Biologics* <1041>). It is a sterile, freeze-dried powder consisting of partially purified Factor IX fraction, as well as concentrated Factors II, VII, and X fractions, of venous plasma obtained from healthy human donors. It contains no preservative. It meets the requirements of the test for potency in having not less than 80 percent and not more than 120 percent of the potency stated on the label in Factor IX Units by comparison with the U.S. Factor IX Standard or with a working reference that has been calibrated with it.

**Packaging and storage**—Preserve in hermetic containers in a refrigerator.

**Expiration date**—The expiration date is not later than 2 years from the date of manufacture.

**Labeling**—Label it with a warning that it is to be used within 4 hours after constitution, and to state that it is for intravenous administration and that a filter is to be used in the administration equipment.

## Famotidine



$C_8H_{15}N_7O_2S_3$  337.45

Propanimidamide, *N*'-(aminosulfonyl)-3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-; [1-Amino-3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]propylidene]sulfamide; 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*'-sulfamoylpropanimidamide [76824-35-6].

### DEFINITION

Famotidine contains NLT 98.5% and NMT 101.0% of famotidine ( $C_8H_{15}N_7O_2S_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>

### ASSAY

#### • PROCEDURE

**Sample:** Dissolve 250 mg of Famotidine in 80 mL of glacial acetic acid.

**Analysis:** Titrate with 0.1 N perchloric acid VS (see *Titrimetry* <541>), using a suitable anhydrous electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 16.87 mg of  $C_8H_{15}N_7O_2S_3$ .

**Acceptance criteria:** 98.5%–101.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS**, *Method II* <231>: NMT 10 ppm

### Change to read:

#### • ORGANIC IMPURITIES

**Buffer:** 1.882 g/L of sodium 1-hexanesulfonate in water, adjusted with acetic acid to a pH of 3.5

**Solution A:** Acetonitrile, methanol, and *Buffer* (94:6:900)

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*. [NOTE—If necessary, adjust the *Mobile phase* to achieve a retention time of 19–23 min for the famotidine peak and a maximum of 48 min for the famotidine related compound E peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	1
23	96	4	1
27	96	4	2
47	78	22	2
48	100	0	2
54	100	0	1

• **Standard stock solution:** 0.5 mg/mL of USP Famotidine RS in *Solution A*

**Standard solution:** 0.5 µg/mL of USP Famotidine RS in *Solution A*

**System suitability stock solution:** 0.25 mg/mL of USP Famotidine Related Compound D RS in methanol

**System suitability solution:** Transfer 1 mL of the *System suitability stock solution* and 0.5 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with *Solution A* to volume. • (ERR 1-Jul-2012)

**Sample solution:** 0.5 mg/mL of Famotidine in *Solution A*

**Identification solution:** 0.5 mg/mL of USP Famotidine RS and 1.5 µg/mL of each of USP Famotidine Related Compound B RS, USP Famotidine Related Compound C RS, USP Famotidine Related Compound D RS, USP Famotidine Related Compound E RS, and USP Famotidine Related Compound F RS in *Solution A*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 50°

**Flow rate:** See *Table 1*.

**Injection volume:** 20 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between famotidine and famotidine related compound D

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Identification solution*

Chromatograph the *Identification solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.

Calculate the percentage of each impurity in the portion of Famotidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of famotidine from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)



$C_U$  = concentration of Famotidine in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

#### Acceptance criteria

Individual impurities: See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Famotidine	1.0	—	—
Famotidine related compound D <sup>a</sup>	1.1	1.0	0.3
Famotidine related compound C <sup>b</sup>	1.2	0.53	0.3
Famotidine cyanoamidine <sup>c</sup>	1.4	0.71	0.2
Famotidine related compound F <sup>d</sup>	1.5	0.59	0.1
Famotidine amidine <sup>e</sup>	1.6	1.0	0.2
Famotidine related compound B <sup>f</sup>	2.0	0.40	0.3
Famotidine related compound E <sup>g</sup>	2.1	1.0	0.3
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

<sup>a</sup> Famotidine propanamide.

<sup>b</sup> Famotidine sulfamoyl propanamide.

<sup>c</sup> *N*-Cyano-3-[[2-(diaminomethyleneamino)thiazol-4-yl]methylthio]propanimidamide.

<sup>d</sup> Famotidine propionic acid.

<sup>e</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanimidamide.

<sup>f</sup> Famotidine dimer.

<sup>g</sup> Famotidine disulfide.

#### SPECIFIC TESTS

- **LOSS ON DRYING (731):** Dry a sample at a pressure not exceeding 5 mm of mercury at 80° for 5 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Famotidine RS

USP Famotidine Related Compound B RS

3,5-Bis[2-[[2-[(diaminomethyleneamino)thiazol-4-yl]methylthio]ethyl]-4*H*-1,2,4,6-thiatriazine 1,1-dioxide.

$C_{16}H_{23}N_{11}O_2S_5$  561.73

USP Famotidine Related Compound C RS

3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*-sulfamoylpropanamide hydrochloride.

$C_8H_{15}ClN_6O_3S_3$  374.88

USP Famotidine Related Compound D RS

3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

$C_8H_{13}N_5OS_2$  259.35

USP Famotidine Related Compound E RS

2,2'-[4,4'-Disulfanediy]bis(methylene)bis(thiazole-4,2-diyl)]diguanidine.

$C_{10}H_{14}N_8S_4$  374.53

USP Famotidine Related Compound F RS

3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.

$C_8H_{12}N_4O_2S_2$  260.34

## Famotidine Injection

### DEFINITION

Famotidine Injection is a sterile, concentrated solution of Famotidine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ). It may contain suitable preservatives.

### IDENTIFICATION

- **A.** The retention time of the famotidine peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 13.8 g/L of monobasic sodium phosphate

**Mobile phase:** Methanol, water, and *Buffer* (5:32:3). Adjust with 1 N sodium hydroxide to a pH of 5.3.

**Diluent:** Dissolve 1.36 g of monobasic potassium phosphate in 800 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.0, and dilute with water to 1 L.

#### Standard solution

**If benzyl alcohol is present:** 0.1 mg/mL of USP Famotidine RS and 0.09 mg/mL of USP Benzyl Alcohol RS in *Diluent*

**If benzyl alcohol is not present:** 0.1 mg/mL of USP Famotidine RS in *Diluent*

**Sample solution:** Transfer a volume of Injection, equivalent to 20 mg of famotidine based on the label claim, to a 200-mL volumetric flask, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L3

**Flow rate:** 1 mL/min

**Injection size:** 30 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0% for the famotidine peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of famotidine from the *Sample solution*

$r_S$  = peak response of famotidine from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of famotidine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### • CONTENT OF BENZYL ALCOHOL (if present)

**Buffer, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability stock solution:** Proceed as directed in the *Organic Impurities* test.

**System suitability solution:** Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask. Add 1 drop (approximately 20 mg) of USP Benzyl Alcohol RS, and dilute with *Diluent* to volume.

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—See Table 1 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.3 between adjacent peaks of benzyl alcohol and famotidine propionic acid; the benzyl alcohol peak is resolved from the solvent front, *System suitability solution*

**Relative standard deviation:** Less than 2.0% for each peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of benzyl alcohol from the *Sample solution*

$r_S$  = peak area of benzyl alcohol from the *Standard solution*

$C_S$  = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

**Acceptance criteria:** The content of benzyl alcohol meets the requirements under *Injections* (1), *Added Substances*.

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, Diluent, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability stock solution:** Transfer 10 mg of USP Famotidine RS to a 50-mL volumetric flask. Add 1 mL of 0.1 N hydrochloric acid. Heat at 80° for 30 min. Allow to cool, add 2 mL of 0.1 N sodium hydroxide, and heat at 80° for an additional 30 min. Allow to cool, and neutralize by adding 1 mL of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume (*Solution A*). Transfer 5 mg of USP Famotidine RS to a separate 50-mL volumetric flask, add 8 mL of methanol, and sonicate to dissolve. Add 10 mL of *Solution A*, and dilute with *Diluent* to volume.

#### System suitability solution

**If benzyl alcohol is present:** Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask. Add 1 drop (approximately 20 mg) of USP Benzyl Alcohol RS, and dilute with *Diluent* to volume.

**If benzyl alcohol is not present:** Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See Table 1 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.3 between adjacent peaks of famotidine propionic acid, famotidine sulfamoyl propanamide, famotidine, and famotidine propanamide for each pair of peaks

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of the total of famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = sum of the peak areas for famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide from the *Sample solution*

$r_T$  = sum of the peak areas for famotidine, famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide from the *Sample solution*

#### Acceptance criteria

**Total impurities:** NMT 5.0%

Table 1

Name	Relative Retention Time
Benzyl alcohol (if present)	0.4
Famotidine propionic acid (famotidine related compound F) <sup>a</sup>	0.7
Famotidine sulfamoyl propanamide (famotidine related compound C) <sup>b</sup>	0.8
Famotidine	1.0
Famotidine propanamide (famotidine related compound D) <sup>c</sup>	1.3

<sup>a</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.

<sup>b</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-N-sulfamoyl-propanamide.

<sup>c</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

### SPECIFIC TESTS

• **STERILITY TESTS** (71): Meets the requirements

• **PH** (791): 5.0–5.6

• **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

• **BACTERIAL ENDOTOXINS TEST** (85): NMT 16.67 USP Endotoxin Units/mg of famotidine

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* (1), *Container Content*.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a refrigerator.

• **LABELING:** It meets the requirements under *Injections* (1), *Labeling*. Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to administration. Label it to indicate the name and the quantity of any added preservative.

• **USP REFERENCE STANDARDS** (11)

USP Benzyl Alcohol RS  
USP Endotoxin RS  
USP Famotidine RS

## Famotidine for Oral Suspension

### DEFINITION

Famotidine for Oral Suspension contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of famotidine (C<sub>8</sub>H<sub>15</sub>N<sub>7</sub>O<sub>2</sub>S<sub>3</sub>) when constituted as directed. It contains one or more suitable buffers, colors, diluents, flavors, and preservatives.

### IDENTIFICATION

• **A.** The retention time of the famotidine peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer A:** Dissolve 13.6 g of sodium acetate trihydrate in 900 mL of water, and adjust with glacial acetic acid to a pH of 6.0 ± 0.1, before final dilution to 1 L.

**Buffer B:** Dissolve 13.6 g of monobasic sodium phosphate in 900 mL of water, adjust with 1 M sodium hydroxide to a pH of  $7.0 \pm 0.1$ , and dilute with water to 1 L.

**Solution A:** Acetonitrile and *Buffer A* (7:93)

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
42	52	48
43	100	0
45	100	0

**Diluent:** Acetonitrile and *Buffer B* (7:93)

**Standard solution:** 0.16 mg/mL of USP Famotidine RS in *Diluent*

**Sample solution:** Transfer to a 100-mL volumetric flask a portion of Famotidine for Oral Suspension, equivalent to about 40 mg of famotidine, freshly mixed and free from air bubbles and constituted as directed in the labeling. Add 10 mL of methanol, sonicate for 5 min, add 70 mL of *Diluent*, sonicate for an additional 5 min, and dilute with *Diluent* to volume. Dilute 10.0 mL of this solution with *Diluent* to 25.0 mL, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 268 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** Greater than 2000 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) in the portion of Famotidine for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of famotidine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements for *Content Uniformity*. For the product in multi-dose containers, the unit is a 5-mL aliquot of the suspension, constituted as directed in the labeling.

#### IMPURITIES

##### ORGANIC IMPURITIES

**Mobile phase, Diluent, Standard solution, Sample solution and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability stock solution:** Transfer 16 mg of famotidine to a 50-mL volumetric flask. Dissolve in 1.0 mL of 1 N hydrochloric acid, heat at 80° for 30 min, and cool to room temperature. Add 2.0 mL of 1 N

sodium hydroxide, heat at 80° for 30 min, and cool to room temperature. Add 1.0 mL of 1 N hydrochloric acid to neutralize, and dilute with *Diluent* to volume.

**System suitability solution:** Transfer 16 mg of famotidine to a 50-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate to dissolve. Add 5 drops of hydrogen peroxide solution, heat at 80° for 15 min, and cool to room temperature. Add 20 mL of *System suitability stock solution*, and dilute with *Diluent* to volume.

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Identify the components of the *System suitability solution* based on the relative retention times listed in *Table 2*.]

#### Suitability requirements

**Resolution:** Greater than 1.5 between famotidine and famotidine propanamide, *System suitability solution*

**Column efficiency:** Greater than 2000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the total of famotidine sulfamoyl propanamide and famotidine propanamide in the portion of Famotidine for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak areas for famotidine sulfamoyl propanamide and famotidine propanamide from the *Sample solution*

$r_S$  = peak area of famotidine from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of famotidine in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Total of famotidine sulfamoyl propanamide and famotidine propanamide:** Less than 2.0%

Table 2

Name	Relative Retention Time
Famotidine sulfoxide <sup>a</sup>	0.3
Famotidine propionic acid (famotidine related compound F) <sup>b</sup>	0.5
Famotidine sulfamoyl propanamide (famotidine related compound C) <sup>c</sup>	0.7
Famotidine	1.0
Famotidine propanamide (famotidine related compound D) <sup>d</sup>	1.2

<sup>a</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylsulfinyl]-N'-sulfamoyl-propanimidamide.

<sup>b</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.

<sup>c</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-N-sulfamoyl-propanamide.

<sup>d</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

#### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count is NMT  $10^2$  cfu/g. The total combined molds and yeasts count is NMT  $10^2$  cfu/g. It meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.
- pH** <791>: 6.5–7.5, in the suspension constituted as directed in the labeling

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)  
USP Famotidine RS

**Famotidine Tablets****DEFINITION**

Famotidine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)  
**Standard solution:** 4 mg/mL of USP Famotidine RS in glacial acetic acid  
**Sample solution:** Transfer a portion of finely powdered Tablets, equivalent to about 40 mg of famotidine, to a 10-mL volumetric flask. Dissolve in glacial acetic acid with the aid of sonication, dilute with glacial acetic acid to volume, and centrifuge to get a clear liquid.  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 10  $\mu$ L  
**Developing solvent system:** Ethyl acetate, methanol, toluene, and ammonium hydroxide (40:25:20:2)  
**Analysis:** Allow the spots to dry, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent system* for about 1 h before use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine the plate under short-wavelength UV light.  
**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in appearance and  $R_f$  value to that of the *Standard solution*.
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**  
**Buffer:** Dissolve 13.6 g of sodium acetate trihydrate in 750 mL of water. Add 1 mL of triethylamine, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 1 L.  
**Mobile phase:** Acetonitrile and *Buffer* (7:93)  
**Diluent:** Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water, adjust with 1 M potassium hydroxide to a pH of 6.0, and dilute with water to 1 L.  
**System suitability stock solution:** Transfer 10 mg of famotidine to a 50-mL volumetric flask. Add 1 mL of 0.1 N hydrochloric acid, heat at 80° for 30 min, and cool to room temperature. Add 2 mL of 0.1 N sodium hydroxide, heat at 80° for 30 min, cool to room temperature, and neutralize by adding 1 mL of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume. Transfer 10 mL of this solution to a separate 50-mL volumetric flask containing 5 mg of famotidine dissolved in 8 mL of methanol. Dilute with *Diluent* to volume. Transfer 25 mL of this solution to a 50-mL volumetric flask, and dilute with *Diluent* to volume. [NOTE—This solution is stable for up to 1 month.]

**System suitability solution:** Transfer 1–1.5 mL of the *System suitability stock solution* to a suitable container, add 1 drop of hydrogen peroxide solution, and mix well.

[NOTE—Prepare fresh daily.]

**Standard solution:** Transfer 10 mg of USP Famotidine RS to a 100-mL volumetric flask, add 20 mL of methanol, and sonicate for 5 min. Dilute with *Diluent* to volume.

**Sample solution:** Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 200 mL of *Diluent*, and swirl to erode the Tablets. Add 200 mL of methanol, and stir by mechanical means at 300 rpm for 1 h. Dilute with *Diluent* to volume, mix, and filter. Quantitatively dilute a portion of the clear filtrate with *Diluent* to obtain a solution containing about 0.1 mg/mL of famotidine.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1.4 mL/min

**Injection size:** 50  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

[NOTE—Identify peaks using *Table 1*.]

**Resolution:** NLT 1.3 between the famotidine sulfamoyl propanamide and famotidine peaks, and NLT 1.3 between the famotidine and famotidine propanamide peaks, *System suitability solution*

**Capacity factor:** NLT 2.0 for the famotidine peak, *System suitability solution*

**Relative standard deviation:** Less than 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of famotidine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

**Medium:** pH 4.5, 0.1 M phosphate buffer (13.6 g/L of monobasic potassium phosphate); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of  $C_8H_{15}N_7O_2S_3$  dissolved using one of the following methods.

**Spectrophotometric method**

**Standard solution:** USP Famotidine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

**Sample solution:** Pass a portion of the sample under test through a suitable filter.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV

Analytical wavelength: 265 nm

#### Chromatographic method

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the Assay.

**Standard solution:** 0.14 mg/mL of USP Famotidine RS in *Medium*. Dilute this solution with *Medium* to obtain a solution containing  $(L/900)$  mg/mL, where  $L$  is the Famotidine Tablet label claim, in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Capacity factor:** Greater than 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$L$  = label claim, mg/Tablet

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) is dissolved.

#### For Tablets labeled as chewable

Proceed as directed for either of the methods specified above, except for the following:

**Time:** 45 min

**Tolerances:** NLT 80% (Q) of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) is dissolved.

#### For Tablets labeled as film-coated

Proceed as directed for either of the methods specified above, except for the following:

**Time:** 30 min

**Tolerances:** NLT 80% (Q) of the labeled amount of famotidine ( $C_8H_{15}N_7O_2S_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of famotidine from the *Standard solution*

$C_S$  = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of famotidine in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Famotidine sulfoxide <sup>a</sup>	0.4	1.0	1.0
Famotidine propionic acid (famotidine related compound F) <sup>b</sup>	0.7	1.0	0.5
Famotidine sulfamoyl propanamide (famotidine related compound C) <sup>c</sup>	0.8	1.0	0.5
Famotidine	1.0	—	—
Famotidine propanamide (famotidine related compound D) <sup>d</sup>	1.2	1.3	0.5
Total impurities	—	—	1.5

<sup>a</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylsulfinyl]-N'-sulfamoylpropanimidamide.

<sup>b</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.

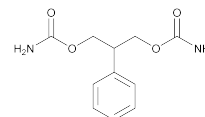
<sup>c</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-N-sulfamoylpropanamide.

<sup>d</sup> 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Famotidine RS

## Felbamate



$C_{11}H_{14}N_2O_4$  238.24  
1,3-Propanediol, 2-phenyl-, dicarbamate;  
2-Phenyl-1,3-propanediol dicarbamate [25451-15-4].

#### DEFINITION

Felbamate contains NLT 98.0% and NMT 102.0% of felbamate ( $C_{11}H_{14}N_2O_4$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, and water (126:84:790)

**System suitability solution:** 0.05 mg/mL of USP Felbamate Related Compound A RS and 0.2 mg/mL of USP Felbamate RS in *Mobile phase*

**Diluent:** Acetonitrile, methanol, and water (222:148:630)

**Standard stock solution:** 1 mg/mL of USP Felbamate RS in methanol and *Diluent* prepared as follows. Dissolve a suitable quantity of USP Felbamate RS in 10% of the flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Diluent*.

**Standard solution:** 0.2 mg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** 1.0 mg/mL of Felbamate in methanol and *Diluent* prepared as follows. Dissolve a suitable quantity of Felbamate in 10% of the flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Diluent*.

**Sample solution:** 0.2 mg/mL of Felbamate from *Sample stock solution* in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.8 mL/min

**Injection size:** 20 μL

**Run time:** 3 times the retention time of felbamate

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of felbamate (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of felbamate from the *Sample solution*

$r_S$  = peak response of felbamate from the *Standard solution*

$C_S$  = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Felbamate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

• **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

#### • ORGANIC IMPURITIES, EARLY ELUTING

**Mobile phase**, **Standard stock solution**, **System suitability solution**, and **Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1 μg/mL of USP Felbamate RS in *Mobile phase* from *Standard stock solution*

**Sample solution:** 1000 μg/mL of Felbamate in *Mobile phase*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 10%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times shown in *Table 1*. Calculate the percentage of each impurity in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of felbamate from the *Standard solution*

$C_S$  = concentration of USP Felbamate RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Felbamate in the *Sample solution* (μg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol <sup>a</sup>	0.43	1.7	0.15
Felbamate related compound A <sup>b</sup>	0.65	1.3	0.15
Felbamate	1.0	—	—
N-Aminocarbonyl felbamate <sup>c</sup>	1.43	0.89	0.15
Felbamate related compound B <sup>d</sup>	2.23	—	—
Individual unspecified impurity	—	1.0	0.1

<sup>a</sup> 2-Phenylpropane-1,3-diol.

<sup>b</sup> 3-Hydroxy-2-phenylpropyl carbamate.

<sup>c</sup> 3-Carbamoyloxy-2-phenylpropyl allophanate.

<sup>d</sup> Phenethyl carbamate, which is quantified in the test for *Organic Impurities*, *Late Eluting*.

#### • ORGANIC IMPURITIES, LATE ELUTING

**Mobile phase:** Acetonitrile, methanol, and water (222:148:630)

**System suitability solution:** 1 μg/mL each of USP Felbamate RS and USP Felbamate Related Compound B RS in *Mobile phase*

**Standard solution:** 1 μg/mL of USP Felbamate RS in *Mobile phase*

**Sample solution:** 1000 μg/mL of Felbamate in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.8 mL/min

**Injection size:** 20 μL

**Run time:** 10 times the retention time of felbamate

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3 between felbamate and felbamate related compound B, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 10%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times shown in *Table 2*. Calculate the percentage of each impurity in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of felbamate from the *Standard solution*

$C_S$  = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Felbamate in the *Sample solution* (µg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Felbamate	1.0	—	—
Felbamate related compound B <sup>a</sup>	1.9	1.29	0.15
Felbamate dimer <sup>b</sup>	9.1	1.0	0.15
Individual unspecified impurity	—	1.0	0.1

<sup>a</sup> Phenethyl carbamate.

<sup>b</sup> 3,3'-Carbonylbis(oxy)bis(2-phenylpropane-3,1-diyl) dicarbamate.

**Total impurities:** NMT 0.75% for the sum of all impurities from *Table 1* and *Table 2*

• **LIMIT OF METHYLCARBAMATE**

**Mobile phase:** Water

**Standard solution:** 0.1 mg/mL of methylcarbamate in water

**Sample solution:** Suspend 1 g of Felbamate in 5 mL of water, and mix on a vortex mixer for 1 min followed by sonication for 5 min. Filter the slurry, and use as the *Sample solution*.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 10%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The peak response for methylcarbamate in the *Sample solution* does not exceed the peak response for methylcarbamate in the *Standard solution* (0.05%).

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Felbamate RS

USP Felbamate Related Compound A RS

3-Hydroxy-2-phenylpropyl carbamate.

C<sub>10</sub>H<sub>13</sub>NO<sub>3</sub> 195.22

USP Felbamate Related Compound B RS

Phenethyl carbamate.

C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> 165.19

## Felbamate Oral Suspension

**DEFINITION**

Felbamate Oral Suspension is a viscous liquid containing NLT 90.0% and NMT 110.0% of the labeled amount of felbamate (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>). The product may contain suitable preservatives.

**IDENTIFICATION**

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 28.5 g/L of tribasic sodium phosphate dodecahydrate in water

**Mobile phase:** Acetonitrile and *Buffer* (23:77). Adjust with phosphoric acid to a pH of 9.9.

**Diluent A:** Methanol and water (1:1)

**Diluent B:** Acetonitrile and water (23:77)

**System suitability solution:** 0.16 mg/mL of USP Felbamate RS and 0.02 mg/mL of USP Felbamate Related Compound A RS in *Diluent B* and methanol prepared as follows. Dissolve the required amounts of the Reference Standards in a suitable volumetric flask in 8% of the flask volume of methanol. Dilute with *Diluent B* to volume.

**Standard solution:** 0.16 mg/mL of USP Felbamate RS prepared as follows. Dissolve a weighed quantity in USP Felbamate RS in a suitable volumetric flask containing 16% of the flask volume of *Diluent A*. Add 50% of the flask volume of *Diluent B*. Sonication may be used to aid dissolution. Dilute with *Diluent B* to volume.

**Sample stock solution:** Nominally 1 mg/mL of felbamate in *Diluent A* prepared as follows. Transfer an amount of the Oral Suspension to a suitable volumetric flask. Add 60% of the flask volume of *Diluent A*, and sonicate for 15 min. Shake mechanically for 30 min, and dilute with *Diluent A* to volume.

**Sample solution:** Nominally 0.16 mg/mL of felbamate from *Sample stock solution* in *Diluent B*. Pass through a filter of 45-µm pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.1-mm × 15-cm; 5-µm packing L21

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of felbamate ( $C_{11}H_{14}N_2O_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of felbamate from the *Sample solution*  
 $r_S$  = peak response of felbamate from the *Standard solution*  
 $C_S$  = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of felbamate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Mobile phase:** Acetonitrile and water (35:65)

**System suitability solution:** Prepare a solution of 0.1 mg/mL of USP Methylparaben RS in methanol. Transfer 10 mL of this solution to a 100-mL volumetric flask containing 40 mg of USP Felbamate RS. Sonicate for 5 min, and dilute with *Medium* to volume.

**Standard solution:** ( $L/1000$ ) mg/mL of USP Felbamate RS in *Medium*, where  $L$  is the Oral Suspension label claim in mg/mL. An amount of methanol, not exceeding 10% of the final volume, can be used to help in solubilizing felbamate.

**Sample solution:** Using a syringe, accurately weigh by difference approximately 5 mL of the well-mixed Oral Suspension. Introduce the sample into the dissolution vessel with the paddles rotating, and avoid getting the sample on the paddle or shaft. At the time specified, withdraw an aliquot of the solution under test, and pass through a suitable filter.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 10- $\mu$ m packing L7

**Flow rate:** 2 mL/min

**Injection size:** 25  $\mu$ L for *System suitability* and 15  $\mu$ L for *Analysis*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for methylparaben and felbamate are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between methylparaben and felbamate, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of felbamate ( $C_{11}H_{14}N_2O_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (D/W) \times V \times 100$$

$r_U$  = peak response of felbamate from the *Sample solution*  
 $r_S$  = peak response of felbamate from the *Standard solution*  
 $C_S$  = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)

$L$  = label claim of the Oral Suspension (mg/mL)  
 $D$  = density of the Oral Suspension (g/mL)  
 $W$  = weight of the Oral Suspension taken (g)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% ( $Q$ ) of the labeled amount of felbamate ( $C_{11}H_{14}N_2O_4$ ) is dissolved.

**IMPURITIES**• **ORGANIC IMPURITIES**

**Mobile phase, Diluent A, Diluent B, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 0.2 mg/mL of USP Felbamate RS and 0.02 mg/mL of USP Felbamate Related Compound A RS in *Diluent B* prepared as follows. Transfer suitable quantities of USP Felbamate RS and USP Felbamate Related Compound A RS into a suitable volumetric flask. Dissolve in 8% of the flask volume of methanol. Dilute with *Diluent B* to volume.

**Standard stock solution:** 0.2 mg/mL of USP Felbamate RS in *Diluent A*

**Standard solution:** 0.2  $\mu$ g/mL of USP Felbamate RS from *Standard stock solution* in *Diluent B* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask containing 0.8% of the flask volume of methanol, and dilute with *Diluent B* to volume.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0 for the felbamate peak, *Standard solution*

**Relative standard deviation:** NMT 10% for the felbamate peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of felbamate from the *Standard solution*  
 $C_S$  = concentration of USP Felbamate RS in the *Standard solution* ( $\mu$ g/mL)  
 $C_U$  = nominal concentration of felbamate in the *Sample solution* ( $\mu$ g/mL)  
 $F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol <sup>a</sup>	0.63	2.6	0.2
Felbamate related compound A <sup>b</sup>	0.78	1.8	0.2
Felbamate	1.0	—	—

<sup>a</sup> 2-Phenylpropane-1,3-diol.

<sup>b</sup> 3-Hydroxy-2-phenylpropyl carbamate.



Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.75

<sup>a</sup> 2-Phenylpropane-1,3-diol.<sup>b</sup> 3-Hydroxy-2-phenylpropyl carbamate.**SPECIFIC TESTS**

- **PH** (791): 4.5–6.0, in the well-stirred Oral Suspension
- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10<sup>2</sup> cfu/mL. The total yeasts and molds count is NMT 10<sup>1</sup> cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Felbamate RS
  - USP Felbamate Related Compound A RS
  - 3-Hydroxy-2-phenylpropyl carbamate.
  - C<sub>10</sub>H<sub>13</sub>NO<sub>3</sub> 195.22
  - USP Methylparaben RS

**Felbamate Tablets****DEFINITION**

Felbamate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of felbamate (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197)
 

**Sample:** Transfer a weighed quantity of finely powdered Tablets, equivalent to 12 mg of felbamate, to a centrifuge tube. Add 10 mL of methanol, and mix on a vortex mixer for 3 min. Centrifuge, and transfer 1 mL of the clear supernatant to a mortar with 200 mg of potassium bromide. Evaporate at 105° in an oven for 15 min. Cool the dried residue in a desiccator, and blend by grinding. Prepare a pellet with 100 mg of the dried residue.

**Standard:** Transfer 1 mL of 1.2 mg/mL of USP Felbamate RS in methanol to a mortar with 200 mg of potassium bromide. Evaporate at 105° in an oven for 15 min. Cool the dried residue in a desiccator, and blend by grinding. Prepare a pellet with 100 mg of the dried residue.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Diluent:** Methanol and water (80:20)

**Mobile phase:** Acetonitrile, methanol, and water (126:84:790)

**System suitability solution:** 0.05 mg/mL of USP Felbamate Related Compound A RS and 0.2 mg/mL of USP Felbamate RS in *Mobile phase*

**Standard stock solution:** 2 mg/mL of USP Felbamate RS in *Diluent*

**Standard solution:** 0.2 mg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Nominally 2 mg/mL of felbamate from NLT 20 finely powdered Tablets, prepared as

follows. Transfer a weighed quantity of the powder to a suitable volumetric flask. Add 50% of the flask volume of the *Diluent*. Sonicate for 30 min with intermittent shaking. Shake the flask vigorously for NLT 30 min. Dilute with *Diluent* to volume. Pass a portion through a suitable membrane filter.

**Sample solution:** 0.2 mg/mL of felbamate from *Sample stock solution* in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.8 mL/min

**Injection size:** 20 μL

**Run time:** 3 times the retention time of felbamate

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of felbamate (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of felbamate from the *Sample solution*

$r_S$  = peak response of felbamate from the *Standard solution*

$C_S$  = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of felbamate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****DISSOLUTION** (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Mobile phase:** Prepare as directed in the *Assay*.

**Standard solution:** (L/1000) mg/mL of USP Felbamate RS, where L is the Tablet label claim, in mg. Transfer a suitable weighed quantity of USP Felbamate RS to a suitable volumetric flask. Add 10% of the flask volume of methanol, and sonicate for 5 min to dissolve. Dilute with *Medium* to volume.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.0-mm × 15-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 50 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of felbamate (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 65% (Q) of the labeled amount of felbamate ( $C_{11}H_{14}N_2O_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Diluent, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability stock solution:** 0.4 mg/mL of USP Felbamate Related Compound A RS and 0.6 mg/mL of USP Felbamate RS in *Diluent*

**System suitability solution:** 0.4 µg/mL of USP Felbamate Related Compound A RS and 0.6 µg/mL of USP Felbamate RS from *System suitability stock solution* in *Mobile phase*

**Standard stock solution:** 0.6 mg/mL of USP Felbamate RS in *Diluent*

**Standard solution:** 0.6 µg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NMT 2 between felbamate related compound A and felbamate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 10%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times shown in *Table 1*. Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response of each impurity from *Sample solution*  
 $r_S$  = peak response of felbamate from the *Standard solution*  
 $C_S$  = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of felbamate in the *Sample solution* (µg/mL)  
 $F$  = relative response factor (see *Table 1*)  
**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol <sup>a</sup>	0.43	1.7	0.2
Felbamate related compound A <sup>b</sup>	0.65	1.3	0.2
Felbamate	1.0	—	—
N-Aminocarbonyl-felbamate <sup>c</sup>	1.43	—	—
Felbamate related compound B <sup>d</sup>	2.23	—	—

<sup>a</sup> 2-Phenylpropane-1,3-diol.

<sup>b</sup> 3-Hydroxy-2-phenylpropyl carbamate.

<sup>c</sup> 3-Carbamoyloxy-2-phenylpropyl allophanate.

<sup>d</sup> Phenylethyl carbamate. No limit. This is a process impurity.

**Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.75

<sup>a</sup> 2-Phenylpropane-1,3-diol.

<sup>b</sup> 3-Hydroxy-2-phenylpropyl carbamate.

<sup>c</sup> 3-Carbamoyloxy-2-phenylpropyl allophanate.

<sup>d</sup> Phenylethyl carbamate. No limit. This is a process impurity.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

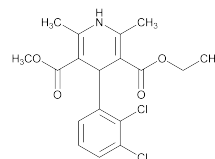
USP Felbamate RS

USP Felbamate Related Compound A RS

3-Hydroxy-2-phenylpropyl carbamate.

$C_{10}H_{13}NO_3$  195.22

## Felodipine



$C_{18}H_{19}Cl_2NO_4$  384.25  
 3,5-Pyridinedicarboxylic acid 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ethyl methyl ester, (±)-;  
 (±)-Ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate [72509-76-3;  
 86189-69-7].

## DEFINITION

Felodipine contains NLT 98.0% and NMT 101.0% of felodipine ( $C_{18}H_{19}Cl_2NO_4$ ), calculated on the dried basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

**Buffer:** Dissolve 6.9 g of monobasic sodium phosphate in 400 mL of water, add 8.0 mL of 1 M phosphoric acid, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (40:20:40)

**System suitability stock solution:** 0.05 mg/mL of USP Felodipine RS and 0.1 mg/mL of felodipine oxidation product in *Mobile phase* prepared as follows. Dissolve 150 mg of Felodipine in a mixture of 50 mL of tertiary butyl alcohol and 1 N perchloric acid (1:1). Add 10 mL of 0.1 M ceric sulfate, and allow to stand for 15 min. Add 3.5 mL of 10 N sodium hydroxide, and neutralize with 2 N sodium hydroxide. Shake the mixture with 25 mL of methylene chloride in a separator. Draw off the lower layer, and evaporate it to dryness under a stream of nitrogen on a water bath. Dissolve and dilute 10 mg of the residue (felodipine oxidation product) and 5 mg of USP Felodipine RS, with *Mobile phase* to 100 mL.

**System suitability solution:** 0.5 µg/mL of USP Felodipine RS and 1 µg/mL of felodipine oxidation product in *Mobile phase* from the *System suitability stock solution*

**Standard solution:** 0.3 mg/mL of USP Felodipine RS in *Mobile phase*. [NOTE—Prepare fresh before analysis.]

**Sample solution:** 0.3 mg/mL of Felodipine in *Mobile phase*. [NOTE—Prepare fresh before analysis.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL for the *System suitability solution* and 40 µL for the *Standard solution* and the *Sample solution*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

Adjust the sensitivity of the system so that the heights of the two peaks in the chromatogram are 20% of the recorder full scale.

#### Suitability requirements

**Resolution:** NLT 2.5 between the first peak (felodipine oxidation product) and the second peak (felodipine), *System suitability solution*

**Capacity factor, *k'*:** NLT 5.0, *Standard solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of felodipine (C<sub>18</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>4</sub>) in the portion of Felodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of felodipine from the *Sample solution*

*r<sub>S</sub>* = peak response of felodipine from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of Felodipine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

- **HEAVY METALS, Method II** <231>: NMT 20 ppm

- **ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, and Sample solution:** Prepare as directed in the *Assay*.

**Chromatographic system:** Proceed as directed in the *Assay*, except use a run time of NLT 2 times the retention time of felodipine.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Felodipine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

*r<sub>U</sub>* = peak response for each impurity

*r<sub>T</sub>* = sum of all the peak responses

#### Acceptance criteria

**Individual impurities:** NMT 1.0%

**Total impurities:** NMT 1.5%

#### SPECIFIC TESTS

- **LOSS ON DRYING** <731>

**Analysis:** Dry the sample at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

- **COLOR OF SOLUTION**

**Sample solution:** 20 mg/mL in methanol

**Instrumental conditions**

**Mode:** Vis

**Cell:** 5 cm

**Analytical wavelength:** 440 nm

**Blank:** Methanol

**Acceptance criteria:** NMT 0.2 absorbance

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS** <11>

USP Felodipine RS

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## Felodipine Extended-Release Tablets

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#### DEFINITION

Felodipine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of felodipine (C<sub>18</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>4</sub>).

#### IDENTIFICATION

- The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

- **PROCEDURE**

**Solution A:** 6.9 mg/mL of monobasic sodium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.05.

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (2:1:2)

**Standard stock solution:** 2 mg/mL of USP Felodipine RS in methanol

**Standard solution:** 0.02 mg/mL from the *Standard stock solution* in *Mobile phase*

**Sample solution:** Dissolve a quantity equivalent to 10 mg of felodipine from powdered Tablets (NLT 10 Tablets), in 40 mL of acetonitrile and 20 mL of methanol in a 100-mL volumetric flask, and sonicate for 5 min. Add about 30 mL of *Solution A*, and shake by mechanical means for 30 min. Allow the solution to cool to room temperature, and dilute with *Solution A* to volume. Centrifuge a portion of the solution at high speed for 15 min. Transfer 10 mL of the supernatant to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, discarding the first 4 mL of the filtrate.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 362 nm**Column:** 4.6-mm × 15-cm; packing L1**Flow rate:** 1 mL/min**Injection size:** 40 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 1500 theoretical plates**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $C_{18}H_{19}Cl_2NO_4$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of felodipine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION <711>****Test 1**

**Medium:** pH 6.5 phosphate buffer with 1% sodium lauryl sulfate (Transfer 206 mL of 1 M monobasic sodium phosphate monohydrate, 196 mL of 0.5 M anhydrous dibasic sodium phosphate, and 50.0 g of sodium lauryl sulfate to a 5000-mL volumetric flask. Add about 4000 mL of water, and mix well. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.5. Dilute with water to volume.); 500 mL

**Apparatus 2:** 50 rpm**Times:** 2, 6, and 10 h

**Buffer solution:** 6.9 mg/mL of monobasic sodium phosphate. Adjust with 1 M phosphoric acid to a pH of  $3.0 \pm 0.05$ .

**Mobile phase:** Acetonitrile, methanol, and *Buffer solution* (2.5:1:2)

**Standard stock solution:** 0.25 mg/mL of USP Felodipine RS in alcohol

**NOTES**

1. Rod and Basket with a Tablet cover placed in the horizontal diagonal of the basket.
2. Basket and Tablet cover material; stainless steel.
3. Basket gauze wire size: 8 mesh.

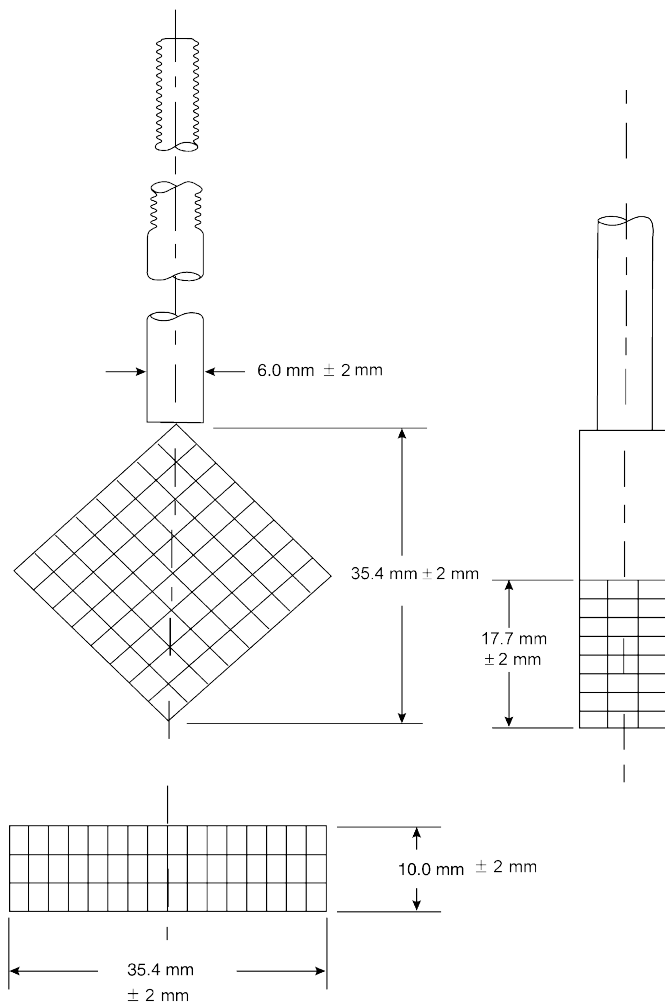


Figure 1. Stationary tablet basket.

**Standard solution:** (0.6 L/500) mg/mL of USP Felodipine RS from the *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet. [NOTE—L equals Label claim]

**Sample solution:** Place each Tablet in a specially made quadrangular basket of stainless steel wire gauze, soldered in one of its upper, narrow sides to the end of a steel rod (see *Figure 1*). Place the tablet cover in the horizontal diagonal of the basket. Put the rod assembly up through the cover of the dissolution vessel, and fix it by means of two teflon nuts, 3.2 cm from the center of the vessel, or by any other appropriate means. Adjust the lower edge of the bottom of the basket to about 1 cm above the top of the paddle blade (see *Figure 2*). Orient the large side of the basket tangentially to the flow stream with the Tablet standing on its edge. Pass a 10-mL portion of the solution under test, obtained at each time interval, through a suitable filter.

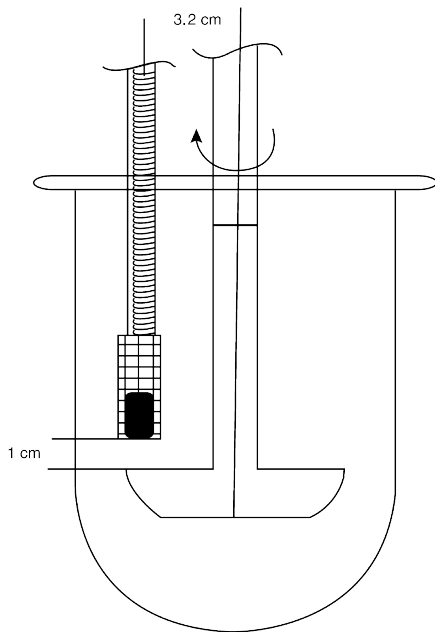


Figure 2. Drug release stationary tablet basket configuration diagram.

**Chromatographic system:** Proceed as directed in the Assay.

**Analysis**

**Injection size:** 100  $\mu$ L

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{18}H_{19}Cl_2NO_4$  dissolved in the *Medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times 100/L$$

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)
- $D$  = dilution factor of the *Sample solution*
- $V$  = volume of the *Medium* (mL), 500
- $L$  = Tablet label claim (mg)

**Tolerances:** The percentages of the labeled amount of  $C_{18}H_{19}Cl_2NO_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	10%–30%
6	42%–68%
10	NLT 75%

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 1% (w/v) polysorbate 80; 500 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 4, and 8 h

**Buffer solution:** Dissolve 6.9 g of monobasic sodium phosphate in 400 mL of water, add 8.0 mL of 1 M phosphoric acid, and dilute with water to 1000.0 mL.

**Mobile phase:** Acetonitrile, methanol, and *Buffer solution* (2:1:2)

**Standard stock solution:** 0.2 mg/mL of USP Felodipine RS in methanol. Sonicate for 2 min, cool, and dilute with methanol to final concentration.

**Standard solution:** Transfer the appropriate volume of *Standard stock solution* into a suitable volumetric flask according to the following table. Dilute with *Medium* to volume.

Tablet Label Claim (mg)	Volume Transferred (mL)	Volumetric Flask (mL)
10	10	100
5	5	100
2.5	2.5	100

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Replace the withdrawn amount with *Medium*.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Capacity factor ( $k'$ ):** NLT 5

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in mg, of  $C_{18}H_{19}Cl_2NO_4$  dissolved:

$$C_U = (r_U/r_S) \times C_S$$

$C_U$  = concentration of felodipine in the sample at each time point

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of felodipine in the *Standard solution* (mg/mL)

Calculate the percentage of  $C_{18}H_{19}Cl_2NO_4$  dissolved, with volume correction:

$$\text{Result} = \frac{[C_N \times (V_M - V_U(n-1))] + [\sum_{i=1}^{n-1} C_i \times V_U]}{L} \times 100$$

$C_N$  = concentration of felodipine in the *Sample solution* at each time point (mg/mL)

$V_M$  = volume of *Medium* (mL), 500

$V_U$  = volume of sample withdrawn at each time point (mL)

$n$  = the time point (at 4 h,  $n + 1$ )

$\sum_{i=1}^{n-1}$  = summation of the concentration of the *Sample solution* from the first to the (n-1)<sup>th</sup> time point (only applicable for n = 2)  
 L = Tablet label claim (mg)

**Tolerances:** The percentages of the labeled amount of felodipine dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	5%–30%
4	45%–70%
8	NLT 80%

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** pH 6.5 phosphate buffer with 1% sodium lauryl sulfate (5.7 g/L of monobasic sodium phosphate monohydrate, 2.8 g/L of dibasic sodium phosphate, and 10 g/L of sodium lauryl sulfate in water, adjusted, if necessary, with diluted sodium hydroxide to a pH of  $6.5 \pm 0.05$ ); 500 mL

**Apparatus 2:** 50 rpm

**Times:** 2, 6, and 10 h

**Buffer solution:** 6.9 g/L of monobasic sodium phosphate monohydrate in water. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.05$ .

**Mobile phase:** *Buffer solution*, acetonitrile, and methanol (30:45:25)

**Standard stock solution:** 0.5 mg/mL of USP Felodipine RS in methanol

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute to volume with *Medium*. This solution is stable for 5 days at room temperature.

**Sample solution:** Place each Tablet in a specially made quadrangular basket of stainless steel wire gauze, soldered in one of its upper, narrow sides to the end of a steel rod (see *Figure 1*, under *Test 1*). Place the tablet cover in the horizontal diagonal of the basket. Put the rod assembly up through the cover of the dissolution vessel, and fix it by means of two teflon nuts 3.2 cm from the center of the vessel, or by any other appropriate means. Adjust the lower edge of the bottom of the basket to approximately 1 cm above the top of the paddle blade (see *Figure 2*, under *Test 1*). Orient the large side of the basket tangentially to the flow stream with the Tablet standing on its edge. Pass a portion of the solution under test, obtained at each time interval, through a suitable filter, discarding the first few mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 362 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 2.0 mL/min

**Injection size:** 40  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of felodipine dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium* (mL), 500

**Tolerances:** The percentages of the labeled amount of felodipine dissolved at the times specified conform to *Acceptance Table 2*.

Time (h)	Amount Dissolved
2	10%–30%
6	50%–80%
10	NLT 80%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### Procedure for content uniformity

**Solution A:** 6.9 mg/mL of monobasic sodium phosphate. Adjust with 1 M phosphoric acid to a pH of  $3.0 \pm 0.05$ .

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (2:1:2)

**Standard stock solution:** 2 mg/mL of USP Felodipine RS in methanol

**Standard solution:** 20  $\mu$ g/mL from the *Standard stock solution* in *Mobile phase*

**Sample solution:** 20  $\mu$ g/mL of felodipine, prepared by combining one Tablet taken in a 100-mL volumetric flask and, 40 mL of acetonitrile, and sonicating for 20 min with occasional swirling. Add 20 mL of methanol, and shake by mechanical means for 30 min. Allow to cool to room temperature, and dilute with *Solution A* to volume. Centrifuge a portion of the solution at high speed for 15 min. Dilute a portion of the supernatant with *Mobile phase* to obtain a final concentration. Pass this solution through a filter of 0.5- $\mu$ m or finer pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 362 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 40  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{18}H_{19}Cl_2NO_4$  in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Felodipine RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of felodipine in the *Sample solution* ( $\mu$ g/mL)

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

**Solution A:** 6.9 mg/mL of monobasic sodium phosphate. Adjust with 1 M phosphoric acid to a pH of  $3.0 \pm 0.05$

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (2:1:2)

**Standard stock solution A:** 0.2 mg/mL of USP Felodipine Related Compound A RS in methanol

**Standard solution A:** 0.02 mg/mL of USP Felodipine Related Compound A RS from the *Standard stock solution A* in *Mobile phase*

**Standard solution B:** 2 mg/mL of USP Felodipine RS in methanol

**System suitability solution:** Combine 15.0 mL of *Standard solution A* and 5.0 mL of *Standard solution B*, and dilute with *Mobile phase* to 100.0 mL.

**Standard solution:** Dilute 10.0 mL of *Standard solution A* with *Mobile phase* to 100.0 mL.

**Sample solution:** Dissolve a quantity equivalent to 10 mg of felodipine from powdered Tablets (NLT 10 Tablets) in a 100-mL volumetric flask. Add 40 mL of acetonitrile and 20 mL of methanol, and sonicate for 5 min. Add about 30 mL of *Solution A*, and shake by mechanical means for 30 min. Allow the solution to cool to room temperature, and dilute with *Solution A* to volume. Centrifuge a portion of the solution at high speed for 15 min, and pass a portion of the supernatant through a filter of 0.5-μm or finer pore size, discarding the first 4 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 40 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE— The relative retention times for felodipine related compound A and felodipine are about 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between felodipine and felodipine related compound A

**Column efficiency:** NLT 1500 theoretical plates

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of felodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of felodipine related compound A from the *Sample solution*  
 $r_S$  = peak response of felodipine related compound A from the *Standard solution*  
 $C_S$  = concentration of USP Felodipine Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of felodipine in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 2.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** <11>
  - USP Felodipine RS
  - USP Felodipine Related Compound A RS
  - Ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.  
 $C_{18}H_{17}Cl_2NO_4$  382.24

Methyl 5-(phenylthio)-2-benzimidazolecarbamate  
 [43210-67-9].

» Fenbendazole contains not less than 98.0 percent and not more than 101.0 percent of  $C_{15}H_{13}N_3O_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards <11>—

USP Fenbendazole RS

USP Fenbendazole Related Compound A RS

Methyl (1*H*-benzimidazole-2-yl)carbamate.

$C_9H_9N_3O_2$  191.19

USP Fenbendazole Related Compound B RS

Methyl [5(6)-chlorobenzimidazole-2-yl]carbamate.

$C_9H_8ClN_3O_2$  225.63

USP Mebendazole RS

#### Identification, Infrared Absorption <197K>.

**Loss on drying** <731>—Dry it at 100° to 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.3%.

**Heavy metals, Method II** <231>: not more than 10 μg per g.

#### Related compounds—

*Solution A*—Prepare a mixture of water, methanol, and acetic acid (70:30:1).

*Solution B*—Prepare a mixture of methanol, water, and acetic acid (70:30:1).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

*Hydrochloric methanol*—Prepare a mixture of methanol and hydrochloric acid (99:1).

*Standard solution 1*—Dissolve an accurately weighed quantity of USP Fenbendazole RS in *Hydrochloric methanol* to obtain a solution having a known concentration of about 5 mg per mL. Dilute 1.0 mL of this solution with methanol to 200.0 mL. Dilute 5.0 mL of this solution with *Hydrochloric methanol* to 10.0 mL. This solution contains about 0.0125 mg of USP Fenbendazole RS per mL.

*Standard solution 2*—Dissolve an accurately weighed quantity of USP Fenbendazole Related Compound A RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Dilute 1.0 mL of this solution with *Hydrochloric methanol* to 10.0 mL, and mix.

*Standard solution 3*—Dissolve an accurately weighed quantity of USP Fenbendazole Related Compound B RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Dilute 1.0 mL of this solution with *Hydrochloric methanol* to 10.0 mL, and mix.

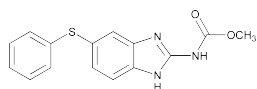
*Resolution solution*—Dissolve 10.0 mg of USP Fenbendazole RS and 10.0 mg of USP Mebendazole RS in 100.0 mL of methanol. Dilute 1.0 mL of this solution with *Hydrochloric methanol* to 10.0 mL, and mix.

*Test solution*—Dissolve 50.0 mg of Fenbendazole in 10.0 mL of *Hydrochloric methanol*.

*Chromatographic system* (see *Chromatography* <621>—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–10	100→0	0→100	linear gradient

## Fenbendazole



$C_{15}H_{13}N_3O_2S$  299.35

Carbamic acid, [5-(phenylthio)-1*H*-benzimidazol-2-yl]-, methyl ester.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
10–40	0	100	isocratic
40–50	0→100	100→0	linear gradient

Chromatograph the *Resolution solution*, and record the peak area responses as directed for *Procedure*: the retention time for fenbendazole is about 19 minutes; the relative retention time is about 0.85 for mebendazole and 1.0 for fenbendazole; and the resolution,  $R$ , between mebendazole and fenbendazole is not less than 1.5. Chromatograph *Standard solution 2* and *Standard solution 3*, and record the peak area responses as directed for *Procedure*: the relative retention times are about 0.25 for fenbendazole related compound A and 0.65 for fenbendazole related compound B.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for all of the peaks. Calculate the percentages of fenbendazole related compound A and of fenbendazole related compound B in the portion of Fenbendazole taken by the formula:

$$0.1(C/W)(r_i / r_{Si})$$

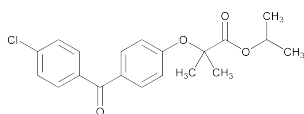
in which  $C$  is the concentration, in mg per mL, of USP Fenbendazole Related Compound A RS or USP Fenbendazole Related Compound B RS in *Standard solution 2* or *Standard solution 3*, as appropriate;  $W$  is the weight, in mg, of Fenbendazole taken to prepare the *Test solution*;  $r_i$  is the peak area response of the relevant related compound obtained from the *Test solution*; and  $r_{Si}$  is the peak area response of the relevant related compound obtained from *Standard solution 2* or *Standard solution 3*, as appropriate. Not more than 0.5% of either related compound is found. Calculate the percentage of any other impurity in the portion of Fenbendazole taken by the formula:

$$0.1(C/W)(r_i / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Fenbendazole RS in *Standard solution 1*;  $W$  is as defined above;  $r_i$  is the peak area response of any impurity other than fenbendazole related compound A or fenbendazole related compound B obtained from the *Test solution*; and  $r_s$  is the peak area response of the main peak in the chromatogram obtained from *Standard solution 1*. Disregard any impurity peak with a response that is less than one-fifth that of  $r_s$ . Not more than 0.5% of any other impurity is found. The sum of all impurities found, including fenbendazole related compound A and fenbendazole related compound B, is not more than 1%.

**Assay**—Dissolve about 200 mg of Fenbendazole, accurately weighed, in 30 mL of glacial acetic acid, warming if necessary to effect solution. Cool, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 29.94 mg of  $C_{15}H_{13}N_3O_2S$ .

## Fenofibrate



$C_{20}H_{21}ClO_4$  360.83  
Isopropyl 2-[p-(p-chlorobenzoyl)phenoxy]-2-methylpropanoate [49562-28-9].

## DEFINITION

Fenofibrate contains NLT 98.0% and NMT 102.0% of  $C_{20}H_{21}ClO_4$ , calculated on the dried basis.

## IDENTIFICATION

- **INFRARED ABSORPTION** (197K)

## ASSAY

### PROCEDURE

**Mobile phase:** Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)

**Standard solution:** 1 mg/mL of USP Fenofibrate RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Fenofibrate in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 4.0-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 5  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 1.0% for six replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{20}H_{21}ClO_4$  in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fenofibrate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%, determined on 1.0 g

- **CHLORIDE AND SULFATE, Chloride** (221)

**Sample solution:** Add 25 mL of water to 5.0 g of Fenofibrate, and heat at 50° for 10 min. Cool, dilute with water to 50.0 mL, filter, and use the filtrate. [NOTE—Retain the remaining portion of the *Sample solution* for the test for *Chloride and Sulfate, Sulfate*.]

**Analysis:** Use 10 mL of the *Sample solution*.

**Acceptance criteria:** It shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid (0.01%).

- **CHLORIDE AND SULFATE, Sulfate** (221)

**Sample:** Use the *Sample solution* prepared in the test for *Chloride and Sulfate, Chloride*.

**Analysis:** Use 10 mL of the *Sample*.

**Acceptance criteria:** It shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.01%).

- **HEAVY METALS, Method II** (231): NMT 20 ppm

### Organic Impurities

#### PROCEDURE

**Mobile phase:** Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)

**Impurity standard solution:** 1  $\mu$ g/mL each of USP Fenofibrate RS, USP Fenofibrate Related Compound A RS, and USP Fenofibrate Related Compound B RS, and 2  $\mu$ g/mL of USP Fenofibrate Related Compound C RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Fenofibrate in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 286 nm

Column: 4.0-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 20 µL

#### System suitability

Sample: Impurity standard solution

#### Suitability requirements

Resolution: NLT 1.5 between fenofibrate related compound A and fenofibrate related compound B

#### Analysis

Samples: Impurity standard solution and Sample solution

Identify the fenofibrate peak and the peaks due to the impurities and degradation products listed in Impurity Table 1.

Measure the responses for the major peaks, and calculate the percentage of each of fenofibrate related compound A, fenofibrate related compound B, and fenofibrate related compound C in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of appropriate fenofibrate related compound from the Sample solution

$r_S$  = peak response of appropriate fenofibrate related compound from the Impurity standard solution

$C_S$  = concentration of the appropriate fenofibrate related compound in the Impurity standard solution (µg/mL)

$C_U$  = concentration of Fenofibrate in the Sample solution (µg/mL)

Calculate the percentage of any other impurity in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of fenofibrate from the Impurity standard solution

$C_S$  = concentration of fenofibrate in the Impurity standard solution (µg/mL)

$C_U$  = concentration of Fenofibrate in the Sample solution (µg/mL)

#### Acceptance criteria

Individual impurities: See Impurity Table 1.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
(4-Chlorophenyl)(4-hydroxyphenyl) methanone <sup>a</sup>	0.34	0.1
2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid (fenofibric acid) <sup>b</sup>	0.36	0.1
(3R)-3-[4-(4-Chlorobenzoyl)phenoxy]butan-2-one	0.50	0.1
Methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoate	0.65	0.1
Ethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoate	0.80	0.1
(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanone	0.85	0.1

<sup>a</sup> Fenofibrate related compound A.

<sup>b</sup> Fenofibrate related compound B.

<sup>c</sup> Fenofibrate related compound C.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate <sup>c</sup>	1.35	0.2
Any other impurity	—	0.1

<sup>a</sup> Fenofibrate related compound A.

<sup>b</sup> Fenofibrate related compound B.

<sup>c</sup> Fenofibrate related compound C.

#### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE**, Class Ia (741): 79°–82°

• **ACIDITY**

Sample: 1.0 g

Analysis: Dissolve the Sample in 50 mL of alcohol previously neutralized to phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS.

Acceptance criteria: NMT 0.2 mL is required to change the color of the indicator to pink.

• **LOSS ON DRYING** (731)

Analysis: Dry a sample in a vacuum over phosphorus pentoxide at 60° to constant weight.

Acceptance criteria: NMT 0.5%

• **COLOR AND ACHROMICITY** (631)

Reference solution: Mix 5 mL of Matching Fluid G and 95 mL of dilute hydrochloric acid (1 in 40).

Sample solution: 50 mg/mL of Fenofibrate in acetone

Analysis: Proceed as directed in the chapter.

Acceptance criteria: The Sample solution is not more intensely colored than the Reference solution.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE**: Preserve in well-closed, light-resistant containers. Store at room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Fenofibrate RS

USP Fenofibrate Related Compound A RS

(4-Chlorophenyl)(4-hydroxyphenyl)methanone.

USP Fenofibrate Related Compound B RS

2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.

USP Fenofibrate Related Compound C RS

1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate.

## Fenofibrate Capsules

#### DEFINITION

Fenofibrate Capsules contains NLT 90.0% and NMT 110.0% of the labeled amount of fenofibrate (C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub>).

#### IDENTIFICATION

• The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

[NOTE—Use Sample stock solution 2 for Capsules labeled to meet the requirements of Dissolution Test 2. For all other products, use Sample stock solution 1.]

**Solution A**: 136 mg/L of monobasic potassium phosphate in water. Adjust with dilute phosphoric acid (1 in 10) to a pH of 2.9 ± 0.05.

**Mobile phase**: Methanol and Solution A (4:1)

**Standard solution**: 67 µg/mL of USP Fenofibrate RS in Mobile phase

**Sample stock solution 1:** Accurately weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the powder, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 min, stir for 15 min, and dilute with *Mobile phase* to volume.

**Sample stock solution 2** (For Capsules labeled to meet the requirements of *Dissolution Test 2*): Weigh the contents of NLT 20 Capsules. Mix the contents, melt in an oven at 80° for NLT 30 min, and homogenize. Allow the sample to solidify. Transfer a weighed portion of the sample, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask, dissolve in 30 mL of methanol with the aid of a mechanical shaker for NLT 4 h, and dilute with *Mobile phase* to volume.

**Sample solution:** 67 µg/mL of fenofibrate from the designated *Sample stock solution*, in *Mobile phase*. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 285 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 6000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub> in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** 0.05 M sodium lauryl sulfate in water; 1000 mL, deaerated

**Apparatus 2:** 75 rpm

**Time:** 40 min

**Solution A and Mobile phase:** Proceed as directed in the Assay.

**Standard solution:** (0.001 × L) mg/mL of USP Fenofibrate RS in *Mobile phase*, where L is the Capsule label claim, in mg

**Sample solution:** Pass a portion of the solution under test through a suitable polyvinylidene difluoride (PVDF) filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 285 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL for Capsules labeled to contain 67 mg; 5 µL for Capsules labeled to contain 134 or 200 mg

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 4000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub> dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (100/L)$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$L$  = Capsule label claim (mg)

**Tolerances:** NLT 70% (Q) of the labeled amount of C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub> is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Phosphate buffer pH 6.8 ± 0.1 containing 0.1% pancreatin and 2% polysorbate 80; 900 mL, deaerated by vacuum

**Apparatus 2:** 75 rpm with sinkers (see *Dissolution* <711>, Figure 2a)

**Time:** 2 h

**Detector:** UV 288 nm

**Standard solution:** (L/1000) mg/mL of USP Fenofibrate RS in *Medium*, where L is the Capsule label claim, in mg. A volume of methanol, not exceeding 10%, can be used in the first dilution to solubilize fenofibrate.

**Sample solution:** Pass 20 mL of the solution under test through a suitable PVDF filter of 0.45-µm pore size, discarding the first 2 mL.

**Path length:** 0.1 cm, flow cell

**Blank:** *Medium*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub> dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = Capsule label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of C<sub>20</sub>H<sub>21</sub>ClO<sub>4</sub> is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** 0.72% sodium lauryl sulfate in water; 1000 mL, deaerated

**Apparatus 2:** 75 rpm, with sinkers with three prongs

**Time:** 30 min

**Standard solution:** L/10 mg/mL of USP Fenofibrate RS in methanol, where L is the Capsule label claim in mg. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, and dilute with *Medium* to volume.

**Sample solution:** Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size. Dilute with *Medium*, if necessary.

**Detection:** UV 290 nm

**Blank:** *Medium*

Calculate the percentage of fenofibrate dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_s$  = concentration of the *Standard solution* (mg/mL)

$L$  = Capsule label claim (mg)

$D$  = dilution for the *Sample solution*

$V$  = volume of the *Medium*, 1000 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of fenofibrate is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### Procedure for content uniformity

**Solution A, Mobile phase, Standard solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed in the *Assay*, except to prepare the *Sample stock solution* and *Sample solution* as follows.

**Sample stock solution:** Place 1 Capsule in a suitable volumetric flask, add *Solution A* to 10%–20% of the final volume, and stir for 20 min to disintegrate the Capsule. Fill the flask to about 80% with methanol, sonicate for 10 min, stir for 15 min, and dilute with methanol to volume to obtain a solution having a known concentration of about 0.4–0.7 mg/mL of fenofibrate, based on the label claim.

**Sample solution:** 60–70 µg/mL of fenofibrate, from the *Sample stock solution*, in *Mobile phase*. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL.

### IMPURITIES

#### Organic Impurities

[NOTE—Use *Sample solution 2* for Capsules labeled to meet the requirements of *Dissolution Test 2*. For all other products, use *Sample solution 1*.]

#### • PROCEDURE

**Solution A:** 136 mg/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid (1 in 10) to a pH of  $2.9 \pm 0.05$ .

**Mobile phase:** Methanol and *Solution A* (4:1)

**Standard solution:** 3.35 µg/mL of USP Fenofibrate RS and 3.35 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*

**System suitability solution:** 0.67 mg/mL of USP Fenofibrate RS and 3.35 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*

**Quantitative limit solution:** 0.67 µg/mL of USP Fenofibrate RS and 0.67 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*, from the *Standard solution*

**Sample solution 1:** Accurately weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the powder, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 min, stir for 15 min, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL. The final concentration based on the label claim is about 0.67 mg/mL.

**Sample solution 2** (For Capsules labeled to meet the requirements of *Dissolution Test 2*): Weigh the contents of NLT 20 Capsules. Mix the contents, melt in an oven at 80° for NLT 30 min, and homogenize. Allow the sample to solidify. Transfer a weighed portion of the sample, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask, dissolve in 30 mL of methanol with the aid of a mechanical shaker for NLT 4 h, and dilute with *Mobile phase* to volume. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 1–2 mL. The final concentration based on the label claim is about 0.67 mg/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 285 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *Standard solution*, *System suitability solution*, and *Quantitative limit solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between fenofibrate and fenofibrate related compound B, *System suitability solution*

**Column efficiency:** NLT 3000 theoretical plates for fenofibrate related compound B, *System suitability solution*

**Tailing factor:** NMT 2.0 for fenofibrate related compound B, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10 for the fenofibrate peak, *Quantitative limit solution*

#### Analysis

**Samples:** *Standard solution* and designated *Sample solution*

Calculate the percentage of fenofibrate related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of fenofibrate related compound B from the *Sample solution*

$r_S$  = peak response of fenofibrate related compound B from the *Standard solution*

$C_S$  = concentration of fenofibrate related compound B in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of fenofibrate from the *Standard solution*

$C_S$  = concentration of fenofibrate in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** NMT 0.5% for fenofibrate related compound B; NMT 0.2% for any other individual impurity

**Total impurities:** NMT 2.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Fenofibrate RS  
USP Fenofibrate Related Compound B RS  
2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.

## Fenofibrate Tablets

### DEFINITION

Fenofibrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fenofibrate ( $C_{20}H_{21}ClO_4$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Acidified water:** Adjust the pH of water with phosphoric acid to  $2.5 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Acidified water* (70:30)

**System suitability stock solution:** 0.1 mg/mL each of USP Fenofibrate Related Compound A RS and USP Fenofibrate Related Compound B RS in acetonitrile

**System suitability solution:** 0.5 µg/mL each of USP Fenofibrate Related Compound A RS and USP Fenofibrate Related Compound B RS in *Mobile phase* from *System suitability stock solution*

**Standard solution:** 0.05 mg/mL of USP Fenofibrate RS in *Mobile phase*

**Sample stock solution:** Prepare a solution containing approximately 2–4 mg/mL of fenofibrate by disintegrating the appropriate number of Tablets with sonication in *Acidified water*, using 30% of the final volume of the flask. Add acetonitrile to approximately 90% of flask volume, and sonicate with periodic swirling. Dilute with acetonitrile to volume.

**Sample solution:** 0.05 mg/mL of fenofibrate in *Mobile phase*, based on the label claim from the *Sample stock solution*. Filter a portion of this solution, discarding the first few mL of the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 4.0-mm × 25-cm or 4.6-mm × 25-cm; 5-µm or 4-µm packing L1

**Column temperature:** 35°

**Flow rate:** 1.2 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between fenofibrate related compound A and fenofibrate related compound B peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenofibrate ( $C_{20}H_{21}ClO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

##### Test 1

**Medium:** 0.025 M sodium dodecyl sulfate in water; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Acidified water:** Adjust the pH of water with phosphoric acid to  $2.5 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Acidified water* (70:30)

**Standard stock solution:** 2.5 mg/mL of USP Fenofibrate RS in acetonitrile

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of about  $(0.001 \times L)$  mg/mL, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 2-mm × 3-cm; 3-µm packing L1

**Column temperature:** 35°

**Flow rate:** 1.2 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NLT 0.9 and NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenofibrate ( $C_{20}H_{21}ClO_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 1000 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of fenofibrate ( $C_{20}H_{21}ClO_4$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.05 M sodium dodecyl sulfate in water; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 136 mg/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of  $2.9 \pm 0.05$ .

**Mobile phase:** Methanol and *Buffer* (80:20)

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

**Standard solution:**  $(0.001 \times L)$  mg/mL of USP Fenofibrate RS in *Mobile phase*, where  $L$  is the label claim, in mg/Tablet

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC  
 Detector: UV 286 nm  
 Column: 4.6-mm × 15-cm; 5-μm packing L1  
 Flow rate: 1.0 mL/min  
 Injection size: 10 μL

**System suitability**

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenofibrate ( $C_{20}H_{21}ClO_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of fenofibrate is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

Acidified water, Mobile phase, System suitability solution, Sample stock solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.5 μg/mL of USP Fenofibrate RS in *Mobile phase*

Sample solution: 0.5 mg/mL of fenofibrate in *Mobile phase*, based on the label claim from the *Sample stock solution*. Filter a portion of this solution, discarding the first few mL of filtrate.

**System suitability**

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between fenofibrate related compound A and fenofibrate related compound B peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of fenofibrate from the *Standard solution*  
 $C_S$  = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

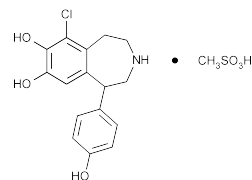
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fenofibrate related compound A	0.34	1.3	0.2
Fenofibrate related compound B	0.36	1.0	0.2
(3 <i>RS</i> )-3-[4-(4-Chlorobenzoyl)phenoxy]butan-2-one	0.50	—	— <sup>a</sup>
Methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate	0.65	—	— <sup>a</sup>
Ethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate	0.80	—	— <sup>a</sup>
(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanone	0.85	—	— <sup>a</sup>
Fenofibrate	1.00	—	—
Fenofibrate related compound C <sup>b</sup>	1.35	—	— <sup>a</sup>
Any unspecified impurity	—	1.0	0.2
Total impurities (includes fenofibrate related compounds A and B, and unspecified impurities)	—	—	0.3

<sup>a</sup> Disregard this impurity. It is a process impurity and is controlled in the drug substance monograph.

<sup>b</sup> 1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
  - USP Fenofibrate RS
  - USP Fenofibrate Related Compound A RS (4-Chlorophenyl)(4-hydroxyphenyl)methanone.  
 $C_{13}H_9ClO_2$  232.66
  - USP Fenofibrate Related Compound B RS 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.  
 $C_{17}H_{15}ClO_4$  318.75

**Fenoldopam Mesylate**

$C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$  401.86

1*H*-3-Benzazepine-7,8-diol, 6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-, methanesulfonate (salt).

6-Chloro-2,3,4,5-tetrahydro-1-(*p*-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol methanesulfonate (salt) [67227-57-0].

» Fenoldopam Mesylate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, protected from moisture. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Fenoldopam Mesylate RS

USP Fenoldopam Related Compound A RS

1-Methyl-3-benzazepine-7,8-diol, 6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-, methanesulfonate (salt).  $C_{17}H_{18}ClNO_3 \cdot CH_4SO_3$  415.89

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method I* (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Limit of iodide**—

*Mobile phase*—Prepare a filtered and degassed solution containing about 0.94 g of sodium bicarbonate, 0.952 g of sodium carbonate, 0.38 g of 4-cyanophenol, and 80 mL of acetonitrile in 4 L of water. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard stock solution*—Transfer about 118.1 mg of sodium iodide, accurately weighed, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix to obtain a solution containing the equivalent of 100 µg of iodide per mL.

*Standard solutions*—Pipet 2.0 mL, 4.0 mL, 6.0 mL, and 8.0 mL of the *Standard stock solution* into separate 100-mL volumetric flasks, dilute with water to volume, and mix to obtain solutions having known concentrations of about 2 µg, 4 µg, 6 µg, and 8 µg of iodide per mL.

*Test solution*—Transfer about 300 mg of Fenoldopam Mesylate, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

*Chromatographic system*—The ion chromatograph is equipped with a conductivity detector, a 4-mm × 3.5-cm anion-exchange guard column, a 4-mm × 15-cm anion-exchange analytical column, and a micromembrane anion suppressor column. The flow rate is about 2.0 mL per minute. The regeneration solution for the suppressor column is a 0.050 M sulfuric acid solution, flowing at a rate of 5 mL per minute. Chromatograph the 6 µg per mL *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 100 µL) of each of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and measure the heights of peak responses. Plot the response of each of the *Standard solutions* versus the concentration, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the quantity of iodide in the portion of Fenoldopam Mesylate taken: not more than 0.2% is found.

**Related compounds**—

*Buffer solution*, *System suitability stock solution*, and *System suitability solution*—Proceed as directed in the *Assay*.

*Solution A*—Use *Mobile phase* as prepared in the *Assay*.

*Solution B*—Use filtered and degassed methanol.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Test solution*—Use the *System suitability stock solution*.

*Chromatographic system*—Proceed as directed in the *Assay*, except to program the chromatograph as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–30	100	0	isocratic
30–60	100→70	0→30	linear gradient

*Procedure*—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fenoldopam Mesylate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 0.3% of fenoldopam related compound A is found; not more than 0.1% of any other individual impurity is found; and not more than 1.0% of total impurities is found.

**Limit of residual solvents**—

*Internal standard solution*—Prepare a solution, in organic-free water, containing 10 mg of *n*-butanol per mL. Transfer 100 µL of this solution to a 10-mL volumetric flask, dilute with dimethylsulfoxide to volume, and mix.

*Standard solution*—Prepare a solution, in organic-free water, containing 10 mg each of *n*-propanol, isopropyl alcohol, and dimethylformamide per mL. Transfer 100 µL of this solution to a 10-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Test solution*—Transfer about 50 mg of Fenoldopam Mesylate, accurately weighed, to a 1-mL volumetric flask. Dilute with *Internal standard solution* to volume, and sonicate to dissolve completely.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm × 30-m fused-silica capillary column coated with a 1.8-µm film of stationary phase G43, and a split injection system. The carrier gas is helium, flowing at a rate of about 1 mL per minute through the column and a split ratio of about 50:1. The injection port and the detector temperatures are maintained at 140° and 260°, respectively. The column temperature is programmed as follows. It is maintained for 12 minutes at 40°, then increased at a rate of 8° per minute to 120°, held for 0.1 minute, then increased at a rate of 25° per minute to 180°, and maintained at that temperature for 8 minutes.

*Procedure*—Separately inject equal volumes (about 1 µL) of the *Standard solution*, dimethylsulfoxide, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Identify, based on retention time, any peaks present in the chromatogram of the *Test solution*. Calculate the response factor,  $F_r$ , for each solvent in the *Standard solution* by the formula:

$$(W_R / W_i)(r_i / r_R)$$

in which  $W_R$  is the weight, in mg, of the solvent of interest;  $W_i$  is the weight, in mg, of the internal standard taken to prepare the *Internal standard solution*; and  $r_i$  and  $r_R$  are the peak responses for the internal standard and the solvent of interest, respectively, obtained from the *Standard solution*.

Calculate the percentage, by weight, of each solvent found in the *Test solution* by the formula:

$$100FD(r_i / r_s)(W_i / W_D)$$

in which *F* is the average response factor for the solvent of interest obtained from all injections of the *Standard solution*; *D* is the dilution factor for the internal standard in the *Test solution* (i.e., 0.0001); *r<sub>i</sub>* and *r<sub>s</sub>* are the peak responses for the solvent of interest and the internal standard, respectively, obtained from the *Test solution*; *W<sub>i</sub>* is the weight, in mg, of the internal standard taken to prepare the *Internal standard solution*; and *W<sub>D</sub>* is the weight, in mg, of Fenoldopam Mesylate taken to prepare the *Test solution*: not more than 0.2% of total residual solvents is found.

#### Assay—

**Buffer solution**—Transfer about 16.33 g of monobasic potassium phosphate and 2 mL of triethylamine to a 2-L volumetric flask, and dissolve in 1800 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (19:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability stock solution**—Transfer about 50 mg of Fenoldopam Mesylate, accurately weighed, to a 50-mL volumetric flask. With the aid of an ultrasonic bath, dissolve in and dilute with *Mobile phase* to volume, and mix.

**System suitability solution**—Transfer about 5 mg of USP Fenoldopam Related Compound A RS, accurately weighed, to a 50-mL volumetric flask. Add about 25 mL of *Mobile phase*, and sonicate to dissolve. Add 5 mL of the *System suitability stock solution*, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fenoldopam Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer 5.0 mL of the *System suitability stock solution*, accurately measured, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.7 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R<sub>s</sub>*, between fenoldopam and fenoldopam related compound A is not less than 1.5; the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of C<sub>16</sub>H<sub>16</sub>ClNO<sub>3</sub> · CH<sub>4</sub>SO<sub>3</sub> in the portion of Fenoldopam Mesylate taken by the formula:

$$500C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Fenoldopam Mesylate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for fenoldopam obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fenoldopam Mesylate Injection

» Fenoldopam Mesylate Injection is a sterile solution of Fenoldopam Mesylate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fenoldopam mesylate (C<sub>16</sub>H<sub>16</sub>ClNO<sub>3</sub> · CH<sub>4</sub>SO<sub>3</sub>).

**Packaging and storage**—Preserve in tight, single-dose Containers for Injections as described under *Injections* <1>, preferably of Type I glass. Store in a refrigerator or at controlled room temperature.

#### USP Reference standards <11>—

USP Endotoxin RS

USP Fenoldopam Mesylate RS

USP Fenoldopam Related Compound B RS

1 *H*-3-Benzazepine-7,8-diol, 2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-, methanesulfonate (salt).  
C<sub>16</sub>H<sub>16</sub>NO<sub>3</sub> · CH<sub>4</sub>SO<sub>3</sub> 366.42

#### Identification—

**A: Thin-Layer Chromatographic Identification Test** <201>—

**Test solution**—Pipet 1.0 mL of Injection into a 10-mL volumetric flask. Dilute with methanol to volume, and mix.

**Standard solution**: 1 mg per mL in methanol.

**Application volume**: 20 μL.

**Developing solvent system**—Prepare a homogeneous mixture of acetone, chloroform, acetic acid, and water (6:2:1:1). Place mixture in a paper-lined chromatographic chamber, and equilibrate for about 15 minutes prior to use.

**Procedure**—Proceed as directed in the chapter, and then dry the plate under a current of warm air until completely dry. Place the plate into a second chromatographic chamber containing iodine crystals, and examine the plate: meets the requirements.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 84 USP Endotoxin Units per mg of fenoldopam mesylate.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 2.8 and 3.8.

**Particulate matter** <788>: meets the requirements for small-volume injections.

#### Related compounds—

**Diluent, Mobile phase, System suitability solution, Standard preparation, Column treatment, and Chromatographic system**—Proceed as directed in the *Assay*.

**Test preparation**—Use the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg per mL, of fenoldopam related compound B (C<sub>16</sub>H<sub>16</sub>NO<sub>3</sub> · CH<sub>4</sub>SO<sub>3</sub>) in the volume of Injection taken by the formula:

$$W_S S(D_S / D_U)(r_U / r_S)$$

in which *W<sub>S</sub>* is the weight, in mg, of USP Fenoldopam Related Compound B RS taken; *S* is the conversion factor from the fenoldopam related compound B to the free base (i.e., 0.77026); *D<sub>S</sub>* and *D<sub>U</sub>* are the dilution factors for the *Standard preparation* and the *Test preparation*, respectively (i.e., 0.0001 and 0.02); and *r<sub>U</sub>* and *r<sub>S</sub>* are the average peak responses for fenoldopam related compound B obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 0.6% is found.

**Content of sodium metabisulfite**—Transfer 10.0 mL of Injection to a glass-stoppered conical flask containing 5.0 mL of 0.1 N iodine VS, and swirl to dissolve. Allow to stand for exactly 5 minutes, protected from light. Add 0.5 mL of hydrochloric acid, and titrate the excess iodine with 0.05 N sodium thiosulfate VS, adding 0.5 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N sodium thiosulfate is equivalent to 2.3763 mg of sodium metabisulfite: not less than 0.25 mg per mL of Injection is found.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Diluent**—Dissolve about 1.38 g of monobasic sodium phosphate monohydrate in 1 L of water, adjust with a phosphoric acid solution (1 in 9) to a pH of 2.5, and mix.

**Mobile phase**—Dissolve about 1.38 g of monobasic sodium phosphate monohydrate and 1.88 g of sodium hexanesulfonate in 1 L of water, adjust with a phosphoric acid solution (1 in 9) to a pH of 2.5, and mix. Prepare a filtered and degassed mixture of this solution and methanol (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Transfer about 2.74 mg of USP Fenoldopam Related Compound B RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Standard preparation**—Transfer about 26.3 mg of USP Fenoldopam Mesylate RS, accurately weighed, to a 100-mL volumetric flask, and dissolve in about 75 mL of *Diluent*. Add, by pipetting, 1.0 mL of the *System suitability solution*, dilute with *Diluent* to volume, and mix.

**Assay preparation**—Accurately pipet about 0.5 mL of Injection into a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Column treatment**—[NOTE—This treatment is required for new columns only.] Pump a solution of cyclam (1 in 1000) through the new column for about 3 hours at a flow rate of about 1 mL per minute, and then pump *Mobile phase* for at least 2 hours at the same flow rate.

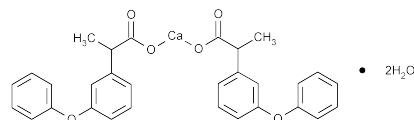
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is between 1 and 3; the column efficiency is not less than 1800 theoretical plates; and the tailing factor is not more than 2.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is between 4 and 6; the column efficiency is not less than 1800 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg per mL, of fenoldopam mesylate ( $C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$ ) in the volume of Injection taken by the formula:

$$W_S(D_S / D_U)(r_U / r_S)$$

in which  $W_S$  is the weight, in mg, of USP Fenoldopam Mesylate RS taken;  $S$  is the conversion factor from the fenoldopam mesylate to the free base (i.e., 0.7610);  $D_S$  and  $D_U$  are the dilution factors for the *Standard preparation* and the *Assay preparation*, respectively (i.e., 0.01 and 0.02); and  $r_U$  and  $r_S$  are the fenoldopam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fenoprofen Calcium



$C_{30}H_{26}CaO_6 \cdot 2H_2O$  558.63

Benzeneacetic acid,  $\alpha$ -methyl-3-phenoxy-, calcium salt dihydrate, ( $\pm$ )-.

Calcium ( $\pm$ )-*m*-phenoxyhydratropate dihydrate [53746-45-5].

Anhydrous 522.61 [34597-40-5].

» Fenoprofen Calcium contains not less than 97.0 percent and not more than 103.0 percent of  $(C_{15}H_{13}O_3)_2Ca$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Fenoprofen Calcium RS

#### Identification—

**A: Infrared Absorption** (197K).

**B:** Heat a 1 in 50 mixture of it with acetic acid, filter, and add 2 mL of ammonium oxalate TS to the filtrate: a white precipitate, which is soluble in 3 N hydrochloric acid, is formed.

**Water, Method I** (921): between 5.0% and 8.0%.

**Heavy metals, Method II** (231): 0.001%.

#### Chromatographic purity—

**Solution A**—Prepare a filtered and degassed mixture of water and acetic acid (98:2).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and acetic acid (98:2).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent:** a mixture of water and acetonitrile (1:1).

**System suitability solution**—Dissolve accurately weighed quantities of 3-phenoxybenzoic acid and USP Fenoprofen Calcium RS in *Diluent* to obtain a solution containing about 0.02 mg of each per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Fenoprofen Calcium RS in *Diluent* to obtain a solution having a known concentration of about 0.02 mg per mL.

**Test solution**—Transfer about 200 mg of Fenoprofen Calcium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a suitable 4.6-mm × 25-cm column<sup>1</sup> that contains 5- $\mu$ m packing L7. The flow rate is 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	70	30	equilibration
0–3	70	30	isocratic
3–41	70→10	30→90	linear gradient
41–42	10	90	isocratic
42–43	10→70	90→30	linear gradient
43–55	70	30	re-equilibration

<sup>1</sup> One suitable column brand is the Zorbax Eclipse XDB-C8.



Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.89 for 3-phenoxybenzoic acid and 1.0 for fenopropfen; the resolution, *R*, between 3-phenoxybenzoic acid and fenopropfen is not less than 9.0; and the tailing factor for the fenopropfen peak is not more than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor for the fenopropfen peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fenopropfen Calcium taken by the formula:

$$10,000(C/W)(r_i/r_s)$$

in which *C* is the concentration, in mg per mL, of USP Fenopropfen Calcium RS in the *Standard solution*; *W* is the quantity, in mg, of Fenopropfen Calcium taken to prepare the *Test solution*; *r<sub>i</sub>* is the response for each impurity peak obtained from the *Test solution*; and *r<sub>s</sub>* is the response of the fenopropfen peak obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

#### Content of calcium—

**Test solution**—Transfer about 750 mg of Fenopropfen Calcium, accurately weighed, to a 50-mL volumetric flask, dissolve in alcohol with the aid of heat, if necessary, cool, dilute with alcohol to volume, and mix.

**Procedure**—In a 150-mL beaker, mix 70 mL of water, 2 mL of sodium hydroxide solution (1 in 10), and about 0.3 g of hydroxy naphthol blue. Add about 1 mL of the *Test solution*, and titrate to the blue endpoint with 0.05 M edetate disodium. Transfer 10.0 mL of the *Test solution* to the solution so obtained, and titrate to the blue endpoint with 0.05 M edetate disodium VS. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of Ca: not less than 7.3% and not more than 8.0% of Ca, calculated on the anhydrous basis, is found.

#### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of acetonitrile, water, and phosphoric acid (50:49.6:0.4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluting solution**—Prepare a mixture of methanol and water (700:300).

**Resolution solution**—Prepare a solution in *Diluting solution* containing about 1 mg of Fenopropfen Calcium and 1 mg of gemfibrozil per mL.

**Standard preparation**—Transfer about 70 mg of USP Fenopropfen Calcium RS, accurately weighed, to a 100-mL volumetric flask, add 0.5 mL of 0.5 N hydrochloric acid and 2 mL of acetone, and dissolve by shaking. Dilute with *Diluting solution* to volume, and mix.

**Assay preparation**—Transfer about 70 mg of Fenopropfen Calcium, accurately weighed, to a 100-mL volumetric flask, add 0.5 mL of 0.5 N hydrochloric acid and 2 mL of acetone, and dissolve by shaking. Dilute with *Diluting solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for fenopropfen and 1.0 for gemfibrozil; the tailing factor for the fenopropfen peak is not more than 2; and the resolution, *R*, between the fenopropfen peak and the gemfibrozil peak is not less than 8. Chromatograph the *Standard prepara-*

*tion*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the fenopropfen peak is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of (C<sub>15</sub>H<sub>13</sub>O<sub>3</sub>)<sub>2</sub>Ca in the portion of Fenopropfen Calcium taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of anhydrous fenopropfen calcium in the *Standard preparation*, as determined from the concentration of USP Fenopropfen Calcium RS, corrected for moisture content by a titrimetric water determination; and *r<sub>U</sub>* and *r<sub>S</sub>* are the fenopropfen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fenopropfen Calcium Capsules

» Fenopropfen Calcium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fenopropfen (C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>).

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Fenopropfen Calcium RS

USP Fenopropfen Sodium RS

#### Identification—

**A:** Transfer a portion of Capsule contents, equivalent to about 85 mg of fenopropfen, to a 125-mL separator containing 5 mL of acetone and 2 mL of 6 N hydrochloric acid. Swirl to dissolve, add 15 mL of water, and extract with three 15-mL portions of chloroform, draining each chloroform extract through a layer of about 2 g of anhydrous sodium sulfate, supported on glass wool and previously washed with chloroform, into a 50-mL volumetric flask. Rinse the sodium sulfate filter with about 2 mL of chloroform, collect the rinsing with the combined chloroform extracts, dilute with chloroform to volume, and mix. Transfer this solution to a suitable flask, and evaporate on a water bath with the aid of a current of air to dryness: the IR absorption spectrum of a film of the liquid residue thus obtained between sodium chloride plates exhibits maxima only at the same wavelengths as that of a similar preparation of USP Fenopropfen Calcium RS.

**B:** Place an amount of Capsule contents, equivalent to about 300 mg of fenopropfen, in a suitable container, and dissolve in 10 mL of acetone. Filter the solution through paper, and collect the filtrate in a crucible. Carefully evaporate to dryness, and ignite the crucible and its contents. Dissolve the residue in 10 mL of 1 N hydrochloric acid, transfer the solution to a beaker, add 2 drops of methyl red TS, neutralize with 6 N ammonium hydroxide, and add 3 N hydrochloric acid dropwise until the solution is acid to the indicator. Upon the addition of ammonium oxalate TS, a white precipitate is formed. The residue so obtained is insoluble in acetic acid but dissolves in hydrochloric acid.

#### Dissolution (711)—

**Medium:** pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

**Apparatus 1:** 10-mesh basket; 100 rpm.

**Time:** 60 minutes.

**Procedure**—Filter 20 mL of the solution under test, and transfer 5.0 mL of the filtrate to a 25-mL volumetric flask. Dilute with *Dissolution Medium* to volume, and mix. Determine the absorbances of this solution and a Standard solution prepared from USP Fenopropfen Sodium RS, in the same medium having a known concentration of about 60 µg per mL, at the wavelength of maximum absorbance at about 270 nm, using *Dissolution Medium* as the blank. Calculate the amount of C<sub>15</sub>H<sub>14</sub>O<sub>3</sub> dissolved, in mg, by the formula:

$$(242.28 / 264.26)(5C)(A_U / A_S)$$

in which 242.28 is the molecular weight of fenopropfen; 264.26 is the molecular weight of anhydrous fenopropfen sodium; C is the concentration of anhydrous fenopropfen sodium in the Standard solution, as determined from the concentration of USP Fenopropfen Sodium RS corrected for moisture content by a titrimetric water determination; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solutions obtained from the substance under test and the USP Reference Standard, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>15</sub>H<sub>14</sub>O<sub>3</sub> is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Diluting solution, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Fenopropfen Calcium*.

**Assay preparation**—Weigh the contents of not fewer than 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 150 mg of fenopropfen, to a 250-mL volumetric flask. Add about 200 mL of *Diluting solution*, and sonicate for about 15 minutes. Allow to cool, dilute with *Diluting solution* to volume, mix, and pass through a suitable filter, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed in the Assay under *Fenopropfen Calcium*. Calculate the quantity, in mg, of fenopropfen (C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>) in the portion of Capsules taken by the formula:

$$(484.55 / 522.61)(1000C)(r_U / r_S)$$

in which 484.55 is two times the molecular weight of fenopropfen; 522.61 is the molecular weight of fenopropfen calcium; and the other terms are as defined therein.

## Fenopropfen Calcium Tablets

» Fenopropfen Calcium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fenopropfen (C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>).

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Fenopropfen Calcium RS

USP Fenopropfen Sodium RS

**Identification**—An amount of finely powdered Tablets responds to the *Identification* tests under *Fenopropfen Calcium Capsules*.

#### Dissolution (711)—

**Medium:** pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

**Apparatus 1:** 10-mesh basket; 100 rpm.

**Time:** 60 minutes.

**Procedure**—Filter 20 mL of the solution under test, and transfer 10.0 mL of the filtrate to a 100-mL volumetric flask. Dilute with *Dissolution Medium* to volume, and mix. Determine the absorbances of this solution and a Standard solution prepared from USP Fenopropfen Sodium RS, in the same medium having a known concentration of about 60 µg per mL at the wavelength of maximum absorbance at about 270 nm, using *Dissolution Medium* as the blank. Calculate the amount of C<sub>15</sub>H<sub>14</sub>O<sub>3</sub> dissolved, in mg, by the formula:

$$(242.28 / 264.26)(10C)(A_U / A_S)$$

in which 242.28 is the molecular weight of fenopropfen; 264.26 is the molecular weight of anhydrous fenopropfen sodium; C is the concentration of anhydrous fenopropfen sodium in the Standard solution, as determined from the concentration of USP Fenopropfen Sodium RS corrected for moisture content by a titrimetric water determination; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solutions obtained from the substance under test and the USP Reference Standard, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>15</sub>H<sub>14</sub>O<sub>3</sub> is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Diluting solution, Resolution solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Fenopropfen Calcium*.

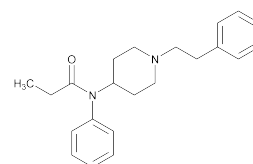
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6000 mg of fenopropfen, to a 250-mL volumetric flask, add 50 mL of 0.5 N hydrochloric acid, and sonicate for 10 minutes, shaking the solution occasionally. Dilute with acetone to volume, sonicate for 10 minutes, and stir for an additional 30 minutes. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.5-µm or finer porosity, discarding the first 8 mL of the filtrate, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed in the Assay under *Fenopropfen Calcium*. Calculate the quantity, in mg, of fenopropfen (C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>) in the portion of Tablets taken by the formula:

$$(484.55 / 522.61)(10,000C)(r_U / r_S)$$

in which 484.55 is two times the molecular weight of fenopropfen; 522.61 is the molecular weight of fenopropfen calcium; and the other terms are as defined therein.

## Fentanyl



C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O

Propanamide, N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl];

336.47

*N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylpropionamide  
[437-38-7].

### DEFINITION

Fentanyl contains NLT 98.0% and NMT 102.0% of the labeled amount of  $C_{22}H_{28}N_2O$ , calculated on the dried basis.

**[CAUTION]**—Great care should be taken to prevent inhaling particles of Fentanyl and exposing the skin to it.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** Add 3 mL of triethylamine to about 950 mL of water in a 1000-mL volumetric flask. Adjust with perchloric acid to a pH of  $2.62 \pm 0.02$ , and dilute with water to volume.

**Solution B:** Acetonitrile

**Diluent:** *Solution A* and *Solution B* (9:1)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
3.5	85	15
5.1	82	18
7.6	72	28
11.5	63	37
15	40	60
19	40	60
20	90	10
25	90	10

**System suitability stock solution:** 0.4 mg/mL of USP Fentanyl Related Compound A RS, and about 0.1 mg/mL each of USP Fentanyl Related Compound B RS, USP Fentanyl Related Compound D RS, USP Fentanyl Related Compound E RS, and USP Fentanyl Related Compound G RS, prepared by dissolving in a suitable volumetric flask 50% filled with acetonitrile and diluting with water to volume

**Standard stock solution:** 1 mg/mL of USP Fentanyl RS prepared by dissolving in a suitable volumetric flask 40% filled with acetonitrile and diluting with water to volume

**Standard solution:** 0.1 mg/mL of USP Fentanyl RS from the *Standard stock solution* in *Diluent*

**System suitability solution:** 0.3 µg/mL each of USP Fentanyl Related Compound B RS, USP Fentanyl Related Compound D RS, USP Fentanyl Related Compound G RS, and USP Fentanyl Related Compound E RS; 1.3 µg/mL of USP Fentanyl Related Compound A RS; and 100 µg/mL of USP Fentanyl RS, prepared by diluting an appropriate amount of *System suitability stock solution* and *Standard stock solution* in *Diluent*

**Sample stock solution:** Transfer 100 mg of Fentanyl to a 100-mL volumetric flask. Add 25 mL of acetonitrile, dissolve by shaking, and dilute with *Diluent* to volume.

**Sample solution:** 0.1 mg/mL of Fentanyl from the *Sample stock solution* in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L7

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 30 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Tailing factor:** NLT 0.5 and NMT 2.0 for fentanyl and all fentanyl related compound peaks

**Resolution:** NLT 1.2 between fentanyl and fentanyl related compound E peaks

**Relative standard deviation:** NMT 0.7% for fentanyl and NMT 10% for all fentanyl related compounds

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fentanyl ( $C_{22}H_{28}N_2O$ ) in the portion of Fentanyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of fentanyl from the *Sample solution*

$r_S$  = peak response of fentanyl from the *Standard solution*

$C_S$  = concentration of fentanyl in the *Standard solution*

$C_U$  = concentration of Fentanyl in the *Sample solution*

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.5%

• **HEAVY METALS**, *Method II* (231): NMT 20 ppm

#### • ORGANIC IMPURITIES

**Solution A, Solution B, Diluent, System suitability solution, Sample stock solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1.2 µg/mL in *Diluent* prepared by dissolving 0.1 mg/mL of USP Fentanyl Related Compound E RS in a suitable volumetric flask 50% filled with acetonitrile and then diluting with water to volume. Dilute an aliquot of this solution with *Diluent* to achieve the desired concentration.

**Standard solution:** 0.024 µg/mL of USP Fentanyl Related Compound E RS from the *Standard stock solution* in *Diluent*

**Sample solution:** 250 µg/mL of Fentanyl from the *Sample stock solution* in *Diluent*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Fentanyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of fentanyl related compound E from the *Standard solution*

$C_S$  = concentration of USP Fentanyl Related Compound E RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Fentanyl in the *Sample solution* (µg/mL)

$F$  = relative response factor of the fentanyl related compounds (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

[NOTE—Identify fentanyl related compound C and fentanyl related compound F by using the relative retention times provided in *Table 2*. Identify the other impurity peaks in the sample by comparing them to those in the *System suitability solution*.]

Table 2

Name	Relative Retention Time <sup>a</sup>	Relative Response Factor <sup>b</sup>	Acceptance Criteria, NMT (%)
Fentanyl related compound B <sup>c</sup>	0.28	0.67	0.015
Fentanyl related compound C <sup>d</sup>	0.56	0.67	0.25
Fentanyl related compound D <sup>e</sup>	0.86	0.97	0.015
Fentanyl related compound G <sup>f</sup>	0.89	0.82	0.25
Fentanyl related compound F <sup>g</sup>	0.92	0.75	0.25
Fentanyl related compound E <sup>h</sup>	0.98	1.0	0.015
Fentanyl	1.00	—	—
Fentanyl related compound A <sup>i</sup>	1.26	0.55	0.25
Individual unspecified impurities	—	—	0.10
Total impurities	—	—	0.5

<sup>a</sup> The relative retention time is calculated based on fentanyl.

<sup>b</sup> The relative response factor (RRF) is calculated based on fentanyl related compound E.

<sup>c</sup> 4-Anilinopiperidine.

<sup>d</sup> N-Phenyl-N-(4-piperidyl)propanamide.

<sup>e</sup> N-Phenyl-1-(phenylmethyl)-4-piperidinamine.

<sup>f</sup> N-Phenyl-N-[1-(2-phenylethyl)-4-piperidyl]acetanilide hydrochloride, or acetyl fentanyl.

<sup>g</sup> N-(1-Benzyl-4-piperidyl)propionanilide.

<sup>h</sup> N-Phenyl-1-(2-phenylethyl)-4-piperidinamine.

<sup>i</sup> (2-Bromoethyl)benzene.

(Organic Impurities postponed indefinitely)

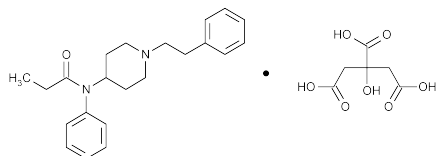
### SPECIFIC TESTS

- **Loss on Drying** (731): Dry a sample in a vacuum at 60° for 2 h: it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tightly closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP Reference Standards** (11)
  - USP Fentanyl RS
  - USP Fentanyl Related Compound A RS
  - USP Fentanyl Related Compound B RS
  - USP Fentanyl Related Compound C RS
  - USP Fentanyl Related Compound D RS
  - USP Fentanyl Related Compound E RS
  - USP Fentanyl Related Compound F RS
  - USP Fentanyl Related Compound G RS

## Fentanyl Citrate



$C_{22}H_{28}N_2O \cdot C_6H_8O_7$  528.59

Propanamide, N-phenyl-N-[1-(2-phenylethyl)-4-piperidyl]-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1).

N-(1-Phenethyl-4-piperidyl)propionanilide citrate (1:1) [990-73-8].

» Fentanyl Citrate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{22}H_{28}N_2O \cdot C_6H_8O_7$ , calculated on the dried basis.

[Caution—Great care should be taken to prevent inhaling particles of Fentanyl Citrate and exposing the skin to it.]

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Fentanyl Citrate RS

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

Solution: 500 µg per mL.

Medium: dilute hydrochloric acid in methanol (1 in 10).

**Loss on drying** (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** (231): 0.002%.

**Ordinary impurities** (466)—

Test solution: a mixture of chloroform and methanol (4:1).

Standard solution: a mixture of chloroform and methanol (4:1) except to eliminate the 0.01 mg per mL solution and add a 0.02 mg per mL solution.

Procedure—Use a thin-layer chromatographic plate coated with chromatographic silica gel with a calcium sulfate binder.

Eluant: a mixture of chloroform, methanol, and formic acid (85:10:5).

Visualization: 3.

**Assay**—Dissolve about 500 mg of Fentanyl Citrate, accurately weighed, in 30 mL of glacial acetic acid. Add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.05 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 26.43 mg of  $C_{22}H_{28}N_2O \cdot C_6H_8O_7$ .

## Fentanyl Citrate Injection

» Fentanyl Citrate Injection is a sterile solution of Fentanyl Citrate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fentanyl ( $C_{22}H_{28}N_2O$ ), present as the citrate.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Fentanyl Citrate RS

**Identification**—The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Bacterial endotoxins** (85)—It contains not more than 50.0 USP Endotoxin Units per mg.

**pH** (791): between 4.0 and 7.5.

**Other requirements**—It meets the requirements under Injections (1).

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture containing 4 volumes of ammonium acetate solution (1 in 100) and 6 volumes of a mixture of methanol, acetonitrile, and glacial acetic acid (400:200:0.6). Adjust this solution to a pH of  $6.6 \pm 0.1$  by the dropwise addition of glacial acetic acid, and make adjustments if necessary (see *System Suitability* under *Chromatography* <621>), to obtain a retention time of about 5 minutes for the fentanyl peak.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fentanyl Citrate RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of about 80 µg per mL.

**Assay preparation**—If necessary, dilute the Injection with water so that each mL contains the equivalent of about 50 µg of fentanyl.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the tailing factor for the fentanyl peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of fentanyl ( $C_{22}H_{28}N_3O$ ) in each mL of the Injection taken by the formula:

$$(336.48 / 528.59)CD(r_U / r_S)$$

in which 336.48 and 528.59 are the molecular weights of fentanyl and fentanyl citrate, respectively; C is the concentration, in µg per mL, of USP Fentanyl Citrate RS in the *Standard preparation*; D is the dilution factor used to obtain the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for the fentanyl peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Ferric Subsulfate Solution**


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$Fe_4(OH)_2(SO_4)_5$  737.71

Basic ferric sulfate solution.

Monse's Solution [1310-45-8].

» Ferric Subsulfate Solution contains, in each 100 mL, basic ferric sulfate equivalent to not less than 20 g and not more than 22 g of iron (Fe). Ferric Subsulfate Solution may be prepared as follows. Add 55 mL of Sulfuric Acid to 800 mL of water in a porcelain dish and heat to nearly 100°, then add 75 mL of nitric acid, and mix. Divide 1045 g of Ferrous Sulfate, coarsely powdered, into 4 portions, and add these portions one at a time to the hot liquid, stirring after each addition until effervescence ceases. If, after the Ferrous Sulfate has dissolved, the solution has a black color, add nitric acid, a few drops at a time, with heating and stirring, until red fumes cease to be evolved. Boil the solution until it assumes a red color and is free from nitrate, as indicated by the test for *Limit of nitrate* below, maintaining the volume at about 1000 mL by the addition of water as needed. Cool, and add enough water to make the solution measure

1000 mL. Filter, if necessary, until the Solution is clear.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at temperatures above 22°.

**Labeling**—The label indicates that crystallization may occur if the Solution is exposed to temperatures below 22°, and that warming will redissolve the crystals. Label it to indicate that it is intended for topical and vaginal use only.

**Identification—**

**A:** A 1 in 20 dilution of the Solution in water yields a brownish-red precipitate with ammonia TS.

**B:** A 1 in 20 dilution of the Solution in water yields a blue precipitate with potassium ferrocyanide TS.

**C:** A 1 in 20 dilution of the Solution in water yields a white precipitate with barium chloride TS that is insoluble in hydrochloric acid.

**Limit of nitrate**—Add a clear crystal of ferrous sulfate to a cooled mixture of equal volumes of sulfuric acid and a 1 in 10 dilution of the Solution in water: the crystal does not become brown, nor does a brownish-black color develop around it.

**Limit of ferrous salts**—Add a few drops of freshly prepared potassium ferricyanide TS to 2 mL of 1 in 20 dilution of the solution in water: a brown color is produced and the solution remains free from even a transient green or greenish-blue color.

**Assay**—Transfer 10.0 mL of the Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a glass-stoppered conical flask, and add 5 mL of hydrochloric acid and 3 g of potassium iodide. Insert the stopper into the flask, and allow the mixture to stand for 15 minutes. Add 15 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

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**Ferric Sulfate**


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$Fe_2(SO_4)_3 \cdot xH_2O$  (anhydrous) 399.88

Ferric persulfate.

Ferric sesquisulfate.

Ferric tersulfate [10028-22-5].

Hydrate [142906-29-4].

» Ferric Sulfate is hydrated  $Fe_2(SO_4)_3$ . It contains not less than 73.0 percent and not more than 80.0 percent of  $Fe_2(SO_4)_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**Labeling**—Label it to indicate that it is intended for use in compounding topical and periodontal dosage forms only.

**Identification**—Dissolve 0.5 g in a mixture of 50 mL of water and 3 mL of hydrochloric acid: this solution meets the requirements for the *Ferric Salts* test under *Iron* <191>.

**Limit of insoluble matter**—Transfer 10 g in a covered beaker, and dissolve in a mixture of 100 mL of water and 5 mL of sulfuric acid. Heat to boiling, and warm on a steam bath for 1 hour. Filter the hot solution through a tared sintered-glass crucible of fine porosity. Wash the beaker and the filter with hot water, dry the crucible at 105°, cool in a desiccator, and weigh: not more than 2 mg of insoluble matter is found (0.02%).

**Limit of chloride**—Dissolve 1 g by warming with a mixture of 10 mL of water and 1 mL of nitric acid, add 4 mL of additional nitric acid, and dilute with water to 50 mL. To 25 mL of this solution add 1 mL of phosphoric acid and 1 mL of silver nitrate TS. Any turbidity does not exceed that

produced in a control containing 0.01 mg of chloride ion (Cl), 1 mL of nitric acid, 1 mL of phosphoric acid, and 1 mL of silver nitrate TS (0.002%).

**Limit of ferrous iron**—Dissolve 4 g by warming with 50 mL of dilute sulfuric acid (1 in 10), cool, and titrate with 0.1 N potassium permanganate: not more than 0.16 mL is required to produce a permanent pink color (0.02% as  $\text{Fe}^{++}$ ).

**Limit of copper and zinc**—[NOTE—If the reagents used in the tests for *Copper* and *Zinc* contain excessive amounts of copper and zinc, then they should first be purified by extracting with *Dithizone Extraction Solution* (see *Lead* (251)).]

**Copper**—Dissolve 1.2 g in 100 mL of water. To 10 mL of this test solution add 50 mL of a solution containing 5 g of ammonium tartrate and 5 mL of ammonium hydroxide. Add 10 mL of *Standard Dithizone Solution* (see *Lead* (251)), shake for 2 minutes, draw off the dithizone layer, and compare the pink color with that in a control containing 6  $\mu\text{g}$  of copper ion (Cu) and treated exactly as the 10-mL portion of test solution. If the color in the test solution is less than that in the control, then less than 0.005% of Cu and of Zn is found and the test for *Zinc* is not necessary. If the color in the test solution is more than that in the control, add 15 mL of dilute hydrochloric acid (1 in 250), and shake for 2 minutes. Draw off the dithizone solution, and shake with a second 15 mL of dilute hydrochloric acid (1 in 250) for 2 minutes. [NOTE—Draw off the dithizone, combine the two acid extracts, and reserve for the test for *Zinc*.] Any pink color in the dithizone solution is not darker than that in the control solution treated exactly as the test solution (0.005%).

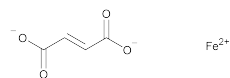
**Zinc**—To the combined acid extracts saved from the test for *Copper*, add sufficient 0.5 M sodium acetate to bring the pH to between 5.0 and 5.5, and then add 1 mL of 0.1 N sodium thiosulfate. Add 10 mL of *Standard Dithizone Solution* (see *Lead* (251)), shake for 2 minutes, and allow the layers to separate. Draw off the dithizone layer, and discard the water layer. Any pink color in the dithizone layer is not greater than that in a control prepared by adding 0.006 mg of zinc ion (Zn) to the combined acid extracts from the control used in the test for *Copper* (0.005%).

**Limit of nitrate**—Dissolve 10 g in 100 mL of dilute sulfuric acid (1 in 100), heat to boiling, and pour, slowly, into a mixture of 140 mL of water and 50 mL of ammonium hydroxide. Pass through a folded filter while still hot, wash the filter with hot water until the volume of filtrate is 300 mL, mix, and cool. To 15 mL of the diluted filtrate add 1 mL of sodium chloride solution (1 in 200), 0.10 mL of indigo carmine TS, and 15 mL of sulfuric acid. The blue color is not completely discharged at the end of 5 minutes (0.01%).

**Substances not precipitated by ammonia**—Evaporate to dryness 30 mL of the diluted filtrate obtained in the test for *Limit of nitrate*, and ignite gently: the weight of residue does not exceed 1 mg (0.1%).

**Assay**—Transfer about 700 mg of Ferric Sulfate, accurately weighed, to a glass-stoppered conical flask. Add a mixture of 50 mL of water and 3 mL of hydrochloric acid, and swirl to dissolve. Add 3 g of potassium iodide, insert the stopper into the flask, and allow to stand in the dark for 30 minutes. Then add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 19.994 mg of ferric sulfate [ $\text{Fe}_2(\text{SO}_4)_3$ ].

## Ferrous Fumarate



$\text{C}_4\text{H}_2\text{FeO}_4$  169.90  
2-Butenedioic acid, (E)-, iron(2+) salt;  
Iron(2+) fumarate [141-01-5].

### DEFINITION

Ferrous Fumarate contains NLT 97.0% and NMT 101.0% of ferrous fumarate ( $\text{C}_4\text{H}_2\text{FeO}_4$ ), calculated on the dried basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION

**Sample:** To 1.5 g of Ferrous Fumarate, add 25 mL of dilute hydrochloric acid (1 in 2). Dilute with water to 50 mL, heat to dissolve, then cool, and filter on a fine-pore size, sintered-glass crucible. Wash the precipitate with dilute hydrochloric acid (3 in 100), saving the filtrate for *Identification* test B, and dry the precipitate at 105°.

**Acceptance criteria:** The IR absorption of a potassium bromide dispersion of the dried precipitate exhibits maxima only at the same wavelengths as that of a similar preparation of USP Fumaric Acid RS.

- B. IDENTIFICATION TESTS—GENERAL, Iron (191):** A portion of the filtrate obtained in *Identification* test A meets the requirements.

### ASSAY

#### PROCEDURE

**Sample:** 500 mg of Ferrous Fumarate

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 500-mL conical flask, and add 25 mL of dilute hydrochloric acid (2 in 5). Heat to boiling, and add, dropwise, a solution of 112 mg/mL of stannous chloride in dilute hydrochloric acid (3 in 10) until the yellow color disappears, then add 2 drops in excess. Cool the solution in an ice bath to room temperature, add 10 mL of 50 mg/mL of mercuric chloride solution, and allow to stand for 5 min. Add 200 mL of water, 25 mL of dilute sulfuric acid (1 in 2), and 4 mL of phosphoric acid. Then add 2 drops of orthophenanthroline TS, and titrate with *Titrant*. Perform a *Blank* determination.

Calculate the percentage of ferrous fumarate ( $\text{C}_4\text{H}_2\text{FeO}_4$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 169.9 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 97.0%–101.0% on the dried basis

### IMPURITIES

#### SULFATE

**Sample solution:** Transfer 1.0 g of Ferrous Fumarate to a 250-mL beaker, add 100 mL of water, and heat on a steam bath, adding hydrochloric acid dropwise until completely dissolved. [NOTE—About 2 mL of the acid will be required.] Filter the solution if necessary, and dilute the filtrate with water to 100 mL. Heat the filtrate

to boiling, add 10 mL of barium chloride TS, warm on a steam bath for 2 h, cover, and allow to stand for 16 h. [NOTE—If crystals of ferrous fumarate form, warm the solution on the steam bath to dissolve them.]

**Analysis:** Pass the *Sample solution* through ashless filter paper, wash the residue with hot water until, with the addition of ammonium sulfide TS, a black precipitate is no longer formed in the filtrate. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents at 600° to constant weight.

**Acceptance criteria:** NMT 0.2%: Each mg of residue is equivalent to 0.412 mg of sulfate (SO<sub>4</sub>).

• **ARSENIC, Method I (211)**

**Test preparation:** To 2.0 g in 10 mL of water add 10 mL of sulfuric acid. Warm to precipitate the fumaric acid completely, cool, add 30 mL of water, and filter into a 100-mL volumetric flask. Wash the precipitate with water, adding the washings to the flask, then add water to volume, and mix. Transfer 50.0 mL of this solution into the arsine generator flask, and dilute with water to 55 mL.

**Analysis:** Proceed as directed in the chapter, except omit the addition of 20 mL of 7 N sulfuric acid specified for the *Procedure*.

**Acceptance criteria:** NMT 3 ppm

• **LIMIT OF FERRIC IRON**

**Sample:** 2 g of Ferrous Fumarate

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis**

**Analysis:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask, add 25 mL of water and 4 mL of hydrochloric acid, and heat on a hot plate until solution is complete. Insert the stopper in the flask, and cool to room temperature. Add 3 g of potassium iodide, insert the stopper in the flask, swirl to mix, and allow to stand in the dark for 5 min. Remove the stopper, add 75 mL of water, and titrate with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a *Blank* determination.

Calculate the percentage of ferric iron in the portion of Ferrous Fumarate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F/W] \times 100\}$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 55.85 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** NMT 2.0%

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

**Ascorbic acid–sodium iodide solution:** 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

**Trioctylphosphine oxide solution:** 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone

**[CAUTION—]**This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

**Standard solution:** Transfer 5.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in *Heavy Metals* (231), to a 100-mL volumetric flask. Dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL beaker. To this beaker add 6 mL of nitric acid and 10 mL of perchloric acid, and evaporate in a hood to dryness. **[CAUTION—]**Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cool, dissolve the residues in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic solvent layer is the *Standard solution*, and it contains 2.0 µg/mL of lead.

**Sample solution:** Transfer 1.0 g of Ferrous Fumarate to a 50-mL beaker, and add 6 mL of nitric acid and 10 mL of perchloric acid. **[CAUTION—]**Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cover with a ribbed watch glass, and heat in a hood until completely dry. Cool, dissolve the residue in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

**Blank:** To a 50-mL beaker add 6 mL of nitric acid and 10 mL of perchloric acid, and evaporate in a hood to dryness. **[CAUTION—]**Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cool, dissolve the residue in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 283.3 nm

**Lamp:** Lead hollow-cathode

**Flame:** Air–acetylene

**System suitability**

**Samples:** *Standard solution* and *Blank*

**Suitability requirements:** The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

**Acceptance criteria:** NMT 10 ppm: The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **MERCURY**

[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

**Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and Diluted dithizone extraction solution:** Prepare as directed in *Mercury* (261), *Method I*.

**Control solution:** Mix 3.0 mL of *Standard mercury solution*, 30 mL of dilute nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution*.

**Test preparation:** Dissolve 1 g of Ferrous Fumarate in 30 mL of dilute nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with dilute nitric acid (1 in 10) and water. To the filtrate add 20 mL of 250 mg/mL of sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride* solution.

**Analysis:** Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide, and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.

Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution*.

**Acceptance criteria:** NMT 3 µg/g: The color developed by the *Sample solution* is not more intense than that developed by the *Control solution*.

### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 16 h: it loses NMT 1.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Fumaric Acid RS

## Ferrous Fumarate Tablets

### DEFINITION

Ferrous Fumarate Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of ferrous fumarate ( $C_4H_2FeO_4$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Iron** (191)

**Sample solution:** To a portion of powdered Tablets, equivalent to 1 g of ferrous fumarate, add 25 mL of dilute hydrochloric acid (1 in 2), mix, and add 25 mL of water. Boil the solution for a few min, cool, and filter.

**Acceptance criteria:** The filtrate meets the requirements.

### ASSAY

- **PROCEDURE**

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets equivalent to 500 mg of ferrous fumarate

**Blank:** Proceed as in the *Analysis* without the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Indirect titration

**Titrant:** 0.1 N sodium thiosulfate VS

**Indicator:** Starch TS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 25 mL of water, 25 mL of nitric acid, and 7.5 mL of perchloric acid. Cover with a ribbed watch glass, and heat to the production of strong fumes. Cool, rinse the watch glass and the sides of the beaker with water, and evaporate in a hood to near-dryness. Wash down the watch glass and the sides of the beaker with 2 mL of hydrochloric acid and then with a small volume of water. Warm slightly, if necessary, to dissolve the residue. Transfer to a glass-stoppered, 250-mL conical flask. Repeat the washing with 2 mL of hydrochloric acid, and complete the transfer to the flask, using NMT 20–25 mL of water for the transfer. Add 4 g of potassium iodide to the flask, insert the stopper, and allow to stand in the dark for 5 min. Add 75 mL of water and titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination. Calculate the percentage of the labeled amount of ferrous fumarate ( $C_4H_2FeO_4$ ) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $N$  = actual normality of the *Titrant* (mEq/mL)  
 $F$  = equivalency factor, 169.9 mg/mEq  
 $W$  = nominal weight of ferrous fumarate in the *Sample* taken (mg)

**Acceptance criteria:** 95.0%–110.0%

### PERFORMANCE TESTS

- **DISSOLUTION** (711)

**Medium:** 0.1 N hydrochloric acid in 0.5% sodium lauryl sulfate; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of iron in the *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 248.3 nm

**Lamp:** Iron hollow-cathode

**Flame:** Air–acetylene

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of ferrous fumarate ( $C_4H_2FeO_4$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

$M_r$  = molecular weight of ferrous fumarate, 169.9  
 $A_r$  = atomic weight of iron, 55.85  
 $C$  = measured concentration of iron in the *Sample solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label amount of ferrous fumarate (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of ferrous fumarate is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets in terms of ferrous fumarate ( $C_4H_2FeO_4$ ) and in terms of elemental iron.



## Ferrous Fumarate and Docusate Sodium Extended-Release Tablets

» Ferrous Fumarate and Docusate Sodium Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ferrous fumarate ( $C_4H_2FeO_4$ ) and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of docusate sodium ( $C_{20}H_{37}NaO_7S$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the Tablets in terms of the content of ferrous fumarate ( $C_4H_2FeO_4$ ) and in terms of the content of elemental iron.

**USP Reference standards** (11)—

USP Docusate Sodium RS

**Dissolution** (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Times: 1 and 3 hours.

Determine the amount of Fe (II) dissolved, on filtered portions of the solution under test, employing the method described under *Assay for ferrous fumarate* with the following modification.

**Standard solution**—Transfer the appropriate amount of *Iron stock solution* to a volumetric flask, and dilute with 0.1 N hydrochloric acid in such a way that the final concentration is similar to that expected in the solution under test.

**Tolerances**—The percentages of the labeled amount of Fe (II) dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 40% and 75%
3	not less than 80%

**Uniformity of dosage units** (905): meet the requirements with respect to iron.

**Assay for ferrous fumarate**—

**6 N Hydrochloric acid**—Slowly add 5 mL of hydrochloric acid to 5 mL of water, and mix.

**Diluting solution**—Add 1 mL of 6 N Hydrochloric acid to 59 mL of water, and mix.

**Phosphoric acid solution**—Dilute 20 mL of phosphoric acid with *Diluting solution* to 200 mL, and mix.

**Iron stock solution**—Transfer about 350 mg of ferrous ammonium sulfate hexahydrate, accurately weighed, to a 1000-mL volumetric flask, dissolve in *Diluting solution*, dilute with *Diluting solution* to volume, and mix to obtain a solution having a known concentration of about 50 µg per mL.

**Standard preparations**—To separate 100-mL volumetric flasks transfer 2.0, 4.0, 6.0, 8.0, and 10.0 mL of *Iron stock solution*. To each flask add 6.0 mL of *Phosphoric acid solution*, dilute with *Diluting solution* to volume, and mix. The *Standard preparations* so obtained contain about 1.0, 2.0, 3.0, 4.0, and 5.0 µg of iron per mL, respectively.

**Blank solution**—Transfer 6.0 mL of *Phosphoric acid solution* to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 g of ferrous fumarate, to a 1000-mL volumetric flask, add 110 mL of 6 N Hydrochloric acid, and boil for 30 minutes. Cool, dilute with water

to volume, mix, and filter. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. Transfer 8.0 mL of this solution to a 100-mL volumetric flask, add 6.0 mL of *Phosphoric acid solution*, dilute with *Diluting solution* to volume, and mix to obtain a solution having a known concentration of about 4 µg of iron per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the iron emission line at 248.3 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with an iron hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard preparations* versus their concentrations, in µg per mL, of iron, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of iron in the *Assay preparation*. Calculate the average quantity, in mg, of ferrous fumarate ( $C_4H_2FeO_4$ ) in each Tablet taken by the formula:

$$(TC/D)(169.90/55.85)$$

in which *T* is the labeled quantity, in mg, of ferrous fumarate in each Tablet; *C* is the concentration, in µg per mL, of iron in the *Assay preparation*; *D* is the concentration, in µg per mL, of ferrous fumarate in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; and 169.90 and 55.85 are the molecular weight of ferrous fumarate and the atomic weight of iron, respectively.

**Assay for docusate sodium**—

**Calcium acetate solution**—Dissolve 4 g of calcium acetate in 2000 mL of water.

**Diluting solution**—Mix 450 mL of acetonitrile and 550 mL of *Calcium acetate solution*.

**Mobile phase**—Add 2 mL of phosphoric acid to 1000 mL of *Diluting solution*, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Filter, and degas.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Docusate Sodium RS in *Diluting solution* to obtain a solution having a known concentration of about 1 mg per mL.

**Sodium benzoate solution**—Dissolve an accurately weighed quantity of sodium benzoate in *Diluting solution*, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 8 µg per mL.

**Resolution solution**—Dissolve a suitable quantity of USP Docusate Sodium RS in *Sodium benzoate solution* to obtain a solution containing about 1 mg per mL of docusate sodium.

**Assay preparation**—Transfer a number of Tablets, equivalent to about 2 g of docusate sodium, to a 2000-mL volumetric flask. Add about 1500 mL of *Diluting solution*, and sonicate with frequent shaking until the Tablets are completely disintegrated. Cool, dilute with *Diluting solution* to volume, and mix. Centrifuge, and use the clear supernatant. If the supernatant is not clear, pass through a membrane filter.

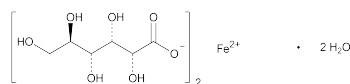
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 30-cm column that contains 3-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between sodium benzoate and docusate sodium is not less than 7.0; the tailing factor is not less than 0.9 and not more than 3.5; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for six replicate injections of the *Standard preparation* is not more than 2.0%. The relative retention times are 1.0 for docusate sodium and 0.25 for sodium benzoate.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the average quantity, in mg, of docusate sodium ( $C_{20}H_{37}NaO_7S$ ) in each of the Tablets taken by the formula:

$$(2000C/N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Docusate Sodium RS in the *Standard preparation*; *N* is the number of Tablets taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ferrous Gluconate



$C_{12}H_{22}FeO_{14} \cdot 2H_2O$  482.17  
D-Gluconic acid, iron(2+) salt (2:1), dihydrate;  
Iron(2+) gluconate (1:2) dihydrate [12389-15-0].  
Anhydrous 446.15 [299-29-6].

### DEFINITION

Ferrous Gluconate contains NLT 97.0% and NMT 102.0% of ferrous gluconate ( $C_{12}H_{22}FeO_{14}$ ), calculated on the dried basis.

### IDENTIFICATION

#### A. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** 10 mg/mL of Ferrous Gluconate, heating in a water bath at 60°, if necessary, to dissolve

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

#### B. FERROUS ION

**Sample solution:** 5 mg/mL in water

**Analysis:** Add potassium ferricyanide TS to the *Sample solution*.

**Acceptance criteria:** The solution yields a dark blue precipitate.

### ASSAY

#### PROCEDURE

**Sample:** 1.5 g of Ferrous Gluconate

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of 75 mL of water and 15 mL of 2 N sulfuric acid in a 300-mL conical flask. Add 250 mg of zinc dust, close the flask with a stopper containing a Bunsen valve, and allow to stand at room temperature for 20 min or until the solution becomes colorless. Pass the solution through a filtering crucible containing a thin layer of zinc dust, and wash the crucible and contents with 10 mL of 2 N sulfuric acid, followed by 10 mL of water. [NOTE—Prepare and use the filtering crucible in a well-ventilated hood.]

Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant*. Perform a *Blank* determination.

Calculate the percentage of ferrous gluconate ( $C_{12}H_{22}FeO_{14}$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F] / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 446.2 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

#### CHLORIDE AND SULFATE, Chloride <221>

**Standard solution:** 1.0 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g

**Acceptance criteria:** NMT 0.07%

#### CHLORIDE AND SULFATE, Sulfate <221>

**Standard solution:** 1.0 mL of 0.020 N sulfuric acid

**Sample:** 1.0 g

**Acceptance criteria:** NMT 0.1%

#### MERCURY <261>

NMT 3 ppm

#### ARSENIC, Method I <211>

**Test preparation:** Transfer 1.0 g of Ferrous Gluconate to a 100-mL round-bottom flask fitted with a 24/40 standard-taper joint. Add 40 mL of 9 N sulfuric acid and 2 mL of potassium bromide solution (3 in 10). Immediately connect to a suitable distillation apparatus that has a reservoir with a water jacket, cooled with circulating ice water, and heat to dissolve the Ferrous Gluconate. Distill, collect 25 mL of distillate, and transfer the distillate to the arsine generator flask. Wash the condenser and reservoir several times with small portions of water, add the washings to the distillate in the generator flask, add bromine TS until the solution is slightly yellow, and dilute with water to 35 mL. Proceed as directed in the chapter.

**Acceptance criteria:** NMT 3 ppm

#### LIMIT OF LEAD

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

**Ascorbic acid–sodium iodide solution:** 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

**Triocetylphosphine oxide solution:** 50 mg/mL of triocetylphosphine oxide in 4-methyl-2-pentanone.

[CAUTION—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

**Standard solution:** Transfer 5.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in the test for *Heavy Metals* (231), to a 100-mL volumetric flask, and dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask, add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.

**Sample solution:** To a 50-mL volumetric flask add 1.0 g of Ferrous Gluconate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

**Blank:** To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 283.3 nm

**Lamp:** Lead hollow-cathode

**Flame:** Air–acetylene

#### System suitability

**Samples:** *Standard solution* and *Blank*

**Suitability requirements:** The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and *Sample solution*. Use the *Blank* to set the instrument to zero.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

#### • LIMIT OF FERRIC IRON

**Sample:** 5 g of Ferrous Gluconate

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of 100 mL of water and 10 mL of hydrochloric acid, and add 3 g of potassium iodide. Shake, and allow to stand in the dark for 5 min. Titrate any liberated iodine with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a *Blank* determination.

Calculate the percentage of ferric iron in the portion of Ferrous Gluconate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant volume consumed by the Sample* (mL)

$V_B$  = *Titrant volume consumed by the Blank* (mL)

$N$  = *Titrant normality* (mEq/mL)

$F$  = *equivalency factor*, 55.85 mg/mEq

$W$  = *Sample weight* (mg)

**Acceptance criteria:** NMT 2.0%

#### • OXALIC ACID

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* in 10 mL of water, add 2 mL of hydrochloric acid, and transfer to a separator. Extract successively with 50 mL and 20 mL of ether. Combine the ether extracts, add 10 mL of water, and evaporate the ether on a steam bath. Add 1 drop of 6 N acetic acid and 1 mL of a 50 mg/mL solution of calcium acetate.

**Acceptance criteria:** No turbidity is produced within 5 min.

#### • REDUCING SUGARS

**Sample:** 500 mg

**Analysis:** Dissolve the *Sample* in 10 mL of water, warm, and render alkaline with 1 mL of 6 N ammonium hydroxide. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the solution to stand for 30 min to coagulate the precipitate. Filter, and wash the precipitate with two successive 5-mL portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 mL of 3 N hydrochloric acid in excess. Boil the solution until the vapors no longer darken lead acetate paper, and continue to boil, if necessary, until it has been concentrated to 10 mL. Cool, add 5 mL of sodium carbonate TS and 20 mL of water, filter, and adjust the volume of the filtrate to 100 mL. To 5 mL of the filtrate add 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

**Acceptance criteria:** No red precipitate is formed within 1 min.

#### SPECIFIC TESTS

• **LOSS ON DRYING** (731): Dry a sample at 105° for 16 h: it loses 6.5%–10.0% of its weight.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Potassium Gluconate RS

## Ferrous Gluconate Capsules

#### DEFINITION

Ferrous Gluconate Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from the contents of the Capsules

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 µL

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

- B. FERROUS ION**

**Sample solution:** Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution of the *Sample solution* obtained in *Identification test A*

**Analysis:** Add potassium ferricyanide TS to the *Sample solution*.

**Acceptance criteria:** The solution yields a dark blue precipitate.

**ASSAY**

- PROCEDURE**

**Buffer:** Dissolve 3.0 g of sodium acetate in 50 mL of water. Add 2.0 mL of glacial acetic acid, dilute with water to 200 mL, and mix. This *Buffer* has a pH of 4.6.

**Color reagent solution:** Dissolve 400 mg of 2,2'-bipyridine in 100 mL of water, using heat if necessary, to dissolve. Cool, and filter.

**Standard stock solution:** 7.022 mg/mL of ferrous ammonium sulfate hexahydrate [ $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ] in water, equivalent to 1000 µg/mL of iron (Fe)

**Standard solution:** 100 µg/mL of iron (Fe) in 0.1 N sulfuric acid from *Standard stock solution*

**Sample solution**

**For hard gelatin capsules:** Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container. Mix and finely powder the combined contents, and transfer a weighed portion of the powder, equivalent to 430 mg of ferrous gluconate dihydrate, to a 500-mL volumetric flask. Add 300 mL of water, heating on a steam bath if necessary to dissolve. Cool, and dilute with water to volume.

**For soft gelatin capsules:** Place a number of Capsules, equivalent to 430 mg of ferrous gluconate dihydrate, in a 500-mL volumetric flask. Add 300 mL of water, heating on a steam bath to dissolve the Capsules. Cool, and dilute with water to volume.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV-Vis

**Analytical wavelength:** 522 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Transfer 3.0 mL of the *Sample solution* to a 100-mL volumetric flask, and add, in the order named, 70 mL of *Buffer*, 10.0 mL of 100 mg/mL of sodium thiosulfate solution, and 5.0 mL of *Color reagent solution*, with mixing following each addition. Heat for 60 min on a steam bath, cool, dilute with *Buffer* to volume, and filter. Prepare reagent blanks for the *Standard solution* and the *Sample solution* by repeating the above procedure, omitting the addition of 5.0 mL of *Color reagent solution*.

Concomitantly determine the absorbances of the reacted solutions against the corresponding reagent blanks.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ( $\text{C}_{12}\text{H}_{22}\text{FeO}_{14} \cdot 2\text{H}_2\text{O}$ ) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_r/A_r) \times 100$$

$A_U$  = absorbance of the *Sample solution* corrected by the absorbance of its reagent blank

$A_S$  = absorbance of the *Standard solution* corrected by the absorbance of its reagent blank

$C_S$  = concentration of iron in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of ferrous gluconate dihydrate in the *Sample solution* (µg/mL)

$M_r$  = molecular weight of ferrous gluconate dihydrate, 482.17

$A_r$  = atomic weight of iron, 55.85

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS**

- DISSOLUTION <711>**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of iron in the *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 248.3 nm

**Lamp:** Iron hollow-cathode

**Flame:** Air-acetylene

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ( $\text{C}_{12}\text{H}_{22}\text{FeO}_{14} \cdot 2\text{H}_2\text{O}$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

$M_r$  = molecular weight of ferrous gluconate dihydrate, 482.17

$A_r$  = atomic weight of iron, 55.85

$C$  = measured concentration of iron in the *Sample solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label amount of ferrous gluconate dihydrate (mg/Capsule)

**Tolerances:** NLT 75% (Q) of the labeled amount of ferrous gluconate dihydrate ( $\text{C}_{12}\text{H}_{22}\text{FeO}_{14} \cdot 2\text{H}_2\text{O}$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Label the Capsules in terms of the content of ferrous gluconate dihydrate ( $\text{C}_{12}\text{H}_{22}\text{FeO}_{14} \cdot 2\text{H}_2\text{O}$ ) and in terms of the content of elemental iron.
- USP REFERENCE STANDARDS <11>**  
USP Potassium Gluconate RS

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**Ferrous Gluconate Oral Solution**


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**DEFINITION**

Ferrous Gluconate Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of ferrous gluconate dihydrate ( $\text{C}_{12}\text{H}_{22}\text{FeO}_{14} \cdot 2\text{H}_2\text{O}$ ).

**IDENTIFICATION**

- A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS in water

**Sample solution:** A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from Oral Solution

**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel**Application volume:** 5 µL**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.**Analysis****Samples:** *Standard solution* and *Sample solution*Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.• **B. FERROUS ION****Sample solution:** Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution in water of the *Sample solution* obtained in *Identification test A***Analysis:** Add potassium ferricyanide TS to the *Sample solution*.**Acceptance criteria:** The solution yields a dark blue precipitate.**ASSAY**• **PROCEDURE****Sample:** An accurately measured volume of Oral Solution, equivalent to 1.2 g of ferrous gluconate dihydrate**Blank:** Proceed as directed in the *Analysis* without the *Sample*.**Titrimetric system**(See *Titrimetry* <541>.)**Mode:** Direct titration**Titrant:** 0.1 N ceric sulfate VS**Indicator:** Orthophenanthroline TS**Endpoint detection:** Visual**Analysis:** Dissolve the *Sample* in a cooled mixture of 80 mL of recently boiled water and 80 mL of 2 N sulfuric acid. Add orthophenanthroline TS, and immediately titrate with *Titrant* until a change in color. Perform a *Blank* determination.Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) in the portion of Oral Solution taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F / W\} \times 100$$

 $V_S$  = *Titrant* volume consumed by the *Sample* (mL) $V_B$  = *Titrant* volume consumed by the *Blank* (mL) $N$  = actual normality of the *Titrant* (mEq/mL) $F$  = equivalency factor, 482.2 mg/mEq $W$  = nominal amount of ferrous gluconate dihydrate in the *Sample* taken (mg)**Acceptance criteria:** 94.0%–106.0%**OTHER COMPONENTS**

- **ALCOHOL DETERMINATION** <611>: 6.3%–7.7% of  $C_2H_5OH$

**SPECIFIC TESTS**

- **pH** <791>: 3.4–3.8

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Oral Solution in terms of the content of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) and in terms of the content of elemental iron.

• **USP REFERENCE STANDARDS** <11>

USP Potassium Gluconate RS

**Ferrous Gluconate Tablets****DEFINITION**Ferrous Gluconate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ).**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY****Standard solution:** 10 mg/mL of USP Potassium Gluconate RS**Sample solution:** A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from powdered Tablets**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel**Application volume:** 5 µL**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.**Analysis****Samples:** *Standard solution* and *Sample solution*Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.• **B. FERROUS ION****Sample solution:** Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution of the *Sample solution* obtained in *Identification test A***Analysis:** Add potassium ferricyanide TS to the *Sample solution*.**Acceptance criteria:** The solution yields a dark blue precipitate.**ASSAY**• **PROCEDURE****Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 1.5 g of ferrous gluconate dihydrate**Blank:** Proceed as directed in the *Analysis* without the *Sample*.**Titrimetric system**(See *Titrimetry* <541>.)**Mode:** Direct titration**Titrant:** 0.1 N ceric sulfate VS**Indicator:** Orthophenanthroline TS**Endpoint detection:** Visual**Analysis:** Dissolve the *Sample* in a mixture of 75 mL of water and 15 mL of 2 N sulfuric acid in a 300-mL conical flask. Add 250 mg of zinc dust, close the flask with a stopper containing a Bunsen valve, and allow to stand at room temperature for 20 min or until the solution becomes colorless. Pass the solution through a filtering crucible containing a thin layer of zinc dust, and wash the crucible and contents with 10 mL of 2 N sulfuric acid, followed by 10 mL of water.

[NOTE—Prepare and use the filtering crucible in a well-ventilated hood.]

Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant* until color change. Perform a *Blank* determination. Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) in the portion of Tablets taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $N$  = actual normality of the *Titrant* (mEq/mL)  
 $F$  = equivalency factor, 482.2 mg/mEq  
 $W$  = nominal amount of ferrous gluconate dihydrate in the *Sample* taken (mg)

Acceptance criteria: 93.0%–107.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

**Medium:** Simulated gastric fluid TS; 900 mL

**Apparatus 2:** 150 rpm

**Time:** 80 min

**Standard solution:** Solution having a known concentration of iron in the *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 248.3 nm

**Lamp:** Iron hollow-cathode

**Flame:** Air–acetylene

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

$M_r$  = molecular weight of ferrous gluconate dihydrate, 482.17  
 $A_r$  = atomic weight of iron, 55.85  
 $C$  = measured concentration of iron in the *Sample solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label amount of ferrous gluconate dihydrate (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>**: Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets in terms of the content of ferrous gluconate dihydrate ( $C_{12}H_{22}FeO_{14} \cdot 2H_2O$ ) and in terms of the content of elemental iron.
- **USP REFERENCE STANDARDS <11>**  
USP Potassium Gluconate RS

## Ferrous Sulfate

$FeSO_4 \cdot 7H_2O$	278.01
$FeSO_4$	151.91
Sulfuric acid, iron(2+) salt (1:1), heptahydrate; Iron(2+) sulfate (1:1) heptahydrate [7782-63-0]. Anhydrous [7720-78-7].	

## DEFINITION

Ferrous Sulfate contains an amount of anhydrous ferrous sulfate ( $FeSO_4$ ) equivalent to NLT 99.5% and NMT 104.5% of ferrous sulfate heptahydrate ( $FeSO_4 \cdot 7H_2O$ ).

## IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Iron, Ferrous Salts <191> and Sulfate <191>**: Meets the requirements

## ASSAY

### • PROCEDURE

**Sample:** 1 g of Ferrous Sulfate

**Blank:** Proceed as in the *Analysis* without the *Sample*.

### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of freshly boiled and cooled water. Add orthophenanthroline TS, and immediately titrate with *Titrant*. Perform a blank determination. Calculate the percentage of ferrous sulfate heptahydrate ( $FeSO_4 \cdot 7H_2O$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)  
 $V_B$  = *Titrant* volume consumed by the *Blank* (mL)  
 $N$  = *Titrant* normality (mEq/mL)  
 $F$  = equivalency factor, 278.0 mg/mEq  
 $W$  = *Sample* weight (mg)

**Acceptance criteria:** 99.5%–104.5% of ferrous sulfate heptahydrate

## IMPURITIES

### • ARSENIC, Method I <211>

**Test preparation:** Transfer 1 g of Ferrous Sulfate to a round-bottomed, 100-mL flask fitted with a glass joint, and add 40 mL of sulfuric acid (1 in 4) and 2 mL of 300 mg/mL potassium bromide solution. Immediately connect the flask to a condenser having a matching glass joint and a reservoir with a water jacket that is cooled by ice water. Heat the flask gently over a low flame until the solid dissolves, then distill until 25 mL of distillate collects in the reservoir. Transfer this distillate to the arsine generator flask, and wash the condenser and the reservoir with several small portions of water, adding the washings to the generator flask. Swirl to mix, add bromine TS until the color of the solution is slightly yellow, and dilute with water to 35 mL.

**Acceptance criteria:** NMT 3 ppm

### • LEAD

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

**Ascorbic acid–sodium iodide solution:** 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

**Trioctylphosphine oxide solution:** 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone

**[CAUTION—** This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.)

**Standard solution:** Transfer 5.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in *Heavy Metals* (231), to a 100-mL volumetric flask. Dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic*

acid-sodium iodide solution and 5.0 mL of Trioctylphosphine oxide solution, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.

**Sample solution:** To a 50-mL volumetric flask add 1.0 g of Ferrous Sulfate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of Ascorbic acid-sodium iodide solution, and 5.0 mL of Trioctylphosphine oxide solution. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

**Blank:** To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of Ascorbic acid-sodium iodide solution, and 5.0 mL of Trioctylphosphine oxide solution. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 283.3 nm

**Lamp:** Lead hollow-cathode

**Flame:** Air-acetylene

#### System suitability

**Samples:** *Standard solution* and *Blank*

**Suitability requirements:** The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

#### • MERCURY

[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

**Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and Diluted dithizone extraction solution:** Prepare as directed in *Mercury* (261), *Method I*.

**Control solution:** Mix 3.0 mL of *Standard mercury solution*, 30 mL of dilute nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution*.

**Test preparation:** Dissolve 1 g of Ferrous Sulfate in 30 mL of dilute nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with dilute nitric acid (1 in 10) and water. To the filtrate add 20 mL of 250 mg/mL sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride solution*.

**Analysis:** Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.

Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of dilute hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL of edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its

outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution*.

**Acceptance criteria:** The color developed by the *Sample solution* is not more intense than that developed by the *Control solution* (NMT 3 µg/g).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is not to be used if it is coated with brownish yellow basic ferric sulfate.

## Ferrous Sulfate Oral Solution

#### DEFINITION

Ferrous Sulfate Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Iron, Ferrous Salts (191) and Sulfate (191):** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Sample:** A volume of Oral Solution equivalent to 625 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  accurately measured

**Blank:** Proceed as in the *Analysis* without the *Sample*.

##### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Indicator:** Orthophenanthroline TS

**Endpoint detection:** Visual

**Analysis:** Add 25 mL of 2 N sulfuric acid and 75 mL of freshly boiled and cooled water to the *Sample*, and shake well. Then add orthophenanthroline TS, and immediately titrate with *Titrant* until the color changes. Perform a blank determination.

Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) in the *Sample* taken:

$$\text{Result} = \left\{ \left[ (V_S - V_B) \times N \times F \right] / W \right\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 278.0 mg/mEq

$W$  = nominal amount of ferrous sulfate heptahydrate in the *Sample* taken (mg)

**Acceptance criteria:** 94.0%–106.0% of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )

#### SPECIFIC TESTS

- **pH (791):** 1.4–5.3

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Oral Solution in terms of the content of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) and in terms of the content of elemental iron.

## Ferrous Sulfate Syrup

### DEFINITION

Ferrous Sulfate Syrup contains, in each 100 mL, NLT 3.75 g and NMT 4.25 g of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), equivalent to NLT 0.75 g and NMT 0.85 g of elemental iron.

Ferrous Sulfate Syrup is prepared as follows.

Ferrous Sulfate	40 g
Citric Acid, hydrous	2.1 g
Peppermint Spirit	2 mL
Sucrose	825 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Ferrous Sulfate*, *Citric Acid*, *Peppermint Spirit*, and 200 g of *Sucrose* in 450 mL of *Purified Water*, and filter the solution until clear. Dissolve the remainder of the *Sucrose* in the clear filtrate, and add *Purified Water* to make 1000 mL. Mix, and filter if necessary, through a pledget of cotton.

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, *Iron*, *Ferrous Salts* <191> and *Sulfate* <191>

**Sample solution:** Dilute 1 mL of Syrup with water to 100 mL.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample:** 25 mL of Syrup

**Blank:** Proceed as in the *Analysis* without the *Sample*.

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Indicator:** Orthophenanthroline TS

**Endpoint detection:** Visual

**Analysis:** Add 15 mL of 2 N sulfuric acid and 100 mL of water to the *Sample*, and shake well. Add 3 drops of orthophenanthroline TS, and titrate with *Titrant* until the color changes. Perform a blank determination. Calculate the amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) in g per each 100 mL of Syrup:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 0.278 g/mEq

$W$  = volume of Ferrous Sulfate Syrup for the *Sample* taken (mL)

**Acceptance criteria:** 3.75–4.25 g in each 100 mL

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight containers.

#### • LABELING: Label the Syrup in terms of the content of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) and in terms of the content of elemental iron.

## Ferrous Sulfate Tablets

### DEFINITION

Ferrous Sulfate Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ). [NOTE—An equivalent amount of Dried Ferrous Sulfate may be used in place of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in preparing Ferrous Sulfate Tablets.]

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, *Iron*, *Ferrous Salts* <191> and *Sulfate* <191>

**Sample solution:** Equivalent to 10 mg/mL of ferrous sulfate heptahydrate from powdered Tablets in water acidified with hydrochloric acid

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample:** Equivalent to 500 mg of ferrous sulfate heptahydrate from finely powdered Tablets (NLT 20)

**Blank:** Proceed as in the *Analysis* without the *Sample*.

#### Titrimetric system

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Indicator:** Orthophenanthroline TS

**Endpoint detection:** Visual

**Analysis:** In a beaker, dissolve the *Sample* in a mixture of 20 mL of 2 N sulfuric acid and 80 mL of freshly boiled and cooled water. Filter the solution rapidly as soon as all soluble ingredients in the Tablets are dissolved, and wash the container and the filter with small portions of a mixture of 20 mL of 2 N sulfuric acid and 80 mL of freshly boiled and cooled water.

Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant* until the color changes. Perform a blank determination.

Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 278.0 mg/mEq

$W$  = nominal weight of ferrous sulfate heptahydrate in the *Sample* taken (mg)

**Acceptance criteria:** 95.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of iron in the *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 248.3 nm

**Lamp:** Iron hollow-cathode

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$



- $M_r$  = molecular weight of ferrous sulfate heptahydrate, 278.02  
 $A_r$  = atomic weight of iron, 55.85  
 $C$  = measured concentration of iron in the *Sample solution* (mg/mL)  
 $D$  = dilution factor for the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets in terms of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) and in terms of elemental iron.

### Dried Ferrous Sulfate

$\text{FeSO}_4 \cdot x\text{H}_2\text{O}$  151.91  
 Sulfuric acid, iron(2+) salt (1:1), hydrate;  
 Iron(2+) sulfate (1:1) hydrate [13463-43-9].  
 Anhydrous [7720-78-7].

#### DEFINITION

Dried Ferrous Sulfate contains NLT 86.0% and NMT 89.0% of anhydrous ferrous sulfate ( $\text{FeSO}_4$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Iron, Ferrous Salts (191) and Sulfate (191):** Meets the requirements

#### ASSAY

##### PROCEDURE

**Sample:** 800 mg of Dried Ferrous Sulfate

**Blank:** Proceed as in the *Analysis* without the *Sample*.

##### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of freshly boiled and cooled water. Add orthophenanthroline TS, and immediately titrate with the *Titrant*. Perform a blank determination. Calculate the percentage of anhydrous ferrous sulfate ( $\text{FeSO}_4$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 151.9 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 86.0%–89.0%

#### IMPURITIES

- **ARSENIC, Method I (211)**

**Test preparation:** Transfer 1 g of Dried Ferrous Sulfate to a round-bottomed, 100-mL flask fitted with a glass joint, and add 40 mL of sulfuric acid (1 in 2) and 2 mL of 300 mg/mL of potassium bromide solution. Immediately connect the flask to a condenser having a matching glass joint and a reservoir with a water jacket that is cooled by ice water. Heat the flask gently over a low flame until the solid dissolves, then distill until 25 mL of distillate collects in the reservoir. Transfer this distillate to the arsine generator flask, and wash the condenser and the reservoir with several small portions of water, adding the washings to the generator flask. Swirl to

mix, add bromine TS until the color of the solution is slightly yellow, and dilute with water to 35 mL.

**Acceptance criteria:** NMT 3 ppm

##### LIMIT OF LEAD

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

**Ascorbic acid–sodium iodide solution:** 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

**Trioctylphosphine oxide solution:** 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone

[**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

**Standard solution:** Transfer 5.0 mL of *Lead Nitrate Stock Solution*, prepared as directed in *Heavy Metals* (231), to a 100-mL volumetric flask. Dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.

**Sample solution:** To a 50-mL volumetric flask add 1.0 g of Dried Ferrous Sulfate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

**Blank:** To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

##### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 283.3 nm

**Lamp:** Lead hollow-cathode

**Flame:** Air–acetylene

##### System suitability

**Samples:** *Standard solution* and *Blank*

**Suitability requirements:** The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

##### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

##### MERCURY

[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

**Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and Diluted dithizone extraction solution:** Prepare as directed in *Mercury* (261), *Method I*.

**Control solution:** Mix 3.0 mL of *Standard mercury solution*, 30 mL of nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution*.

**Test preparation:** Dissolve 1 g of Dried Ferrous Sulfate in 30 mL of nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with nitric acid (1 in 10) and water. To the filtrate, add 20 mL of 250 mg/mL of sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride solution*.

**Analysis:** Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.

Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL of edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution*.

**Acceptance criteria:** The color developed by the *Sample solution* is not more intense than that developed by the *Control solution* (NMT 3 ppm).

#### • INSOLUBLE SUBSTANCES

**Sample:** 2.0 g of Dried Ferrous Sulfate

**Analysis:** Dissolve the *Sample* in 20 mL of freshly boiled dilute sulfuric acid (1 in 100), heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°.

**Acceptance criteria:** The weight of the insoluble residue does not exceed 1 mg (NMT 0.05%).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Ferumoxides Injection

» Ferumoxides Injection is a sterile colloidal suspension of superparamagnetic iron oxide associated with dextran in Water for Injection. It contains not less than 95 percent and not more than 105 percent of the labeled amount of iron. It contains in each mL not less than 5.6 mg and not more than 9.1 mg of dextran. It contains in each mL not less than 0.25 mg and not more than 0.53 mg of citrate. It also contains mannitol. It contains no antimicrobial agents. Ferumoxides is a nonstoichiometric iron oxide magnetite of average formula  $\text{FeO}_{1.44}$ , with particles having a diameter between 100 and 250 nm.

**Packaging and storage**—Preserve in single-dose containers of Type I glass, and store at controlled room temperature. Avoid freezing.

**Labeling**—Label it to indicate that it is to be administered through a 5- $\mu\text{m}$  filter and that it is not to be used if there are indications that the package has been exposed to freezing temperatures.

#### USP Reference standards (11)—

USP Dextran T-10 RS

USP Dextrose RS

USP Endotoxin RS

**Identification**—Transfer about 1 mL of the Injection to a test tube, and add 2 drops of ammonium hydroxide: no precipitate is formed. Add 2 mL of hydrochloric acid, mix, and add 2 mL of ammonium hydroxide: a brown precipitate is formed.

**Specific gravity** (841): between 1.031 and 1.041.

**Bacterial endotoxins** (85)—It contains not more than 12.5 USP Endotoxin Units per mL.

**pH** (791): between 5.0 and 9.0.

#### Colloidal particle size—

**Apparatus**—Use a submicron laser light-scattering instrument.<sup>1</sup>

**Standards**—Use 90- and 270-nm NIST-traceable polystyrene monospheres.

**Standard dilutions**—Transfer 3 mL of 0.2- $\mu\text{m}$  filtered water to each of two clear acrylic cuvettes. Add a sufficient amount of the 90-nm monospheres to one of the cuvettes and a sufficient amount of the 270-nm monospheres to the other cuvette to make the dilutions slightly turbid, place in the *Apparatus*, and then measure the particle sizes: the particle size in the *Standards* is within 10% of the certified diameter.

**Procedure**—Transfer about 40  $\mu\text{L}$  of the Injection to a clear acrylic cuvette, and add 3 mL of 0.2- $\mu\text{m}$  filtered water. Cover, invert to mix (without shaking), place in the *Apparatus*, and measure the intensity-weighted effective diameter. [NOTE—Several minutes are necessary for the sample to reach equilibrium.] The intensity-weighted effective diameter is between 100 and 250 nm.

#### Magnetic susceptibility—

**Apparatus:** a calibrated magnetic susceptibility balance.

**Standard solutions**—Transfer 118.85 g of nickel chloride hexahydrate, accurately weighed, to a 500-mL volumetric flask, dilute with water to volume, and mix to obtain 1 M nickel chloride. Transfer 356.55 g of nickel chloride hexahydrate, accurately weighed, to a separate 500-mL volumetric flask, dilute with water to volume, and mix to obtain 3 M nickel chloride.

**Balance constant**—[NOTE—Each magnetic susceptibility balance has a *Balance constant*,  $K$ , that is determined each time the balance is moved to a new location. The *Balance constant* is referenced to the known magnetic susceptibility of nickel chloride hexahydrate.] Measure the length,  $L$ , in cm, of the large-bore susceptibility tube, from the bottom of the inside of the tube to the bottom of the black band. Tare the susceptibility tube on a balance, fill it with water to the bottom of the black band, and weigh again. Record the weight,  $W$ , in g, of the water in the tube, zero the susceptibility balance, and then place the tube containing water in the susceptibility balance. Zero the balance again. Rinse the tube with 1 M nickel chloride, and then fill the tube with fresh 1 M nickel chloride. Insert the tube into the susceptibility balance, and record the magnetic susceptibility,  $R$ , in

<sup>1</sup> Brookhaven Instruments 90Plus or equivalent.

cgs units, of 1 M nickel chloride. Calculate the *Balance constant*,  $K$ , by the formula:

$$(4.24 \times 10^{-6})(W \times 10^9)/RL$$

in which  $4.24 \times 10^{-6}$  is the magnetic susceptibility, in cgs units, of nickel chloride hexahydrate; and the other terms are as defined above.

**Tube constant**—[NOTE—For each susceptibility tube, a tube constant,  $C_T$ , is determined.] Measure the length of the tube,  $L$ , in cm, from the bottom of the inside of the tube to the bottom of the black band. Tare the tube on a balance, fill it with water to the bottom of the black band, and weigh again. Record the weight,  $W$ , in g, of the water in the tube. Calculate the tube constant,  $C_T$ , by the formula:

$$KL/(W \times 10^9)$$

in which  $K$  is the *Balance constant* as obtained above; and  $L$  and  $W$  are as defined above.

**Diluted sample**—Transfer about 0.2 g of the Injection, accurately weighed, to a 10-mL volumetric container, dilute with water to volume, mix, and accurately weigh the contents.

**Procedure**—[NOTE—Use the same susceptibility tube throughout the procedure.] Fill the susceptibility tube with water to the black band. Insert the tube into the susceptibility balance, and adjust to zero. Remove the tube from the balance, pour the water from the tube, rinse the tube with 1 M nickel chloride, and then fill the tube with fresh 1 M nickel chloride. Insert the tube into the balance, and record the magnetic susceptibility in cgs units. Similarly, measure the magnetic susceptibility of 3 M nickel chloride. The balance readings are within 5% of the expected values, which are  $(4.24 \times 10^{-6})/C_T$  for 1 M nickel chloride and  $(12.72 \times 10^{-6})/C_T$  for 3 M nickel chloride. Rinse the tube with well-mixed Injection, then fill the tube with the Injection to the black band, and weigh. Insert the tube into the susceptibility balance, and proceed as directed for the *Standard solutions*. Calculate the magnetic susceptibility, in cgs units, per g of iron in the Injection by the formula:

$$1000C_T RT/WI$$

in which  $C_T$  is the *Tube constant* as obtained above;  $R$  is the balance reading, in cgs units;  $T$  is the weight, in g, of the *Diluted sample*;  $W$  is the weight, in g, of the ferumoxides in the *Diluted sample*; and  $I$  is the concentration of iron, in mg per g, in the Injection, obtained by using specific gravity to convert the concentration of iron, in mg per mL, as determined in the *Assay for iron*, to mg per g. The magnetic susceptibility is not less than  $17,100 \times 10^{-6}$  in cgs units per g of iron.

**Osmolality** (785): between 325 and 365 mOsmol per kg.  
**Other requirements**—It meets the requirements for *Injections* (1) with the exception of *Foreign Matter* and *Particulate Matter*.

#### Assay for iron—

**Iron standard solution**—Use a NIST-traceable iron standard containing 1000 µg per mL (1000 ppm).

**Standard preparations**—Pipet 5.0, 10.0, 15.0, and 20.0 mL of the *Iron standard solution* into separate 1000-mL volumetric flasks, dilute each with water to volume, and mix to obtain solutions having known concentrations of 5 µg per mL, 10 µg per mL, 15 µg per mL, and 20 µg per mL, respectively.

**Assay preparation**—Accurately weigh 100 µL of the Injection, and transfer to a test tube. Add 2 mL of hydrochloric acid, and mix. [NOTE—The Injection dissolves to yield a medium yellow solution.] Add 2 mL of water, and then transfer the contents of the tube to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Using an atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with an iron hollow-cathode lamp and an air-acetylene flame, set the instrument to zero with water, and measure the absorbance,  $A_{20}$ , of the *Standard preparation* containing 20 µg per mL at the iron emission line of 296.7 nm. Concomitantly determine the absorbances of the *Standard preparations*. Calculate the iron concentration, in µg per mL, of each *Standard preparation* by the formula:

$$20(A_S / A_{20})$$

in which  $A_S$  is the absorbance of the relevant *Standard preparation*. The reading for each *Standard preparation* is within 0.3 µg per mL of its nominal concentration. Measure the absorbance of the *Assay preparation*, and calculate the content of iron, in mg per mL, in the Injection by the formula:

$$2(A_U / A_{20})(S/W)$$

in which  $A_U$  is the absorbance of the *Assay preparation*;  $A_{20}$  is the absorbance of the *Standard preparation* containing 20 µg per mL;  $S$  is the specific gravity of the Injection; and  $W$  is the weight, in g, of the volume of Injection taken to prepare the *Assay preparation*.

#### Assay for dextran—

**Control preparation**—Transfer about 50 mg of USP Dextrose RS, accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter.

**Standard preparation**—Transfer about 50 mg of USP Dextran T-10 RS, accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter.

**Assay preparation**—Transfer 0.25 g of the Injection, accurately weighed, to a test tube, add about 0.25 mL of hydrochloric acid, then add about 9 mL of water, and mix. Remove the free iron from this solution by passing it through a cation-exchange column into a 25-mL volumetric flask. Rinse the column with about 9 mL of water, collecting the washings in the 25-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—To each of four test tubes, separately add 0.2 mL of the *Assay preparation*, the *Standard preparation*, the *Control preparation*, and water (to be used as the blank). Add 0.2 mL of a 5% phenol solution to each test tube. Mix each tube briefly on a vortex mixer, rapidly add 1.0 mL of sulfuric acid to each test tube, and again mix briefly on a vortex mixer. [Caution—Reaction is exothermic.] Cover the test tubes, and allow to stand at room temperature for at least 15 minutes. [NOTE—The resultant solution is orange-yellow in color and free of any solid material.] Mix each tube on a vortex mixer. Using a suitable spectrophotometer, determine the absorbances of the solutions from the *Standard preparation*, the *Control preparation*, and the *Assay preparation* against the blank at the wavelength of maximum absorbance at about 490 nm. Calculate the percent recovery of dextran in the *Control preparation* by the formula:

$$100(1.11)(C/C_D)(A_C / A_S)$$

in which 1.11 is a correction factor (to account for dextrose being a monomer of dextran);  $C$  is the concentration, in mg per mL, of USP Dextran T-10 RS in the *Standard preparation*;  $C_D$  is the concentration, in mg per mL, of USP Dextrose RS in the *Control preparation*; and  $A_C$  and  $A_S$  are the absorbances of the solutions from the *Control preparation* and the *Standard preparation*, respectively: not less than 90% to 110% is found. Calculate the quantity, in mg per mL, of dextran in the volume of Injection taken by the formula:

$$25S(C/W)(A_U / A_S)$$

in which  $S$  is the specific gravity of the Injection;  $C$  is the concentration, in mg per mL, of USP Dextran T-10 RS in the *Standard preparation*;  $W$  is the weight, in g, of the portion

of the Injection taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for citrate—

**Mobile phase**—Prepare a filtered and degassed 0.0375 N sodium hydroxide solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard stock solution**—Transfer 0.7776 g of trisodium citrate dihydrate to a 100-mL volumetric flask, dilute with water to volume, filter, and refrigerate. This solution contains 5000 µg of citrate per mL (5000 ppm).

**Standard preparations**—Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0, 2.0, and 4.0 mL of this solution to separate 100-mL volumetric flasks, add 0.8 mL of hydrochloric acid to each flask, dilute with water to volume, and mix to obtain solutions having known concentrations of 5 µg per mL, 10 µg per mL, and 20 µg per mL.

**System suitability solution**—Use the filtered *Standard preparation* containing 5 µg per mL.

**Assay preparation**—Transfer about 0.5 mL of the Injection, accurately weighed, to a test tube, add 0.2 mL of hydrochloric acid and about 9 mL of water, and mix. Remove the free iron from this solution by passing the solution through a cation-exchange column<sup>2</sup> into a 25-mL volumetric flask. Rinse the column with about 9 mL of water, collecting the washings in the flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph<sup>3</sup> is equipped with an ion detector with suppressed conductivity at 30 µS, a 4-mm × 25-cm separator column<sup>4</sup> that contains 15-µm packing L48, a 4-mm × 50-mm guard column,<sup>5</sup> and integrators. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparations* and the *Assay preparation* into the chromatograph, record the chromatograms for about 16 minutes, and measure the responses for the major peaks. Prepare a standard curve by plotting the conductivities of the *Standard preparations* versus their concentrations, in µg per mL. Determine the concentration,  $C$ , in µg per mL, of citrate in the *Assay preparation* by extrapolation from the standard curve. Calculate the quantity, in mg per mL, of citrate in the volume of Injection taken by the formula:

$$0.025C(S/W)$$

in which  $S$  is the specific gravity of the Injection; and  $W$  is the weight, in g, of the volume of Injection taken to prepare the *Assay preparation*.

## Ferumoxsil Oral Suspension

» Ferumoxsil Oral Suspension is an aqueous suspension of silicone-coated superparamagnetic iron oxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of iron (Fe). It contains a preservative and a thickening agent. It may contain suitable

<sup>2</sup> AG 50W-X8 (H+), available from Biorad.

<sup>3</sup> DX-500, available from Dionex Corporation or equivalent source.

<sup>4</sup> HPLC IonPac AS5 or equivalent

<sup>5</sup> HPLC IonPac AG5 or equivalent

colors, flavors, and sweetening agents. Ferumoxsil is poly[*N*-(2-aminoethyl)-3-aminopropyl]siloxane-coated nonstoichiometric magnetite [ $\text{FeO}_x(\text{C}_5\text{H}_{13}\text{N}_2\text{SiO}_2)_y$ ].

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label it to indicate that it is to be well-shaken for 1 minute before use, and that it is not to be used if there are indications that the package has been exposed to freezing temperatures.

**Viscosity—Capillary Viscometer Methods** <911> and **Rotational Rheometer Methods** <912>: between 11 and 60 centipoises.

**Osmolarity** <785>: between 230 and 270 mOsmol per kg.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Escherichia coli*. The total aerobic microbial count does not exceed 100 cfu per mL.

**pH** <791>: between 5.5 and 9.0.

#### Magnetic susceptibility—

**Apparatus, Standard solutions, Balance constant, and Tube constant**—Proceed as directed for *Magnetic susceptibility* under *Ferumoxides Injection*.

**Procedure**—Proceed as directed for *Magnetic susceptibility* under *Ferumoxides Injection* except to use undiluted Oral Suspension whenever Injection is indicated. Calculate the magnetic susceptibility, in cgs units, per g of iron in the Oral Suspension by the formula:

$$1000C_T/R/I$$

in which  $C_T$  is the *Tube constant*;  $R$  is the balance reading, in cgs units; and  $I$  is the concentration, in mg per g, of iron in the Oral Suspension, as determined in the *Assay for iron*. The magnetic susceptibility is not less than  $22,500 \times 10^{-6}$  in cgs units per g of iron.

**Settling**—Prepare a mixture of Oral Suspension and water (1:5). Mix by gentle inversion, and determine the absorbance at 500 nm in a suitable spectrophotometer, using water as the blank. Cover the cell, and allow to stand undisturbed for 4 hours at room temperature. Without mixing, determine the absorbance again. Calculate the percentage of iron remaining in solution after settling by the formula:

$$100(A_4 / A_0)$$

in which  $A_4$  is the absorbance of the solution after standing for 4 hours; and  $A_0$  is the initial absorbance of the solution: not less than 80% is found.

#### Uniformity of dosage units <905>—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume <698>—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

#### Assay for iron—

**Iron standard solution and Standard preparations**—Proceed as directed in the *Assay for iron* under *Ferumoxides Injection*.

**Assay preparation**—Transfer about 5 g of well-mixed Oral Suspension, accurately weighed, to a 100-mL volumetric flask. Add 2 mL of nitric acid, and mix. Transfer the flask to a boiling water bath, and boil for 30 minutes to obtain a yellowish solution. Allow to cool to room temperature, dilute with water to volume, and mix.

**Procedure**—Proceed as directed in the *Assay for iron* under *Ferumoxides Injection*. Calculate the iron concentrations, in

µg per mL, of the three *Standard preparations* by the formula:

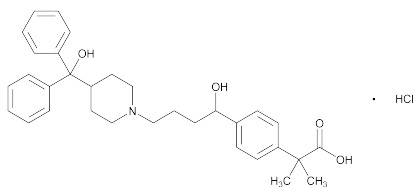
$$20(A_5 / A_{20})$$

in which  $A_5$  is the absorbance of the relevant *Standard preparation*; and  $A_{20}$  is the absorbance of the *Standard preparation* containing 20 µg per mL. The reading for each *Standard preparation*,  $A_5$ , is within 0.5 µg per mL of its nominal concentration. Measure the absorbance of the *Assay preparation*, and calculate the content of iron, in µg per mL, in the Oral Suspension by the formula:

$$2000(A_U / A_{20})(1.01/W)$$

in which  $A_U$  is the absorbance of the *Assay preparation*;  $A_{20}$  is the absorbance as defined above; 1.01 is the specific gravity of the Oral Suspension; and  $W$  is the weight, in g, of Oral Suspension taken to prepare the *Assay preparation*.

## Fexofenadine Hydrochloride



$C_{32}H_{39}NO_4 \cdot HCl$  538.12  
Benzeneacetic acid, 4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]- $\alpha,\alpha$ -dimethyl-, hydrochloride, ( $\pm$ );  
( $\pm$ )-*p*-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)piperidinyl]butyl]- $\alpha$ -methylhydratropic acid, hydrochloride [138452-21-8].

### DEFINITION

Fexofenadine Hydrochloride contains NLT 98.0% and NMT 102.0% of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B.** The retention time of the fexofenadine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**  
**Analysis:** Examine the precipitate formed in *Other Components* for the *Content of Chloride* test.  
**Acceptance criteria:** A white precipitate is observed.

### ASSAY

#### • PROCEDURE

**Buffer:** 6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.  
**Diluent:** Acetonitrile and *Buffer* (1:1)  
**Mobile phase:** Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine.  
**Standard solution:** 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*  
**Sample stock solution:** 1.0 mg/mL of Fexofenadine Hydrochloride in *Diluent*  
**Sample solution:** 0.06 mg/mL of Fexofenadine Hydrochloride in *Mobile phase* from the *Sample stock solution*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L11

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 10 between fexofenadine and fexofenadine related compound A

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for fexofenadine and NMT 3.0% for fexofenadine related compound A

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine

Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fexofenadine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### OTHER COMPONENTS

#### • CONTENT OF CHLORIDE

**Sample:** 300 mg of Fexofenadine Hydrochloride

**Blank:** Methanol

**Titrimetric system:**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.1 N silver nitrate VS

**Endpoint detection:** Potentiometrically

**Analysis:** Dissolve the *Sample* in 50 mL of methanol.

Each mL of 0.1 N silver nitrate VS is equivalent to 3.545 mg of chloride.

**Acceptance criteria:** 6.45%–6.75% on the anhydrous basis

### IMPURITIES

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

• **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

• **LIMIT OF FEXOFENADINE RELATED COMPOUND B**

**Buffer:** Glacial acetic acid and water (2.3: 2000). Adjust with 6 N ammonium hydroxide to a pH of  $4.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (20:80)

**System suitability solution:** Add 1.2 mg of USP Fexofenadine Related Compound B RS to a 5-mL volumetric flask. Dilute with *Mobile phase* to volume. Transfer 2.0 mL of the solution into a 100-mL volumetric flask. Add 25 mg of USP Fexofenadine Hydrochloride RS, and dilute with *Mobile phase* to volume.

**Standard solution:** 2.5 µg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase* from the *System suitability solution*

**Sample solution:** 0.25 mg/mL of Fexofenadine Hydrochloride in *Mobile phase*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L45

**Flow rate:** 0.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for fexofenadine related compound B and fexofenadine are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between fexofenadine and fexofenadine related compound B

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fexofenadine related compound B in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for fexofenadine related compound B from the *Sample solution*

$r_S$  = peak response for fexofenadine from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of fexofenadine in the *Sample solution* (mg/mL)

$F$  = relative response factor for fexofenadine related compound B relative to fexofenadine, 0.8

**Acceptance criteria:** NMT 0.2%

• **OTHER ORGANIC IMPURITIES**

**Buffer, Diluent, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Use the *Sample stock solution* in the *Assay*.

**Reference solution:** Use the *Sample solution* in the *Assay*.

**Samples:** *Standard solution*, *Sample solution*, *Reference solution*, and *Mobile phase* (used as the blank)

Measure the peak areas, excluding the peaks corresponding to those from the *Mobile phase*.

Calculate the percentage of fexofenadine related compound A in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for fexofenadine related compound A from the *Sample solution*

$r_S$  = peak response for fexofenadine related compound A from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of fexofenadine in the *Sample solution* (mg/mL)

Calculate the percentage of decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2, in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the decarboxylated degradant from the *Sample solution*

$r_S$  = peak response of fexofenadine from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of fexofenadine in the *Sample solution* (mg/mL)

$F$  = relative response factor for the decarboxylated degradant relative to fexofenadine, 1.1

Calculate the percentage of other impurities in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for any other impurity from the *Sample solution*

$r_S$  = peak response of fexofenadine from the *Reference solution*

$C_S$  = concentration of fexofenadine in the *Reference solution* (mg/mL)

$C_U$  = concentration of fexofenadine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Acceptance Criteria, NMT (%)
Fexofenadine related compound A <sup>a</sup>	0.2
Decarboxylated degradant <sup>b</sup>	0.15
Any other individual, unidentified impurity	0.1
Total impurities	0.5

<sup>a</sup> Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.

<sup>b</sup> (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method 1c (921):** NMT 2.0% for the anhydrous form; 6.0%–10.0% for the hydrate form. [NOTE—"Hydrate" refers to a mixture of dihydrate and trihydrate forms of fexofenadine hydrochloride.]

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Where it is the hydrate form, the label so indicates.
- **USP REFERENCE STANDARDS (11)**
  - USP Fexofenadine Hydrochloride RS
  - USP Fexofenadine Related Compound A RS
  - Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl. C<sub>32</sub>H<sub>37</sub>NO<sub>4</sub> 499.65
  - USP Fexofenadine Related Compound B RS
  - 3-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl benzeneacetic acid hydrochloride. C<sub>32</sub>H<sub>39</sub>NO<sub>4</sub> · HCl 538.12

## Fexofenadine Hydrochloride Capsules

**DEFINITION**

Fexofenadine Hydrochloride Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of fexofenadine hydrochloride (C<sub>32</sub>H<sub>39</sub>NO<sub>4</sub> · HCl).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. INFRARED ABSORPTION** (197K)

**Sample solution:** Empty an equivalent of 60 mg of fexofenadine hydrochloride, from the contents of several Capsules, into a suitable capped tube. Add 10 mL of a mixture of acetonitrile and methanol (10:1), and shake until the sample is dispersed. Allow to settle. Decant, filter, and collect the supernatant in a suitable beaker. Evaporate the solvent to near dryness by using a stream of nitrogen and with gentle heating from an appropriate source (steam, low-temperature hot plate). While still warm, add 5 mL of water and 5 drops of diluted hydrochloric acid, and stir to induce precipitation. Chill in an ice bath for about 30 min. Pass through a 10- to 15- $\mu$ m filtering crucible with fritted disk. Dry the precipitate in an air oven for 1 h at 105°.

**Acceptance criteria:** Meet the requirements

**ASSAY**

• **PROCEDURE**

**Buffer:** 6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Mobile phase:** Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine.

**Standard solution:** 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*

**Sample stock solution:** Remove, as completely as possible, the contents of NLT 20 Capsules, mix the combined contents, and finely powder by using a mortar and pestle. Transfer a portion of the powder, equivalent to about 50 mg of fexofenadine hydrochloride, to a 50-mL volumetric flask. Add 40 mL of *Diluent*, and shake by mechanical means for 60 min. Sonicate for about 2 min. Allow to cool to room temperature, and dilute with *Diluent* to volume.

**Sample solution:** Transfer 3.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L11

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 10 between fexofenadine and fexofenadine related compound A

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% and 3.0% for fexofenadine and fexofenadine related compound A, respectively

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response of fexofenadine from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–105.0%

**PERFORMANCE TESTS**

• **DISSOLUTION** (711)

**Test 1**

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 15 and 45 min

**Buffer:** 1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of phosphoric acid in 300 mL of water

**Mobile phase:** Acetonitrile and *Buffer* (7:3)

**System suitability stock solution:** 0.44 mg/mL of USP Fexofenadine Related Compound A RS in water.

[NOTE—A small amount of glacial acetic acid, not to exceed 5% of the total volume, may be used if necessary to dissolve USP Fexofenadine Related Compound A RS.]

**System suitability solution:** Prepare a solution of USP Fexofenadine Hydrochloride RS in the *System suitability stock solution* containing 0.01 mg/mL of USP Fexofenadine Related Compound A RS and 0.06 mg/mL of USP Fexofenadine Hydrochloride RS.

**Standard solution:** 0.07 mg/mL of USP Fexofenadine Hydrochloride RS in water. [NOTE—A small amount of methanol, not to exceed 0.5% of the total volume, may be used if necessary to dissolve USP Fexofenadine Hydrochloride RS.]

**Sample solution:** Filtered portions of the solution under test

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between fexofenadine and fexofenadine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) dissolved.

**Tolerances:** NLT 50% (Q) of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) is dissolved in 15 min; NLT 75% (Q) of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) is dissolved in 45 min.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium, Apparatus, Buffer, Mobile phase, System suitability stock solution, System suitability solution, Chromatographic system, and Analysis:** Proceed as directed for *Test 1*.

**Time:** 45 min

**Tolerances:** NLT 75% (Q) of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES**

• **ORGANIC IMPURITIES**

**Buffer:** 6.64 mg/mL of monobasic sodium phosphate and 0.84 mg/mL of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Mobile phase:** Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine

**Standard solution:** 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*

**Sample solution:** Use the *Sample stock solution* as prepared in the *Assay*.

**Reference solution:** 0.06 mg/mL of fexofenadine hydrochloride in *Mobile phase* from the *Sample solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L11

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10 between fexofenadine and fexofenadine related compound A

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% and 3.0% for fexofenadine and fexofenadine related compound A, respectively

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Reference solution*

Calculate the percentage of fexofenadine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for fexofenadine related compound A from the *Sample solution*

$r_S$  = peak response for fexofenadine related compound A from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

Calculate the percentage of decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the decarboxylated degradant from the *Sample solution*

$r_S$  = peak response of fexofenadine from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

$F$  = response factor for the decarboxylated degradant relative to fexofenadine, 1.1

Calculate the percentage of other impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for any other impurity from the *Sample solution*

$r_S$  = peak response of fexofenadine from the *Reference solution*

$C_S$  = concentration of fexofenadine in the *Reference solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Acceptance Criteria, NMT (%)
Fexofenadine related compound A <sup>a</sup>	0.4
Decarboxylated degradant <sup>b</sup>	0.2
Any other individual, unidentified impurity	0.2
Total impurities	0.5

<sup>a</sup> Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.

<sup>b</sup> (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

#### • USP REFERENCE STANDARDS <11>

USP Fexofenadine Hydrochloride RS

USP Fexofenadine Related Compound A RS

Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.

C<sub>32</sub>H<sub>37</sub>NO<sub>4</sub> 538.12

## Fexofenadine Hydrochloride Tablets

#### DEFINITION

Fexofenadine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of fexofenadine hydrochloride (C<sub>32</sub>H<sub>39</sub>NO<sub>4</sub> · HCl).

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION <197K>

**Standard solution:** Transfer 60 mg of USP Fexofenadine Hydrochloride RS to a suitable capped tube and add 10 mL of a mixture of acetonitrile and methanol (10:1).

**Sample solution:** Transfer an equivalent to 60 mg of fexofenadine hydrochloride, from a sufficient number of weighed and finely powdered Tablets, to a suitable capped tube, and add 10 mL of a mixture of acetonitrile and methanol (10:1).

**Analysis:** Shake or mix the *Standard solution* and *Sample solution* on a vortex mixer for 1–2 min to disperse the sample. Allow the solution to stand for 10 min, or centrifuge for 2–3 min. Pass the liquid into a 50-mL beaker using a 0.45-µm polytetrafluoroethylene syringe filter. Evaporate the solvent until about 0.5 mL remains, using a stream of nitrogen with gentle heating (do not exceed 75°). Add 5 mL of water and 5 drops of dilute hydrochloric acid, and stir to induce precipitation. Chill in an ice bath for 30 min. Filter the solution through a 10- to 15-µm sintered-glass crucible. Dry the precipitate in an air oven for 1 h at 105° oven for 1 h at 105°. Prepare a bromide dispersion from the residue.

**Acceptance criteria:** The IR absorption spectrum of the potassium bromide dispersion of the residue from the sample exhibits maxima only at the same wavelengths as that of a potassium bromide dispersion from the Standard.

• **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Solution A:** Glacial acetic acid and water (17:983). Dilute 100 mL of this solution with water to 1 L.

**Solution B:** Dilute 15 mL of a solution containing acetonitrile and triethylamine (1:1) with *Solution A* to 1 L. Adjust with phosphoric acid to a pH of 5.25.

**Diluent:** Acetonitrile and *Solution A* (3:1)

**Mobile phase:** Acetonitrile and *Solution B* (9:16)

**Standard stock solution:** 0.25 mg/mL of USP Fexofenadine Hydrochloride RS in *Diluent*

**Standard solution:** 0.015 mg/mL from the *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Transfer a sufficient number of whole Tablets (NLT 10) to a suitable volumetric flask, add *Solution A* (equivalent to 20% of the total flask volume), and shake by mechanical means at a high speed for 30 min or until the Tablets are fully disintegrated and finely dispersed. Add acetonitrile (equivalent to 80% of the total flask volume), and shake by mechanical means for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a polytetrafluoroethylene filter having a 0.45- $\mu$ m or finer pore size, and use the filtrate. Dilute, if necessary, with *Diluent* to obtain a solution containing an equivalent to 1.2 mg/mL of fexofenadine hydrochloride.

**Sample solution:** 0.018 mg/mL from the *Sample stock solution* in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L11

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{32}H_{39}NO_4 \cdot HCl$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>****Test 1**

**Medium:** 0.001 N hydrochloric acid; 900 mL, deaerated

**Apparatus 2:** 50 rpm

**Time:** 10 and 30 min

Determine the percentages of the labeled amount of  $C_{32}H_{39}NO_4 \cdot HCl$  dissolved by using the following method.

**Solution A:** 1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of concentrated phosphoric acid in 300 mL of water

**Mobile phase:** Acetonitrile and *Solution A* (7:3)

**Standard solution:** USP Fexofenadine Hydrochloride RS in *Medium* to obtain a solution having a known concentration similar to that expected for the solution

under test. [NOTE—A small amount of methanol, not exceeding 0.5% of the total volume, can be used to dissolve fexofenadine hydrochloride.]

**System suitability solution:** 0.44 mg/mL of USP Fexofenadine Related Compound A RS in water. Transfer 1.0 mL of this solution into a vial, and add 40 mL of the *Standard solution*. [NOTE—A small amount of acetic acid, not exceeding 5% of the total volume, can be used to dissolve fexofenadine related compound A.]

**Sample solution:** Pass portions of the solution under test through a glass fiber filter having a 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 2–3  $\mu$ g column load of fexofenadine hydrochloride

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between fexofenadine and fexofenadine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{32}H_{39}NO_4 \cdot HCl$  dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 60% (Q) of the labeled amount of  $C_{32}H_{39}NO_4 \cdot HCl$  is dissolved in 10 min; NLT 80% (Q) of the labeled amount of  $C_{32}H_{39}NO_4 \cdot HCl$  is dissolved in 30 min.

**Test 2**

If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** 0.001 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm. Use paddles and shafts coated with Teflon.

**Time:** 30 min

Determine the percentages of the labeled amount of  $C_{32}H_{39}NO_4 \cdot HCl$  dissolved by using the following method.

**Solution A:** 7 mg/mL of ammonium acetate in water. Adjust with glacial acetic acid to a pH of  $4.0 \pm 0.05$ .

**Mobile phase:** Acetonitrile and *Solution A* (2:3)

**Standard solution 1:** Transfer 20 mg of USP Fexofenadine Hydrochloride RS to a 100-mL volumetric flask. Add 3.0 mL of methanol, and mix. Dilute with *Medium* to volume.

**Standard solution 2:** Transfer 15.0 mL of *Standard solution 1* to a 50-mL volumetric flask. Dilute with *Medium* to volume.

**Standard solution 3:** Transfer 7.5 mL of *Standard solution 1* to a 50-mL volumetric flask. Dilute with *Medium* to volume.

**Sample solution:** Pass portions of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm × 15-cm; packing L11

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL for *Standard solution 1* and 30 µL for *Standard solutions 2* and *3*

#### System suitability

**Sample:** Any of the *Standard solutions*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solutions 1, 2, and 3* and the *Sample solution*

Calculate the percentage of  $C_{32}H_{39}NO_4 \cdot HCl$  dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of the appropriate *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = Tablet label claim (mg)

**Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{32}H_{39}NO_4 \cdot HCl$  is dissolved.

#### Test 3

If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** 0.001 N hydrochloric acid; 900 mL for Tablets labeled to contain 30 mg or 60 mg, and 1800 mL for Tablets labeled to contain 180 mg

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Buffer solution:** 6.64 g/L of monobasic sodium phosphate monohydrate and 0.84 g/L of sodium perchlorate monohydrate in water. Add 4 mL/L of triethylamine. Adjust with phosphoric acid to a pH of  $2.3 \pm 0.1$ .

**Mobile phase:** *Buffer solution* and acetonitrile (65:35)

**Standard stock solution:** 0.5 mg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*. This solution is stable for 3.5 months under refrigeration or for 18 days at room temperature.

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.07 mg/mL of USP Fexofenadine Hydrochloride RS. This solution is stable for 8 days under refrigeration or for 24 h at room temperature.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 10-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 2.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of fexofenadine hydrochloride dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 900 or 1800 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of fexofenadine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE

**Solution A, Solution B, Diluent, Mobile phase, Standard stock solution, Sample stock solution, and Sample solution:** Prepare as directed in the *Assay*.

**Standard solution:** 0.015 mg/mL of fexofenadine hydrochloride and 0.0045 mg/mL of fexofenadine related compound A from *Quantitative limit solution* and the *Standard stock solution* in *Mobile phase*

**System suitability stock solution:** Dilute 4.0 mL of the *Standard stock solution* with *Mobile phase* to 100 mL.

**System suitability solution:** Dilute 6.0 mL of the *System suitability stock solution* with *Mobile phase* to 100 mL.

**Quantitative limit solution:** 0.05 mg/mL of USP Fexofenadine Related Compound A RS in *Diluent*

##### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE— For the relative retention times, see *Impurity Table 1*.]

##### Suitability requirements

**Resolution:** NLT 7 between fexofenadine and fexofenadine related compound A, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 6%, *System suitability solution*; NMT 2.0% and NMT 3.0% for fexofenadine and fexofenadine related compound A, *Standard solution*

#### Analysis

**Samples:** *Standard solution*, *Sample stock solution*, and *Sample solution*

Calculate the percentage of fexofenadine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of fexofenadine related compound A in the *Sample stock solution*

$r_S$  = peak area of fexofenadine related compound A in the *Standard solution*

$C_S$  = concentration of fexofenadine related compound A in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fexofenadine hydrochloride in the *Sample stock solution*

Calculate the percentage of the decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene] in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of the decarboxylated degradant in the *Sample stock solution*

$r_S$  = peak area of fexofenadine in the *Standard solution*

$C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of fexofenadine hydrochloride in the *Sample stock solution*  
 $F$  = relative response factor (see *Impurity Table 1*)  
 Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = r_U / (F \times r_s + r_T) \times 100$$

$r_U$  = peak area for each individual unknown impurity in the *Sample stock solution*  
 $F$  = difference in concentration between the *Sample stock solution* and the *Sample solution*, 66.7  
 $r_s$  = peak area response for fexofenadine in the *Sample solution*  
 $r_T$  = sum of the peak areas of all unknown impurities in the *Sample stock solution*

[NOTE— Disregard any peak below 0.05%.]

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.5%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fexofenadine related compound A	1.6	—	0.4
Decarboxylated degradant	6.7	1.1	0.15
Fexofenadine	1.0	—	—
Any individual other impurity	—	1.0	0.2

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
 USP Fexofenadine Hydrochloride RS  
 USP Fexofenadine Related Compound A RS  
 Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]- $\alpha,\alpha$ -dimethyl.  
 $C_{32}H_{37}NO_4$  499.65

## Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets

#### DEFINITION

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amounts of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

#### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**  
**Standard solution A:** 6 mg/mL of USP Fexofenadine Hydrochloride RS in methanol  
**Standard solution B:** 12 mg/mL of USP Pseudoephedrine Hydrochloride RS in methanol

**Sample solution:** Transfer the equivalent of 30 mg of fexofenadine hydrochloride and 60 mg of pseudoephedrine hydrochloride from finely powdered Tablets (NLT 4) into a suitable vessel, and add 5 mL of methanol. Cap the vessel, and shake vigorously for 2 min. Pass the resulting suspension through a suitable filter of 0.45- $\mu$ m pore size. Use the filtrate.

**Adsorbent:** 0.2-mm layer of high-performance thin-layer chromatographic silica gel mixture. Dry the plate at 105° for 1 h before use.

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Toluene, dehydrated alcohol, and ammonium hydroxide (50:45:5)

**Analysis:** Proceed as directed, using the *Developing solvent system*. After removal of the plate, mark the solvent front, and allow the plate to air-dry. Heat the plate at 105° until the odor of ammonia disappears (about 5 min). Allow the plate to cool, and examine under UV light at 254 nm. [NOTE—The  $R_F$  values for fexofenadine and pseudoephedrine are 0.17 and 0.39, respectively.]

**Acceptance criteria:** The  $R_F$  value of fexofenadine hydrochloride in the *Sample solution* is comparable to that of fexofenadine hydrochloride in *Standard solution A*. The  $R_F$  value of pseudoephedrine hydrochloride in the *Sample solution* is comparable to that of pseudoephedrine hydrochloride in *Standard solution B*.

#### ASSAY

##### • PROCEDURE

**Solution A:** Dissolve 6.8 g of sodium acetate and 16.22 g of sodium 1-octanesulfonate in water and dilute with water to 1 L. Adjust with glacial acetic acid to a pH of 4.6.

**Mobile phase:** Methanol and *Solution A* (13:7)

**Diluent:** Methanol and *Solution A* (3:2)

**System suitability solution:** Transfer 40 mg of USP Pseudoephedrine Hydrochloride RS to a 50-mL volumetric flask. Add 5 mL of *tert*-butylhydroperoxide solution, and sonicate. Cover the flask opening with aluminum foil, and place the flask in an oven at 90° for 60 min. Remove from the oven, and allow to cool. Add 35 mL of *Mobile phase*, and cool to room temperature. Dilute with *Mobile phase* to volume. The degradation of pseudoephedrine hydrochloride by this process produces the related compound ephedrone.

**Related compounds stock solution:** Dissolve quantities of USP Fexofenadine Related Compound A RS and decarboxylated degradant<sup>1</sup> in a volume of methanol, and dilute with *Solution A* to obtain a ratio of methanol to *Solution A* of 3:2. Dilute with *Diluent* to obtain a solution having concentrations of 0.2 mg/mL for each component.

**Related compounds solution:** 0.02 mg/mL each of USP Fexofenadine Related Compound A RS and decarboxylated degradant from *Related compounds stock solution* diluted with *Mobile phase*

**Standard stock solution:** 0.4 mg/mL of fexofenadine hydrochloride and 0.8 mg/mL of pseudoephedrine hydrochloride from USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS, respectively, in *Mobile phase*

**Standard solution:** Dilute 6.0 mL of the *Standard stock solution* and 15.0 mL of the *Related compounds solution* with *Mobile phase* to 50 mL to obtain a solution having known concentrations of 0.096 mg/mL of pseudoephedrine hydrochloride, 0.048 mg/mL of fexofenadine hydrochloride, 0.006 mg/mL of fexofenadine related compound A, and 0.006 mg/mL of decarboxylated degradant.

**Sample stock solution:** Nominally equivalent to 1.2 mg/mL of fexofenadine hydrochloride and 2.4 mg/mL of pseudoephedrine hydrochloride. To prepare, transfer NLT 10 whole Tablets to a 500-mL volumetric flask.

<sup>1</sup> Available from USP as USP Fexofenadine Related Compound C AS, Cat# 1270446.

Add 300 mL of methanol, and shake by mechanical means at high speed for 60 min. Sonicate the flask for 60 min at 40°. Add 150 mL of *Solution A*, and sonicate for 60 min at 40°. Vent the flask, and vigorously shake the flask by hand at 15-min intervals during the mechanical shaking and sonication steps. Cool to room temperature, and dilute with *Solution A* to volume to obtain a final concentration. Pass a portion of this solution through a filter of 0.45- $\mu$ m or finer pore size, and use the filtrate.

**Sample solution:** 0.048 mg/mL and 0.096 mg/mL of fexofenadine hydrochloride and pseudoephedrine hydrochloride, respectively, from the *Sample stock solution* diluted with *Mobile phase*. [NOTE—Alternatively, centrifuge the *Sample stock solution* and use the supernatant to prepare the *Sample solution*. Filter the *Sample solution* before analysis.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  5-cm; 5- $\mu$ m packing L6 connected in series to a 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L11

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for pseudoephedrine and ephedrone are 1.0 and 1.2, respectively (*System suitability solution*); and for fexofenadine, fexofenadine related compound A, and decarboxylated degradant are 1.0, 1.2, and 3.1, respectively (*Standard solution*).]

#### Suitability requirements

**Resolution:** NLT 1.5 between pseudoephedrine and ephedrone, *System suitability solution*; NLT 2.0 between fexofenadine and fexofenadine related compound A, *Standard solution*

**Relative standard deviation:** NMT 1.0% for replicate injections based on the pseudoephedrine peak, *System suitability solution*; NMT 1.0% for replicate injections based on the fexofenadine peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate separately the percentage of the labeled amount of fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of either fexofenadine or pseudoephedrine from the *Sample solution*  
 $r_S$  = peak response of either fexofenadine or pseudoephedrine from the *Standard solution*  
 $C_S$  = concentration of either USP Fexofenadine Hydrochloride RS or USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of either fexofenadine hydrochloride or pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

###### Test 1

**Medium:** 0.001 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

###### Times

**Fexofenadine hydrochloride:** 15 and 45 min

**Pseudoephedrine hydrochloride:** 45 min; 3, 5, and 12 h

**Solution A:** 7.0 mg/mL of monobasic sodium phosphate monohydrate in water. Adjust with 85% phosphoric acid to a pH of  $2.00 \pm 0.05$ .

**Mobile phase:** Acetonitrile and *Solution A* (9:11)

**Standard solution:** Dissolve quantities of USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in *Medium*, and dilute to obtain a solution containing known concentrations similar to those expected in the *Sample solution*. [NOTE—A small amount of methanol, NMT 0.5% of the total volume, can be used to dissolve the fexofenadine hydrochloride.]

**Sample solution:** Pass a portion of the solution under test through a suitable nylon filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L6

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Resolution:** NLT 3.0 between fexofenadine and pseudoephedrine

**Tailing factor:** NMT 1.5 for fexofenadine and pseudoephedrine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of  $C_{32}H_{39}NO_4 \cdot HCl$  and  $C_{10}H_{15}NO \cdot HCl$  dissolved.

#### Tolerances

**Fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ):** NLT 65% (Q) of the labeled amount is dissolved in 15 min and NLT 80% (Q) of the labeled amount is dissolved in 45 min.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 1*.

**Table 1**

Time	Amount Dissolved
45 min	NMT 36%
3 h	45%–69%
5 h	61%–80%
12 h	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** 0.001 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

###### Times

**Fexofenadine hydrochloride:** 45 min

**Pseudoephedrine hydrochloride:** 30 min; 2, 4, and 12 h

**Solution A:** 2.7 mg/mL of monobasic potassium phosphate and 2.2 mg/mL of sodium 1-octanesulfonate in

water. Adjust with phosphoric acid to a pH of  $2.50 \pm 0.05$ .

**Mobile phase:** Methanol, acetonitrile, and *Solution A* (3:3:4)

**Fexofenadine standard stock solution:** Transfer 66 mg of USP Fexofenadine Hydrochloride RS to a 100-mL volumetric flask. Add 10 mL of methanol, and swirl until dissolved. Add 50 mL of *Medium*, and mix. Allow the solution to equilibrate to room temperature, and dilute with *Medium* to volume.

**Pseudoephedrine standard stock solution:** Transfer 66 mg of USP Pseudoephedrine Hydrochloride RS to a 100-mL volumetric flask. Add 10 mL of methanol, and swirl until dissolved. Add 50 mL of *Medium*, and mix. Allow the solution to equilibrate to room temperature, and dilute with *Medium* to volume.

**Standard solution:** 66 µg/mL of USP Fexofenadine Hydrochloride RS and 132 µg/mL of USP Pseudoephedrine Hydrochloride RS from a mixture of *Fexofenadine standard stock solution* and *Pseudoephedrine standard stock solution* diluted with *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between fexofenadine and pseudoephedrine

**Tailing factor:** NMT 2.0 for fexofenadine and NMT 2.5 for pseudoephedrine

**Relative standard deviation:** NMT 2.0% for both peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentages of  $C_{32}H_{39}NO_4 \cdot HCl$  and  $C_{10}H_{15}NO \cdot HCl$  dissolved.

#### Tolerances

**Fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ):** NLT 80% (Q) of the labeled amount is dissolved in 45 min.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 2*.

**Table 2**

Time	Amount Dissolved
30 min	NMT 35%
2 h	38%–58%
4 h	56%–76%
12 h	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** 0.001 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

#### Times

**Fexofenadine hydrochloride:** 30 min

**Pseudoephedrine hydrochloride:** 0.5, 2, 4, and 12 h

**Buffer solution:** 6.64 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of  $2.50 \pm 0.05$ .

**Mobile phase:** *Buffer solution* and acetonitrile (3:2)

**Standard solution:** [NOTE—A small amount of methanol, not exceeding 0.5% of the final total volume, can be used to dissolve fexofenadine hydrochloride.] Prepare a solution in *Medium* containing known concentrations of USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS similar to those expected in the solution under test.

**Sample solution:** Pass a portion of the solution under test through a suitable PVDF or nylon filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 2.5 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for fexofenadine and pseudoephedrine

**Relative standard deviation:** NMT 2.0% for both peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of  $C_{32}H_{39}NO_4 \cdot HCl$  and  $C_{10}H_{15}NO \cdot HCl$  dissolved.

#### Tolerances

**Fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ):** NLT 80% (Q) of the labeled amount is dissolved in 30 min.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 3*.

**Table 3**

Time (h)	Amount Dissolved
0.5	13%–33%
2	35%–55%
4	50%–70%
12	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in <711>.

**Test 4:** For products labeled with a dosing interval of 24 h. If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

**Medium:** 0.001 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

#### Times

**Fexofenadine hydrochloride:** 30 min

**Pseudoephedrine hydrochloride:** 3, 7, and 23 h

Determine the percentages of the labeled amounts of fexofenadine hydrochloride and of pseudoephedrine hydrochloride dissolved by using the chromatographic procedure described in *Test 1*.

#### Tolerances

**Fexofenadine hydrochloride ( $C_{32}H_{39}NO_4 \cdot HCl$ ):** NLT 80% (Q) of the labeled amount is dissolved in 30 min.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 4*.

**Table 4**

Time (h)	Amount Dissolved
3	10%–30%
7	35%–65%
23	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### IMPURITIES

[NOTE—On the basis of knowledge of the product, perform either: (a) *Organic Impurities, Procedure 1* or (b) *Organic Impurities, Procedure 2, Organic Impurities, Procedure 3, and Organic Impurities, Procedure 4.*]

#### • ORGANIC IMPURITIES, PROCEDURE 1

**Solution A, Mobile phase, Diluent, System suitability solution, Related compounds stock solution, Related compounds solution, Standard stock solution, Standard solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sample solution:** Use the *Sample stock solution*, prepared as directed in the Assay.

**Reference solution:** Use the *Sample solution*, prepared as directed in the Assay.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for pseudoephedrine and ephedrone are 1.0 and 1.2, respectively (*System suitability solution*); and for fexofenadine, fexofenadine related compound A, and decarboxylated degradant are 1.0, 1.2, and 3.1, respectively (*Standard solution*).]

#### Suitability requirements

**Resolution:** NLT 1.7 between pseudoephedrine and ephedrone, *System suitability solution*; NLT 2.0 between fexofenadine and fexofenadine related compound A, *Standard solution*

**Relative standard deviation:** NMT 1.0% for replicate injections based on the pseudoephedrine peak, *System suitability solution*; NMT 1.0% for replicate injections based on the fexofenadine peak and NMT 3.0% based on the individual peaks for fexofenadine related compound A and decarboxylated degradant, *Standard solution*

#### Analysis

**Samples:** *Sample solution* and *Reference solution*

Calculate the percentage of fexofenadine related compound A and decarboxylated degradant in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = individual peak area response of either fexofenadine related compound A or decarboxylated degradant from the *Sample solution*

$r_S$  = peak area response of fexofenadine related compound A or decarboxylated degradant from the *Standard solution*

$C_S$  = concentration of either USP Fexofenadine Related Compound A RS or decarboxylated degradant in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of ephedrone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak height response of ephedrone from the *Sample solution*

$r_S$  = peak height response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for ephedrone, 0.394  
Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = r_U/(F \times r_S + r_T) \times 100$$

$r_U$  = individual peak area response of an individual unknown impurity from the *Sample solution*

$F$  = difference in concentration between the *Sample solution* and the *Reference solution*, 25

$r_S$  = peak area response of fexofenadine hydrochloride from the *Reference solution*

$r_T$  = sum of the peak area responses of all unknown impurities from the *Sample solution*

[NOTE—Disregard any peak below 0.05%.]

**Acceptance criteria:** See *Table 5*.

**Table 5**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pseudoephedrine	1.0	—
Ephedrone	1.2 <sup>a</sup>	0.2
Fexofenadine	1.0	—
Fexofenadine related compound A	1.2 <sup>b</sup>	0.4
Decarboxylated degradant <sup>c</sup>	3.1 <sup>b</sup>	0.2
Tertiary dehydrated impurity <sup>d</sup>	1.8	0.2
Any other individual impurity	—	0.2
Total impurities	—	0.8

<sup>a</sup> Relative to pseudoephedrine.

<sup>b</sup> Relative to fexofenadine.

<sup>c</sup> (±)-4-(1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

<sup>d</sup> 4-[4-(Diphenylmethylene)-1-piperidinyl]-1-hydroxybutyl]-2,2-dimethyl phenyl acetic acid.

#### • ORGANIC IMPURITIES, PROCEDURE 2

**Solution A:** Dissolve 2.7 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of  $2.50 \pm 0.05$ .

**Mobile phase:** Methanol and *Solution A* (3:2)

**Standard stock solution:** 0.18 mg/mL USP Fexofenadine Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.0108 mg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*, prepared from the *Standard stock solution*

**Sensitivity solution:** 0.54 µg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*, prepared from the *Standard solution*

**Sample solution:** Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Mobile phase*. Sonicate for 10 min, and add an additional 100 mL of *Mobile phase*. Shake by mechanical means for 30 min, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

[NOTE—The run time is six times the retention time of fexofenadine.]

**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Signal-to-noise:** NLT 10, *Sensitivity solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the amount of each impurity as a percentage of the label claim of fexofenadine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of individual impurities from the *Sample solution* $r_S$  = peak response of fexofenadine from the *Standard solution* $C_S$  = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor for each impurity (see *Table 6*)**Acceptance criteria:** See *Table 6*.**Table 6**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fexofenadine	1.0	1.0	—
Meta fexofenadine	1.14	1.0	0.2
Fexofenadine related compound A	1.38	0.83	0.4
Tertiary dehydrated impurity <sup>a</sup>	2.25	1.3	0.2
Individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.5

<sup>a</sup> 4-[4-(Diphenylmethylene)-1-piperidinyl]-1-hydroxybutyl]-2,2-dimethyl phenyl acetic acid.**• ORGANIC IMPURITIES, PROCEDURE 3****Solution A:** 4 mg/mL of ammonium acetate**Mobile phase:** Methanol and *Solution A* (19:1)**Diluent:** Methanol and water (1:1)**Standard stock solution:** 0.18 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent***Standard solution:** 0.0216 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*, prepared from the *Standard stock solution***Sensitivity solution:** 1.08 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*, prepared from the *Standard solution***Sample solution:** Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Diluent*. Sonicate for 10 min, and add an additional 100 mL of *Diluent*. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-µm packing L3**Flow rate:** 1 mL/min**Injection size:** 20 µL**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Signal-to-noise:** NLT 10, *Sensitivity solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the amount of each impurity as a percentage of the label claim of pseudoephedrine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of individual impurities from the *Sample solution* $r_S$  = peak response of pseudoephedrine from the *Standard solution* $C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor, equal to 0.52 for ephedrone (RRT, 0.85 relative to the pseudoephedrine peak) and 1 for all other impurities**Acceptance criteria****Individual impurities:** NMT 0.2% of ephedrone; NMT 0.1% for any individual unspecified impurity**• ORGANIC IMPURITIES, PROCEDURE 4****Solution A:** Dissolve 2.7 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of  $2.50 \pm 0.05$ .**Solution B:** Methanol and *Solution A* (2:3)**Solution C:** Methanol and *Solution A* (7:3)**Mobile phase:** See *Table 7*.**Table 7**

Time (min)	Solution B (%)	Solution C (%)
0	100	0
40	100	0
41	0	100
65	0	100
66	100	0
90	100	0

**Diluent:** Methanol and water (1:1)**Standard stock solution:** 0.18 mg/mL of USP Benzoic Acid RS in *Diluent***Standard solution:** 0.0216 mg/mL of USP Benzoic Acid RS in *Diluent*, prepared from the *Standard stock solution***Sensitivity solution:** 1.08 µg/mL of USP Benzoic Acid RS in *Diluent*, prepared from the *Standard solution***Sample solution:** Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Diluent*. Sonicate for 10 min, and add an additional 100 mL of *Diluent*. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Signal-to-noise:** NLT 10, *Sensitivity solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the amount of each impurity as a percentage of the label claim of pseudoephedrine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of individual impurities from the *Sample solution* $r_S$  = peak response of benzoic acid from the *Standard solution* $C_S$  = concentration of USP Benzoic Acid RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor for each impurity (see *Table 8*)**Acceptance criteria****Individual impurities:** See *Table 8*.**Total impurities:** The combined total impurities from *Procedure 3* and *Procedure 4* is NMT 0.3%.**Table 8**

Name	Relative Retention Time	Relative Response Factor <sup>a</sup>	Acceptance Criteria, NMT(%)
Benzaldehyde	0.43	0.40	0.1
Benzoic acid	0.55	1.0	0.1
Ephedrone <sup>b</sup>	0.97	—	—
Pseudoephedrine	1.0	0.52	—
Individual unspecified impurity	—	0.52 <sup>c</sup>	0.1

<sup>a</sup> Response factors relative to benzoic acid.<sup>b</sup> Ephedrone is not quantitated in this method. A separate method is used for the quantitation of this impurity.<sup>c</sup> The response factor of pseudoephedrine relative to that of benzoic acid is used in the calculation of individual unspecified impurities.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

- **LABELING:** When more than one *Dissolution Test* is given, the labeling states the test used only if *Test 1* is not used. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states with which *Procedures* the article complies.

- **USP REFERENCE STANDARDS** <11>

USP Benzoic Acid RS

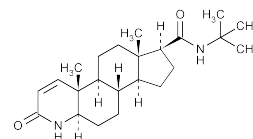
USP Fexofenadine Hydrochloride RS

USP Fexofenadine Related Compound A RS

Benzenecetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidiny]butyl]-α,α-dimethyl.

C<sub>32</sub>H<sub>37</sub>NO<sub>4</sub> 499.65

USP Pseudoephedrine Hydrochloride RS

**Finasteride**C<sub>23</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub> 372.544-Azaandrost-1-ene-17-carboxamide, *N*-(1,1-dimethylethyl)-3-oxo-, (5α,17β)-.*N*-*tert*-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide [98319-26-7].» Finasteride contains not less than 98.5 percent and not more than 101.0 percent of C<sub>23</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub>, calculated on the anhydrous basis.**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.**USP Reference standards** <11>—

USP Finasteride RS

**Identification**—**A:** *Infrared Absorption* <197M>.**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Specific rotation** <781S>: between −56.0° and −60.0°, determined at 405 nm.*Test solution:* 10 mg per mL, in methanol.**Water, Method I** <921>: not more than 0.3%.**Residue on ignition** <281>: not more than 0.1%.**Heavy metals, Method II** <231>: 0.001%.**Chromatographic purity**—*Mobile phase*—Prepare a filtered and degassed mixture of water, tetrahydrofuran, and acetonitrile (8:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).*Diluting solution*—Prepare a solution of water and acetonitrile (1:1).*Standard solution*—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 1.0 mg per mL.*Test solution*—Transfer about 100 mg of Finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 30-cm column that contains 4-μm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 60°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 10,000 theoretical plates; and the tailing factor is not more than 1.3.*Procedure*—Inject a volume (about 15 μL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Finasteride taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the sum of the responses of all peaks: not more than 0.5%



of any individual impurity is found; and not more than 1.0% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and tetrahydrofuran (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluting solution**—Prepare a solution of water and acetonitrile (1:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 200 µg per mL.

**Assay preparation**—Transfer about 20 mg of Finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 3.0-mm × 3.0-cm column that contains 3-µm packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1800 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{23}H_{36}N_2O_2$  in the portion of Finasteride taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Finasteride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Finasteride Tablets

» Finasteride Tablets contain not less than 95.0 percent and not more than 105.0 percent of finasteride ( $C_{23}H_{36}N_2O_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Finasteride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution <711>—

**Medium**: water; 900 mL.

**Apparatus 2**: 50 rpm.

FOR PRODUCTS LABELED AS 5-MG TABLETS—

**Time**: 45 minutes.

Determine the amount of  $C_{23}H_{36}N_2O_2$  dissolved by employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (29:21). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluting solution**—Prepare a solution of acetonitrile and water (7:3).

**Standard solution**—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, and dilute

quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration approximately equivalent to the sample under test.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 5-cm column that contains packing L1. The column temperature is maintained at 45°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 2.0; the column efficiency is greater than 1000 theoretical plates; the tailing factor is less than 2; and the relative standard deviation for replicate injections is less than 2.0%.

**Procedure**—Separately inject equal volumes (about 200 µL) of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of  $C_{23}H_{36}N_2O_2$  dissolved.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{23}H_{36}N_2O_2$  is dissolved in 45 minutes.

FOR PRODUCTS LABELED AS 1-MG TABLETS—

**Time**: 30 minutes.

**Mobile phase**—Prepare a degassed mixture of acetonitrile and water (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluting solution**—Prepare a solution of water and acetonitrile (7:3).

**Standard solution**—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, to obtain a solution having a known concentration of 0.1 mg per mL. Dilute this solution quantitatively, and stepwise if necessary, in 0.5% sodium lauryl sulfate to obtain a solution containing 0.001 mg of finasteride per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L11. The column temperature is maintained at 45°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of finasteride ( $C_{23}H_{36}N_2O_2$ ) dissolved.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{23}H_{36}N_2O_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of 2.5 mM phosphoric acid and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluting solution**—Prepare a solution of acetonitrile and water (7:3).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 100 µg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an amount of powder equivalent to about 10 mg of finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector

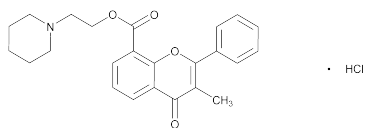
and a 4.6-mm × 10.0-cm column that contains packing L1. The column temperature is maintained at 45°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 2.0; the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of finasteride ( $C_{23}H_{36}N_2O_2$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Finasteride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flavoxate Hydrochloride



$C_{24}H_{25}NO_4 \cdot HCl$  427.92  
4*H*-1-Benzopyran-8-carboxylic acid, 3-methyl-4-oxo-2-phenyl-, 2-(1-piperidinyl)ethyl ester, hydrochloride; 2-Piperidinoethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate hydrochloride [3717-88-2].

### DEFINITION

Flavoxate Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{24}H_{25}NO_4 \cdot HCl$ , calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A solution of 10 mg/mL meets the requirements of the silver nitrate precipitate.

### ASSAY

#### PROCEDURE

**Solution A:** Mixture of 1.0 g of sodium hexanesulfonate in 650 mL of water, 1 mL of triethylamine, and 1.0 mL of phosphoric acid

**Mobile phase:** Add 350 mL of acetonitrile to *Solution A*.

**Standard solution:** 50 µg/mL of USP Flavoxate Hydrochloride RS in *Mobile phase*

**Sample solution:** 50 µg/mL of Flavoxate Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 293 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of flavoxate is about 4.3 min.]

#### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{24}H_{25}NO_4 \cdot HCl$  in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS**, *Method II* (231): NMT 10 ppm

#### Organic Impurities

##### PROCEDURE

**Solution A:** 0.05% (v/v) trifluoroacetic acid in water

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	68	32
15	68	32
25	20	80
35	20	80
36	68	32
41	68	32

**Diluent:** Methanol and 0.02% trifluoroacetic acid (1:1)

**Standard stock solution 1:** 2 mg/mL of USP Flavoxate Hydrochloride RS in *Diluent*

**Standard stock solution 2:** 0.05 mg/mL of USP Flavoxate Related Compound A RS in *Diluent*

**Standard stock solution 3:** 0.05 mg/mL of USP Flavoxate Related Compound B RS in methanol

**Standard stock solution 4:** 0.05 mg/mL of USP Flavoxate Related Compound C RS prepared as follows: Dissolve USP Flavoxate Related Compound C RS first in methanol using 10% of final volume, then fill 10% of final volume with 0.02% trifluoroacetic acid. Dilute with *Diluent* to volume.

**Standard solution:** 1 mg/mL of USP Flavoxate Hydrochloride RS and 5 µg/mL each of USP Flavoxate Related Compound A RS, USP Flavoxate Related Compound B RS, and USP Flavoxate Related Compound C RS in *Diluent* from *Standard stock solutions* 1, 2, 3, and 4, respectively

**Sample solution:** 1 mg/mL of Flavoxate Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 0.8 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between the flavoxate related compound B and flavoxate related compound C peaks

**Tailing factor:** NMT 1.5 for the flavoxate related compounds A, B, and C peaks

**Relative standard deviation:** NMT 2.0% for the flavoxate peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of flavoxate related compounds A, B, and C in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of flavoxate from the *Standard solution*

$C_S$  = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other unspecified degradation product in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each unspecified impurity from the *Sample solution*

$r_T$  = sum of responses of all peaks from the *Sample solution*

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flavoxate	1.0	—	—
Flavoxate related compound A <sup>a</sup> *	2.8	1.6	0.3
Flavoxate related compound B <sup>b</sup> *	3.5	1.5	0.1
Flavoxate related compound C <sup>c</sup> *	3.7	1.4	0.1
Any single unspecified degradation product	—	1.0	0.1

<sup>a</sup> 3-Methylflavone-8-carboxylic acid.

<sup>b</sup> 3-Methylflavone-8-carboxylic acid methyl ester.

<sup>c</sup> 3-Methylflavone-8-carboxylic acid ethyl ester.

\* For identification and system suitability purposes only.

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light; and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Flavoxate Hydrochloride RS
  - USP Flavoxate Related Compound A RS
    - 3-Methylflavone-8-carboxylic acid.
    - $C_{17}H_{12}O_4$  280.27
  - USP Flavoxate Related Compound B RS
    - 3-Methylflavone-8-carboxylic acid methyl ester.
    - $C_{18}H_{14}O_4$  294.30
  - USP Flavoxate Related Compound C RS
    - 3-Methylflavone-8-carboxylic acid ethyl ester.
    - $C_{19}H_{16}O_4$  308.33

## Flavoxate Hydrochloride Tablets

» Flavoxate Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of  $C_{24}H_{25}NO_4 \cdot HCl$ , based on the label claim.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

#### USP Reference standards (11)—

USP Flavoxate Hydrochloride RS

USP Flavoxate Related Compound A RS

3-Methylflavone-8-carboxylic acid.

$C_{17}H_{12}O_4$  280.27

#### Identification—

**A:** *Infrared Absorption* (197K)—

*Test specimen*—Grind at least 4 Tablets into a fine powder. Use an amount of powder equivalent to about 100 mg of flavoxate hydrochloride. Add 30 mL of methanol, and stir for about 10 minutes. Pass through a Whatman #1 filter paper, and collect the filtrate. Evaporate the methanol to dryness on a steam bath with the help of nitrogen gas. Dry the residue in vacuum at 60° for about 30 minutes. Mix 1 to 2 mg of dried residue with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL, deaerated.

*Apparatus 1:* 100 rpm.

*Time:* 30 minutes.

*Standard solution*—Transfer an accurately weighed quantity of USP Flavoxate Hydrochloride RS to a suitable volumetric flask, dissolve in a small amount of methanol (not more than 2% of the final volume), and dilute with *Medium* to volume to obtain a solution having a known concentration of about 0.11 mg per mL.

*Test solution*—Pass a portion of the solution under test through a suitable filter having a porosity of 45  $\mu m$ .

*Procedure*—Determine the amount of  $C_{24}H_{25}NO_4 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 294 nm, in portions of the *Test solution* in comparison with the *Standard solution*, using a cell with a path length of 0.2 cm and using *Medium* as the blank. Calculate the percentage of  $C_{24}H_{25}NO_4 \cdot HCl$  dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times L}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $L$  is the Tablet label claim, in mg.

*Tolerances*—Not less than 70% (Q) of the labeled amount of  $C_{24}H_{25}NO_4 \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Related compounds—

*Mobile phase*—Prepare as directed in the *Assay*.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Flavoxate Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with

*Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*USP Flavoxate Related Compound A RS stock solution*—Dissolve an accurately weighed quantity of USP Flavoxate Related Compound A RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg per mL.

*Standard solution*—Quantitatively dilute suitable volumes of *Standard stock solution* and *USP Flavoxate Related Compound A RS stock solution* with *Mobile phase* to obtain a solution having a final known concentration of about 0.001 mg per mL of flavoxate hydrochloride and about 0.003 mg per mL of flavoxate related compound A.

*Test solution*—Filter the *Assay stock preparation*, prepared as directed in the *Assay*, through a 0.45- $\mu$ m filter (polyvinylidene fluoride [PVDF] or equivalent). Use the filtrate.

*Chromatographic system* (see *Chromatography* <621>)—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the resolution,  $R$ , between flavoxate hydrochloride and flavoxate hydrochloride related compound A is not less than 10.0; the column efficiency is not less than 3000 plate counts; the tailing factor is not more than 2.0 for flavoxate hydrochloride; and the relative standard deviation for five replicate injections is not more than 2.0% for both peaks.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. Measure the peak areas of all the peaks in the *Test solution*. Identify the peaks using the relative retention times as given in *Table 1*.

Table 1

Component Name	Relative Retention Time	Relative Response Factor	Limit (%)
Flavoxate hydrochloride	1.0	—	—
Flavoxate related compound A*	2.5	1.5	0.3
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	1.0

\*3-Methylflavone-8-carboxylic acid.

Calculate the percentage of each impurity relative to flavoxate hydrochloride in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_i / r_S)(1/F)$$

in which  $C_S$  is the concentration, in mg per mL, of flavoxate hydrochloride in the *Standard solution*;  $C_U$  is the nominal concentration, in mg per mL, of flavoxate hydrochloride, based on label claim, in the *Test solution*;  $r_i$  is the peak response of each individual impurity;  $r_S$  is the response of the flavoxate hydrochloride peak obtained from the *Standard solution*; and  $F$  is the relative response factor for each of the impurities relative to flavoxate hydrochloride, as shown in *Table 1*.

#### Assay—

*Mobile phase*—Dissolve 1.0 g of sodium hexanesulfonate, 1.0 mL of triethylamine, and 1.0 mL of phosphoric acid in 650 mL of water. Filter and degas. To the filtrate, add 350 mL of acetonitrile. Mix well. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Flavoxate Hydrochloride RS, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

*Assay stock preparation*—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg of flavoxate hydrochloride based on label claim, and transfer to a 100-mL volumetric flask. Add about 80% volume of *Mobile phase*. Sonicate for 10 minutes, and stir for 15 minutes. Dilute with *Mobile phase* to volume.

*Assay preparation*—Dilute the *Assay stock preparation* quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution with a nominal concentration of about 0.05 mg per mL of flavoxate hydrochloride, based on label claim. Filter the solution through a 0.45- $\mu$ m filter (polyvinylidene fluoride [PVDF] or equivalent). Use the filtrate.

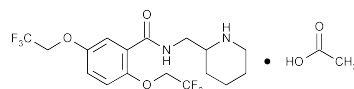
*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 293-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000; the tailing factor is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the flavoxate hydrochloride peak whose retention time is about 4.0 minutes. Calculate the percentage of the label claim of flavoxate hydrochloride ( $C_{24}H_{25}NO_4 \cdot HCl$ ) in the portion of the Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of flavoxate hydrochloride in the *Standard preparation*;  $C_U$  is the nominal concentration of flavoxate hydrochloride in the *Assay preparation*, based on the label claim; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flecainide Acetate



$C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  474.39

Benzamide, *N*-(2-piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)-, monoacetate.

*N*-(2-Piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide monoacetate [54143-56-5].

» Flecainide Acetate contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards <11>—

USP Flecainide Acetate RS

USP Flecainide Related Compound A RS

3-[2,5-Bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8a-hexahydroimidazo-[1,5a]pyridine hydrochloride.

$C_{17}H_{18}F_6N_2O_2 \cdot HCl$  432.8

**Clarity of solution**—Prepare a test solution by placing 250 mg of it in a test tube about 18 mm in diameter, adding 10 mL of water and 1 drop of glacial acetic acid, and

shaking to dissolve it. The solution is colorless and is not less clear than an equal volume of water in a similar test tube.

#### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 130 µg per mL.

*Medium:* alcohol.

Absorptivities at 298 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 146° and 152°, but the range between beginning and end of melting does not exceed 3°.

**Loss on drying** (731)—Dry it in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): not more than 0.002%.

#### Chromatographic purity—

**Mobile phase**—Prepare a mixture of water, acetonitrile, glacial acetic acid, and 1.0 N tetrabutylammonium hydroxide in methanol (710:290:10:5). Adjust with ammonium hydroxide to a pH of 5.8 ± 0.05. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of water and acetonitrile (71:29).

**Standard solution**—Prepare a solution of USP Flecainide Acetate RS and USP Flecainide Related Compound A RS in *Diluent* having known concentrations of about 0.1 mg of each per mL.

**Test solution**—Transfer about 250 mg of Flecainide Acetate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column containing 5-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative retention times are 1.0 for flecainide and about 1.7 for flecainide related compound A; the resolution, *R*, between flecainide and flecainide related compound A is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of flecainide related compound A in the portion of Flecainide Acetate taken by the formula:

$$2500(C_A / W_U)(r_{UA} / r_{SA})$$

in which  $C_A$  is the concentration, in mg per mL, of USP Flecainide Related Compound A RS in the *Standard solution*;  $W_U$  is the weight, in mg, of Flecainide Acetate taken to prepare the *Test solution*; and  $r_{UA}$  and  $r_{SA}$  are the flecainide related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 0.5% is found. Calculate the percentage of any other impurity in the portion of Flecainide Acetate taken by the formula:

$$2500(C_F / W_U)(r_i / r_F)$$

in which  $C_F$  is the concentration, in mg per mL, of USP Flecainide Acetate RS in the *Standard solution*;  $W_U$  is the weight, in mg, of Flecainide Acetate taken to prepare the *Test solution*;  $r_i$  is the peak response for any individual impurity observed in the chromatogram obtained from the *Test solution*; and  $r_F$  is the flecainide peak response obtained from

the *Standard solution*. Not more than 0.5% is found, and the sum of all impurities found is not greater than 2.0%.

**Content of acetate**—Dissolve about 600 mg of Flecainide Acetate, accurately weighed, in about 100 mL of dimethylformamide, and stir by mechanical means until dissolved. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically (see *Titrimetry* (541)). Perform a blank determination and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 6.005 mg of  $C_{21}H_{20}F_6N_2O_3$ : between 12.4% and 12.8%, calculated on the dried basis, is found.

**Assay**—Dissolve about 600 mg of Flecainide Acetate, accurately weighed, in about 20 mL of glacial acetic acid, and stir by mechanical means until dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* (541)). Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.44 mg of  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ .

## Flecainide Acetate Oral Suspension

### DEFINITION

Flecainide Acetate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of flecainide acetate ( $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ ).

Prepare Flecainide Acetate Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)):

Flecainide Acetate	2 g
Vehicle: a mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF (1:1), a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number in a suitable mortar, and comminute to a fine powder with a pestle, or use *Flecainide Acetate* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a flecainide acetate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the liquid *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and 0.06% phosphoric acid solution (40:60)

**Standard stock solution:** 1.0 mg/mL of USP Flecainide Acetate RS in *Mobile phase*

**Standard solution:** Transfer 2 mL of *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing about 200 µg/mL of flecainide acetate.

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of about 200 µg/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of flecainide acetate ( $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = nominal concentration of flecainide acetate in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of flecainide acetate in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 3.8–4.8

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, protected from light, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or when stored at controlled cold temperature
- **USP REFERENCE STANDARDS** (11)  
USP Flecainide Acetate RS

## Flecainide Acetate Tablets

» Flecainide Acetate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flecainide acetate ( $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ ).

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**USP Reference standards** (11)—

USP Flecainide Acetate RS

USP Flecainide Related Compound A RS

3-[2,5-Bis(2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8a-hexahydroimidazo-[1,5a]pyridine hydrochloride.

$C_{17}H_{18}F_6N_2O_2 \cdot HCl$  432.8

**Identification, Ultraviolet Absorption** (197U)—

**Spectral range:** 250 to 400 nm.

**Solution:** Use the test solution obtained in the test for *Uniformity of dosage units*.

**Dissolution** (711)—

**Medium:** 0.075 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes for Tablets each containing 50 mg of flecainide acetate, or 60 minutes for 100-, 150-, or 200-mg Tablets.

**Procedure**—Determine the amount of  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  dissolved from UV absorbances at the wavelength

of maximum absorbance at about 296 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Flecainide Acetate RS in the same *Medium*.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  is dissolved from 50-mg Tablets in 30 minutes; or from 100-, 150-, or 200-mg Tablets in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 Tablet to a volumetric flask of such capacity that the concentration of flecainide acetate should be in the range of 0.1 to 1 mg per mL, dilute with lactic acid solution (2 mg per mL) to volume, mix, and sonicate for about 30 minutes, shaking at about 10-minute intervals. Allow to cool, and pass a portion of this stock solution through a glass-fiber filter. If necessary, quantitatively dilute an accurately measured volume of the clear filtrate with the lactic acid solution (2 mg per mL) to obtain a test solution having a concentration of about 0.1 mg of flecainide acetate per mL. Dissolve an accurately weighed quantity of USP Flecainide Acetate RS in the lactic acid solution (2 mg per mL), sonicating if necessary to achieve dissolution, to obtain a Standard solution having a known concentration of about 0.1 mg per mL. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 296 nm, with a spectrophotometer, using the lactic acid solution (2 mg per mL) as the blank. Calculate the quantity, in mg, of  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  in the Tablet taken by the formula:

$$(CT / D)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Flecainide Acetate RS in the Standard solution; T is the labeled quantity, in mg of flecainide acetate per Tablet; D is the concentration, in mg per mL, of flecainide acetate in the test solution on the basis of the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the test solution and the Standard solution, respectively.

**Assay**—

**Mobile phase**—Prepare a mixture of water, acetonitrile, glacial acetic acid, and 1.0 N tetrabutylammonium hydroxide in methanol (710:290:10:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of water and lactic acid (980:20).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Flecainide Acetate RS in *Diluent* to obtain a solution having a known concentration of about 2 mg per mL.

**Resolution solution**—Prepare a solution in *Diluent* containing about 2 mg of USP Flecainide Acetate RS and 0.8 mg of USP Flecainide Related Compound A RS per mL.

**Assay preparation**—Transfer not fewer than 20 Tablets, accurately counted, to a volumetric flask of such capacity that the concentration of flecainide acetate is about 2 mg per mL after dilution to volume as directed below. Fill the flask to about 90% of its capacity with *Diluent*, and sonicate for about 30 minutes, swirling periodically. Allow to cool, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a of 0.5-μm or finer porosity, discarding the first 2 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are 1.0 for flecainide and about 1.5 for flecainide related compound A; and the resolution, R, between flecainide and the flecainide re-

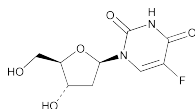
lated compound A is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flecainide acetate ( $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ ) in each Tablet taken by the formula:

$$(CT / D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flecainide Acetate RS in the *Standard preparation*; T is the labeled quantity, in mg, of flecainide acetate per Tablet; D is the concentration, in mg per mL, of flecainide acetate in the *Assay preparation* on the basis of the labeled quantity per Tablet and the extent of dilution; and  $r_U$  and  $r_S$  are the flecainide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Floxuridine



$C_9H_{11}FN_2O_5$  246.19  
Uridine, 2'-deoxy-5-fluoro-  
2'-Deoxy-5-fluorouridine [50-91-9].

» Floxuridine contains not less than 98.5 percent and not more than 101.0 percent of  $C_9H_{11}FN_2O_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected for further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—  
USP Floxuridine RS

### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 20  $\mu$ g per mL.

*Medium:* 0.1 N potassium hydroxide.

Absorptivities at 268 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Dissolve about 200 mg in 10 mL of water, and add a few drops of bromine TS: the bromine color is discharged.  
**Melting range**, *Class I* (741): between 145° and 153°, but the range between beginning and end of melting does not exceed 2°.

**Specific rotation** (781S): between +36° and +39°.

*Test solution:* 10 mg per mL, in water.

**Loss on drying** (731)—Dry it in vacuum over silica gel at 60° for 4 hours: it loses not more than 0.2% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Limit of fluoride ions**—[NOTE—Use plasticware throughout this test.]

*pH 5.25 Buffer*—Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volu-

metric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix: the pH of this solution is between 5.0 and 5.5.

*Standard stock solution*—Transfer 221 mg of sodium fluoride, previously dried at 150° for 4 hours, to a 100-mL volumetric flask, add about 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ions. Store in a tightly closed, plastic container.

*Standard solutions*—Dilute portions of the *Standard stock solution* quantitatively and stepwise with *pH 5.25 Buffer* to obtain 100-mL solutions having concentrations of 1, 3, 5, and 10  $\mu$ g per mL.

*Test solution*—Transfer 1.0 g of Floxuridine to a 100-mL volumetric flask, dissolve in and dilute with *pH 5.25 Buffer* to volume, and mix.

*Procedure*—Concomitantly measure the potential (see *Titrimetry* (541)), in mV, of the *Standard solutions* and the *Test solution*, with a pH meter capable of a minimum reproducibility of  $\pm 0.2$  mV, equipped with a glass-sleeved calomel-fluoride specific-ion electrode system. [NOTE—When taking measurements, immerse the electrodes in the solution, which has been transferred to a 150-mL beaker containing a polytetrafluoroethylene-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.] Plot the logarithm of the fluoride-ion concentrations, in  $\mu$ g per mL, of the *Standard solutions* versus potential, in mV. From the measured potential of the *Test solution* and the standard curve, determine the concentration, in  $\mu$ g per mL, of fluoride ions in the *Test solution*: not more than 0.05% is found.

**Heavy metals**, *Method II* (231): 0.002%.

**Other requirements**—Where the label states that Floxuridine is sterile, it meets the requirements for *Sterile Solids* under *Injections* (1) and for *Pyrogen* under *Floxuridine for Injection*. Where the label states that Floxuridine must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Pyrogen* under *Floxuridine for Injection*.

**Assay**—Dissolve about 800 mg of Floxuridine, accurately weighed, in 80 mL of dimethylformamide, and titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 24.62 mg of  $C_9H_{11}FN_2O_5$ .

## Floxuridine for Injection

» Floxuridine for Injection is lyophilized Floxuridine suitable for intraarterial infusion. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_9H_{11}FN_2O_5$ .

**Packaging and storage**—Preserve in *Containers for Injections* as described under *Injections* (1), protected from light. Store containers of constituted Floxuridine for Injection under refrigeration for not more than 2 weeks.

**USP Reference standards** (11)—

USP Floxuridine RS

**Constituted solution**—It meets the requirements for *Constituted Solutions under Injections* (1).**Identification**—**A:** *Ultraviolet Absorption* (197U)—*Solution*—Use *Assay preparation*.**B:** It responds to *Identification test C* under *Floxuridine*.**Pyrogen**—It meets the requirements of the *Pyrogen Test* (151), the test dose being 0.50 mL per kg of a solution prepared by dilution of Floxuridine for Injection with Sodium Chloride Injection to a concentration of 100 mg per mL.**Uniformity of dosage units** (905): meets the requirements.**pH** (791): between 4.0 and 5.5, in a solution (1 in 50).**Other requirements**—It meets the requirements under *Injections* (1).**Assay**—Transfer about 100 mg of Floxuridine for Injection, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with potassium hydroxide solution (1 in 180) to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with potassium hydroxide solution (1 in 180) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Floxuridine RS, in the same medium having a known concentration of about 20 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using potassium hydroxide solution (1 in 180) as the blank. Calculate the quantity, in mg, of  $C_9H_{11}FN_2O_5$  in the portion of Floxuridine for Injection taken by the formula:

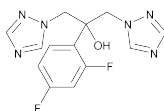
$$5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Floxuridine RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from Floxuridine for Injection and the Standard solution, respectively.

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**Fluconazole**

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$C_{13}H_{12}F_2N_6O$  306.27  
 1*H*-1,2,4-Triazole-1-ethanol, 1-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-ylmethyl)-;  
 2,4-Difluoro-1',1'-bis(1*H*-1,2,4-triazol-1-ylmethyl)benzyl alcohol [86386-73-4].

**DEFINITION**Fluconazole contains NLT 98.0% and NMT 102.0% of  $C_{13}H_{12}F_2N_6O$ , calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)• **B. ULTRAVIOLET ABSORPTION** (197U)*Sample solution:* 200 µg/mL*Medium:* Alcohol**ASSAY**• **PROCEDURE***Sample solution:* Dissolve 200 mg of Fluconazole in 100 mL of glacial acetic acid.*Analysis:* Titrate with 0.1 N perchloric acid VS, using a suitable anhydrous electrode system. Perform a blank determination, and make any necessary correction. EachmL of 0.1 N perchloric acid is equivalent to 15.31 mg of fluconazole ( $C_{13}H_{12}F_2N_6O$ ).**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES**[NOTE—On the basis of information regarding the manufacturing process, perform either: (a) *Organic Impurities, Procedure 1* or (b) *Organic Impurities, Procedure 2* and *Organic Impurities, Procedure 3*.]• **ORGANIC IMPURITIES, PROCEDURE 1****Mobile phase:** Acetonitrile and water (20:80)**Standard solution:** 10 µg/mL each of USP Fluconazole RS, USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS, dissolved in acetonitrile, and then diluted in *Mobile phase***Sample solution:** 3 mg/mL of Fluconazole in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 260 nm**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1**Column temperature:** 40°**Flow rate:** 0.5 mL/min**Injection size:** 20 µL**System suitability****Sample:** *Standard solution*

[NOTE—The retention times for fluconazole related compound A, fluconazole related compound B, fluconazole related compound C, and fluconazole are about 4.9, 8.0, 8.5, and 9.9 min, respectively.]

**Suitability requirements****Resolution:** NLT 1.5 between fluconazole related compound B and fluconazole related compound C  
**Relative standard deviation:** NMT 5.0% for each peak**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fluconazole related compound A, fluconazole related compound B, and fluconazole related compound C in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of fluconazole related compound A, fluconazole related compound B, or fluconazole related compound C from the *Sample solution* $r_S$  = average peak response of fluconazole related compound A, fluconazole related compound B, and fluconazole related compound C for replicate injections of the *Standard solution* $C_S$  = concentration of USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Fluconazole in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurities in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of any other impurity from the *Sample solution* $r_S$  = average peak response of fluconazole for replicate injections of the *Standard solution* $C_S$  = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Fluconazole in the *Sample solution* (mg/mL)



Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluconazole related compound A	0.5	0.2
Fluconazole related compound B	0.81	0.1
Fluconazole related compound C	0.86	0.2
Fluconazole	1.0	—
Specified impurity	0.6	1.0
Any other individual impurity	—	0.1
Total unknown impurities	—	0.3
Total impurities	—	1.5

• **ORGANIC IMPURITIES, PROCEDURE 2**

**Solution A:** 0.01 M anhydrous sodium acetate solution. Adjust with 1 N acetic acid to a pH of 5.0, filter, and degas.

**Solution B:** Acetonitrile

**Solution C:** Methanol

**Mobile phase:** See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	80	5	15
10	80	5	15
20	30	55	15
23	30	55	15
25	80	5	15
30	80	5	15

**Diluent:** Methanol and *Solution A* (16:84)

**Standard solution:** 0.01 mg/mL of USP Fluconazole RS in *Diluent*

**System suitability solution:** 0.02 mg/mL of USP Fluconazole RS and 6 µg/mL of USP Desacetyl Diltiazem Hydrochloride RS in *Diluent*

**Sample solution:** 2 mg/mL of Fluconazole in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 261 nm

**Column:** 4.0-mm × 10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for fluconazole and desacetyl diltiazem are 1.0 and 1.2, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 10.0 between fluconazole and desacetyl diltiazem hydrochloride, *System suitability solution*

**Column efficiency:** NLT 30,000 theoretical plates for the fluconazole peak, *System suitability solution*

**Tailing factor:** NMT 1.4 for the fluconazole peak, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of fluconazole from the *Standard solution*

$C_S$  = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fluconazole in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Specified impurity	0.17–0.37	0.72	0.1
Specified impurity	0.48–0.60	0.85	0.1
Specified impurity	0.67–0.79	1.21	0.1
Specified impurity	1.14–1.18	0.96	0.1
Specified impurity	1.20–1.32	0.97	0.1
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

• **ORGANIC IMPURITIES, PROCEDURE 3**

**Standard solution A:** 1 mg/mL of USP Fluconazole RS in methanol (2.0%)

**Standard solution B:** 0.1 mg/mL of USP Fluconazole RS from *Standard solution A* in methanol (0.2%)

**Standard solution C:** 0.05 mg/mL of USP Fluconazole RS from *Standard solution A* in methanol (0.1%)

**Sample solution:** 50 mg/mL of Fluconazole in methanol

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Chloroform, methanol, and ammonium hydroxide (80:20:1)

**Spray reagent A:** 1.7 mg/mL of silver nitrate in water

**Spray reagent B (potassium iodoplatinate solution):** 375 mg of chloroplatinic acid in 5 mL of 1 N hydrochloric acid. Dissolve 5 g of potassium iodide in 50 mL of water, and store in a light-resistant container. Prepare a mixture of water, potassium iodide solution, and chloroplatinic acid solution (20:9:1).

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Spray the dry plate with *Spray reagent A*, and expose the plate to 365-nm UV light for 10–20 min. Dry the plate for 20 min between 80° and 90°, then spray the plate with *Spray reagent B*. Allow the plate to dry. Examine the plate and compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots in the *Standard solutions*.

**Acceptance criteria:** No spot from the *Sample solution* with an  $R_f$  value between 0.10–0.25 and 0.27–0.41 is larger or more intense than that from *Standard solution B* (0.2%).

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

**Sample:** 0.5 g

• **IRON** <241>

**Sample solution:** Transfer 0.5 g of the sample into a test tube. Dissolve in 5 mL of alcohol, and add 5 mL of distilled water.

**Acceptance criteria:** NMT 20 ppm

**SPECIFIC TESTS**

• **LOSS ON DRYING** <731>: Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

• **CLARITY AND COLOR OF SOLUTION**

**Sample solution:** Dissolve a sample in methanol to obtain a 5-in-100 solution (w/v).

**Acceptance criteria:** The solution is clear and colorless.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 30°.
- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure(s) the article complies.
- **USP REFERENCE STANDARDS** (11)
  - USP Desacetyl Diltiazem Hydrochloride RS  
 $C_{20}H_{24}N_2O_3S \cdot HCl$  408.95
  - USP Fluconazole RS
  - USP Fluconazole Related Compound A RS  
2-[2-Fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol.
  - USP Fluconazole Related Compound B RS  
2-(4-Fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol.
  - USP Fluconazole Related Compound C RS  
1,1'-(1,3-Phenylene)di(1*H*-1,2,4-triazole).

## Fluconazole Injection

**DEFINITION**

Fluconazole Injection is a sterile solution of Fluconazole in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 0.82 g/L of sodium acetate in water. Adjust with 1 N acetic acid solution to a pH of 5.0.

**Diluent:** Methanol and *Buffer* (20:80)

**Solution A:** Methanol and *Buffer* (5:95)

**Solution B:** Acetonitrile and methanol (60:40)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
9	80	20
15	15	85
18	80	20
25	80	20

**System suitability solution:** 0.04 mg/mL each of benzyl alcohol and USP Fluconazole RS in *Diluent*

**Standard solution:** 0.2 mg/mL of USP Fluconazole RS in *Diluent*

**Sample solution:** Equivalent to 0.2 mg/mL of fluconazole from Injection in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 261 nm

**Column:** 4.0-mm × 10-cm; 3-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 100 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, from the *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*

**Tailing factor:** NMT 1.5 for the fluconazole peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES**

**Organic Impurities**

[NOTE—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2*, or (b) *Procedure 3*, or (c) *Procedure 4*. *Procedure 3* is recommended if bis-triazole ketone and epoxyfluconazole (see *Table 4*) are potential impurities. *Procedure 4* is recommended if fluconazole bromohydrine and epoxyfluconazole (see *Table 5*) are potential impurities.]

• **PROCEDURE 1: FOR NONPOLAR IMPURITIES**

**Buffer and Diluent:** Prepare as directed in the *Assay*.

**Solution A:** Methanol and *Buffer* (5:95)

**Solution B:** Acetonitrile and methanol (60:40)

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	77	23
5	77	23
30	40	60
43	77	23
50	77	23

**System suitability solution:** 2.4 and 20 μg/mL, respectively of 1,4-benzoquinone and USP Fluconazole RS in *Diluent*

**Standard solution:** 2 μg/mL of USP Fluconazole RS in *Diluent*

**Diluted standard solution:** 0.2 μg/mL of USP Fluconazole RS in *Diluent* from the *Standard solution*

**Sample solution:** Equivalent to 1.0 mg/mL of fluconazole from Injection in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 261 nm

**Column:** 4.0-mm × 10-cm; 3-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 100 μL

**System suitability**

**Samples:** *System suitability solution*, *Standard solution*, and *Diluted standard solution*

[NOTE— The relative retention times for 1,4-benzoquinone and fluconazole are about 0.5 and 1.0, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 5.0 between 1,4-benzoquinone and fluconazole, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates for the fluconazole peak, *System suitability solution*

**Tailing factor:** NMT 1.5 for the fluconazole peak, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Ratio of average peak area:** Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—For the following calculations, do not include peaks eluting before fluconazole and do not include impurities at relative retention times of 2.00–2.12 and 3.14–3.26. The disregarded impurities at the specified relative retention times are process impurities monitored in the drug substance. Furthermore, disregard any peak due to an excipient or any peak less than 0.02%. This test is for determination of the late-eluting peaks, and hence the early-eluting peaks are not quantitated using this procedure.]

Calculate the percentage of the single largest unknown nonpolar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the largest impurity from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of fluconazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Calculate the percentage of unknown nonpolar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak areas of the unknown peaks from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Largest unknown nonpolar impurity:** NMT 0.1%

**Total unknown nonpolar impurities:** NMT 0.5%

• **PROCEDURE 2: FOR POLAR IMPURITIES**

**Buffer, Diluent, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 2 µg/mL of USP Fluconazole RS in *Diluent*

**Diluted standard solution:** 0.2 µg/mL of USP Fluconazole RS in *Diluent* from the *Standard solution*

**Sample solution:** Equivalent to 0.2 mg/mL of fluconazole from Injection in *Diluent*

**System suitability**

**Samples:** *System suitability solution*, *Standard solution*, and *Diluted standard solution*

[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*

**Tailing factor:** NMT 1.5 for the fluconazole peak, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Ratio of average peak area:** Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times of known related compounds versus fluconazole are included in Table 3.]

Calculate the percentage of the single largest unknown polar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the largest impurity from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of fluconazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Calculate the quantity, as a percentage, of the unknown polar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = total peak area response of all the unknown peaks from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of fluconazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Largest unknown polar impurity:** NMT 0.1%

**Total unknown polar impurities:** NMT 0.5%

**Total unknown polar and nonpolar impurities:** NMT 1.0% (sum of results from Procedure 1 and Procedure 2)

[NOTE—Disregard any peak due to an excipient or any peak less than 0.03%.]

**Table 3**

Name	Relative Retention Time
Hydroxymethylfurfural (if dextrose is present) <sup>a</sup>	0.22–0.28
Aminofluconazole quaternary salt <sup>b</sup>	0.30–0.36
Unidentified impurity (if dextrose is present) <sup>c</sup>	0.37–0.43
Fluconazole isomer <sup>d</sup>	0.47–0.59
Fluconazole diol <sup>e</sup>	0.68–0.74
Cyclohexanone <sup>f</sup>	0.77–0.83
Fluconazole	1.0

<sup>a</sup> 5-Hydroxymethylfurfural.

<sup>b</sup> 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-4H-1,2,4-triazolium bromide.

<sup>c</sup> Dextrose-related compound.

<sup>d</sup> 2-(2,4-Difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-3-(4H-1,2,4-triazol-4-yl)propan-2-ol.

<sup>e</sup> 2-(2,4-Difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propane-1,2-diol.

<sup>f</sup> A process impurity associated with drug product packaged in bags.

[NOTE—Disregard aminofluconazole quaternary salt, fluconazole isomer, and fluconazole diol impurities from *Ta*-

ble 3 in the following calculation because these are process impurities that are monitored in the drug substance.]

• **PROCEDURE 3**

**Buffer:** 0.63 g/L of ammonium formate in water

**Mobile phase:** Acetonitrile and *Buffer* (14:86)

**Standard solution:** 2 mg/mL of USP Fluconazole RS in *Buffer*. [NOTE—Use approximately 14% of the total volume as acetonitrile, and sonicate if necessary to facilitate dissolution.]

**Sensitivity solution:** 1 µg/mL of USP Fluconazole RS in *Mobile phase* from the *Standard solution*

**Sample solution:** Equivalent to 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *Sensitivity solution*

[NOTE—The retention time for fluconazole is between 12 and 14 min.]

**Suitability requirements**

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Column efficiency:** NLT 3000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of fluconazole from the *Standard solution*

$C_S$  = concentration of fluconazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim, in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See *Table 4*.

**Total impurities:** NMT 0.5%

**Table 4**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bistriazole ketone <sup>a</sup>	0.13	0.2
Fluconazole isomer <sup>b</sup>	0.5	0.2
Fluconazole	1.0	—
Epoxyfluconazole <sup>c</sup>	2.6	0.2
Any other individual impurity	—	0.2

<sup>a</sup> 1,3-Bis(1*H*-1,2,4-triazol-1-yl)propan-2-one.

<sup>b</sup> 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl)propan-2-ol.

<sup>c</sup> 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1,2,4-triazole.

**Procedure 4**

**Buffer:** 13.4 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 7.0.

**Mobile phase:** Acetonitrile and *Buffer* (26:74)

**Standard solution:** 2 mg/mL of USP Fluconazole RS in *Mobile phase*

**Sample solution:** Equivalent to 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 0.5 mL/min

**Injection size:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of fluconazole from the *Standard solution*

$C_S$  = concentration of fluconazole in the *Standard solution* (mg/mL)

$C_U$  = concentration of fluconazole, based on the label claim, in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See *Table 5*.

**Total impurities:** NMT 0.5%

**Table 5**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amino fluconazole quaternary salt <sup>a</sup>	0.57	0.74	0.1
Fluconazole isomer <sup>b</sup>	0.68	0.93	0.1
Fluconazole diol <sup>c</sup>	0.91	1.3	0.1
Fluconazole	1.0	1.0	—
Fluconazole bromohydrine <sup>d</sup>	2.58	1.1	0.1
Epoxyfluconazole <sup>e</sup>	2.59	0.90	0.1
Any other individual impurity	—	1.0	0.1

<sup>a</sup> 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-4*H*-1,2,4-triazol-1-ium bromide.

<sup>b</sup> 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl)propan-2-ol.

<sup>c</sup> 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.

<sup>d</sup> 1-Bromo-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol.

<sup>e</sup> 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1,2,4-triazole.

**SPECIFIC TESTS**

• **STERILITY TESTS** <71>: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.416 USP Endotoxin Unit/mg of fluconazole

• **OTHER REQUIREMENTS:** Meets the requirements under *Injections* <1>

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Store at controlled room temperature.

• **LABELING:** Label to indicate the vehicle used. If a test for *Organic Impurities* other than *Procedure 1* and *Procedure 2* is used, then the labeling states with which *Organic Impurities* test the article complies.

• **USP REFERENCE STANDARDS** <11>

USP Endotoxin RS  
USP Fluconazole RS

## Fluconazole Tablets

### DEFINITION

Fluconazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

• **PROCEDURE**

**Buffer:** 0.01 M anhydrous sodium acetate solution. Adjust with glacial acetic acid to a pH of 5.0.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (20:10:70)

**Standard stock solution:** 1.0 mg/mL of USP Fluconazole RS dissolved in water, and diluted with *Mobile phase* to volume. Sonicate the solution, if necessary. [NOTE—The target ratio is about 5% water to 95% *Mobile phase*.]

**Standard solution:** 0.2 mg/mL of USP Fluconazole RS in *Mobile phase* prepared from the *Standard stock solution*

**Sample solution:** Weigh NLT 5 Tablets, and disperse in a suitable quantity of water. Sonicate the solution, if necessary. Add a sufficient quantity of *Mobile phase*, sonicate for 5 min, and shake for 30 min. Dilute with *Mobile phase* to volume to obtain a concentration between about 1 and 4 mg/mL, and mix. [NOTE—The target ratio is about 5% water to 95% *Mobile phase*.] Centrifuge a suitable portion of the mixture. Filter and quantitatively dilute a portion of the supernatant with *Mobile phase* to obtain a solution of about 0.2 mg/mL of fluconazole.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 261 nm

**Column:** 3.9-mm × 150-mm; 4-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1100 theoretical plates

**Tailing factor:** NMT 3.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

• **DISSOLUTION** <711>

**Test 1**

**Medium:** Water; 500 mL (900 mL for Tablets labeled to contain more than 100 mg)

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Buffer, Mobile phase, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 2 mg/mL of USP Fluconazole RS in *Medium*. Sonicate the solution to facilitate dissolution, if necessary. Quantitatively dilute a portion of this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*, making any necessary modifications.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 500 or 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{13}H_{12}F_2N_6O$  is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets *Dissolution Test 2*.

**Medium:** Water; 900 mL (for all Tablet strengths)

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Mobile phase:** Water and acetonitrile (4:1)

**Standard stock solution:** 1.1 mg/mL of USP Fluconazole RS in methanol

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of ( $L/900$ ) mg/mL, where  $L$  is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of the labeled amount of fluconazole ( $C_{13}H_{12}F_2N_6O$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of fluconazole is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
USP Fluconazole RS

## Flucytosine



$C_4H_4FN_3O$  129.09  
Cytosine, 5-fluoro-  
5-Fluorocytosine [2022-85-7].

» Flucytosine contains not less than 98.5 percent and not more than 101.0 percent of  $C_4H_4FN_3O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Flucytosine RS  
USP Fluorouracil RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 8 µg per mL.

*Medium:* dilute hydrochloric acid (1 in 100).

Absorptivities at 285 nm, calculated on the dried basis, do not differ by more than 2.0%.

**B:** The  $R_f$  value of the principal spot in the specimen chromatogram in the test for *Fluorouracil* corresponds to that obtained with the solution of USP Flucytosine RS.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Fluoride ions**—[NOTE—All glassware and/or plasticware used in this test should be scrupulously clean and even free from trace amounts of fluoride. The use of plasticware to contain the solutions while the potential is measured is recommended.]

**Buffer solution**—To 110 g of sodium chloride in a 2-L volumetric flask add 1 g of sodium citrate and 700 mL of water, and dissolve with shaking. Carefully add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and while stirring, cautiously add 450 mL of glacial acetic acid. Cool to room temperature, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix. The pH of this solution is between 5.0 and 5.5.

**Standard stock solution**—Accurately weigh 2.211 g of sodium fluoride, previously dried at 150° for 4 hours, into a 1-L volumetric flask, and dissolve in about 200 mL of water. Add 1.0 mL of sodium hydroxide solution (1 in 250), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ion. Store the solution in a closed plastic container.

**Standard preparations**—Dilute a portion of *Standard stock solution* quantitatively and stepwise with *Buffer solution* to obtain a *Standard preparation* having a fluoride concentration of 1 µg per mL. Prepare the final dilution in a 100-mL volumetric flask. In the same manner, prepare additional *Standard preparations* having fluoride concentrations of 3 µg per mL, 5 µg per mL, and 10 µg per mL, respectively.

**Test preparation**—Place 1 g of Flucytosine, accurately weighed, in a 100-mL volumetric flask, and dissolve in and dilute with *Buffer solution* to volume.

**Procedure**—Concomitantly measure the potential (see *Titrimetry* (541)), in mV, of the *Standard preparations* and the *Test preparation*, with a suitable pH meter equipped with a fluoride-specific ion electrode and a glass-sleeved calomel reference electrode that has been modified in the following manner. Mix 70 mL of freshly prepared saturated potassium chloride solution with 30 mL of isopropyl alcohol, fill the electrode with the clear supernatant, and allow the electrode to remain in the mixture for not less than 2 hours prior to use, or preferably overnight.

When taking the measurements, transfer the solution to a 150-mL beaker, and immerse the electrodes. Insert a polytetrafluoroethylene-coated stirring bar into the beaker, place the beaker on a magnetic stirrer having an insulated top, and allow to stir until equilibrium is attained (about 1 to 2 minutes). Rinse and dry the electrodes between measurements, taking care not to scratch the crystal in the specific ion electrode.

Measure the potential of each *Standard preparation*, and plot the fluoride concentration, in mg per 100 mL, versus the potential, in mV, on semilogarithmic paper. Measure the potential of the *Test preparation*, and determine from the standard curve the fluoride concentration, in mg per 100 mL. Calculate the percentage of fluoride in the portion of Flucytosine taken by the formula:

$$C / 10$$

in which C is the fluoride concentration, in mg per 100 mL, from the standard curve: not more than 0.05% of fluoride is found.

**Fluorouracil**—Dissolve 250 mg in 10 mL of a mixture of glacial acetic acid and water (4:1). Apply 20 µL of this solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.5-mm layer of chromatographic silica gel mixture. To the same plate apply 20 µL, in 10-µL increments, of a 0.025 mg per mL solution of USP Fluorouracil RS in a mixture of glacial acetic acid and water (4:1). Develop the chromatogram in a mixture of chloroform and glacial acetic acid (13:7) until the solvent front has moved not less than 14 cm from the origin. Remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by observing under short-wavelength UV radiation: any spot from the solution under test is not greater in size and intensity than the spot at the respective  $R_f$  produced by the *Standard solution*, corresponding to not more than 0.1% of fluorouracil.

**Assay**—Place about 400 mg of Flucytosine, accurately weighed, in a 250-mL beaker, add 150 mL of a mixture of 2 volumes of glacial acetic acid and 1 volume of acetic anhydride, and dissolve, warming gently if necessary. Titrate potentiometrically with 0.1 N perchloric acid VS, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 12.91 mg of  $C_4H_4FN_3O$ .

## Flucytosine Capsules

» Flucytosine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flucytosine ( $C_4H_4FN_3O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Flucytosine RS

**Identification—**

**A:** The UV absorption spectrum of the solution from the Capsule contents obtained in the Assay exhibits maximum absorption at the same wavelength as that of the Standard solution, and the two spectra are similar between 260 nm and 350 nm.

**B:** Shake a portion of the contents of Capsules, equivalent to about 500 mg of flucytosine, with 10 mL of water. Filter, and to 2 mL of the filtrate add 1 mL of sodium pentacyanoaminoferate reagent [prepared by dissolving 100 mg of sodium (tri)pentacyanoaminoferate in 20 mL of sodium carbonate solution (1 in 100)] and 1 mL of 3 percent hydrogen peroxide: on standing, a darker green is produced than that produced by a blank.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 60 minutes.

**Procedure—**Determine the amount of  $C_4H_4FN_3O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Flucytosine RS in the same *Medium*.

**Tolerances—**Not less than 80% (Q) of the labeled amount of  $C_4H_4FN_3O$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**Weigh the contents of not fewer than 20 Capsules, and determine the average weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 250 mg of flucytosine, to a 250-mL volumetric flask. Add about 50 mL of 0.1 N hydrochloric acid, shake by mechanical means for 30 minutes, then add 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute 10 mL of the clear filtrate with 0.1 N hydrochloric acid to 250 mL. Dilute 10.0 mL of this solution with 0.1 N hydrochloric acid to 50 mL. Dissolve an accurately weighed quantity of USP Flucytosine RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 8 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of flucytosine ( $C_4H_4FN_3O$ ) in the portion of Capsule contents taken by the formula:

$$31.25C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Flucytosine RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Capsule contents and the Standard solution, respectively.

**Flucytosine Oral Suspension****DEFINITION**

Flucytosine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of flucytosine ( $C_4H_4FN_3O$ ). Prepare Flucytosine Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Flucytosine	1.0 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Empty the contents of the required number of capsules into a suitable mortar, or add *Flucytosine* powder to the mortar. Add 10 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Solution A:** 1 g of ammonium acetate and 1 mL of diisopropylamine in 1 L of water. Adjust with glacial acetic acid to a pH of 7.5.

**Mobile phase:** Methanol and *Solution A* (50:50). Filter, and degas.

**Standard solution:** 50 µg/mL of USP Flucytosine RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 0.5 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 200-mm; 5-µm packing L3

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for flucytosine is about 3 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of flucytosine ( $C_4H_4FN_3O$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of flucytosine in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of flucytosine in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

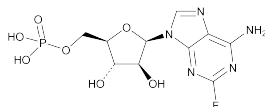
#### SPECIFIC TESTS

- **PH** (791): 4.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a cold place.
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded
- **USP REFERENCE STANDARDS** (11)  
USP Flucytosine RS

## Fludarabine Phosphate



$\text{C}_{10}\text{H}_{13}\text{FN}_5\text{O}_7\text{P}$  365.21  
9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono- $\beta$ -D-arabinofuranosyl)-;  
9- $\beta$ -D-Arabinofuranosyl-2-fluoroadenine 5'-(dihydrogen phosphate) [75607-67-9].

#### DEFINITION

Fludarabine Phosphate contains NLT 98.0% and NMT 102.0% of fludarabine phosphate ( $\text{C}_{10}\text{H}_{13}\text{FN}_5\text{O}_7\text{P}$ ), calculated on the anhydrous, solvent-free basis.

[**CAUTION**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

#### ASSAY

##### PROCEDURE

**Solution A:** 10 mM of monobasic potassium phosphate

**Mobile phase:** Methanol and *Solution A* (6:94)

**Standard solution:** 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*

**Sample solution:** 0.02 mg/mL of Fludarabine Phosphate in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fludarabine phosphate ( $\text{C}_{10}\text{H}_{13}\text{FN}_5\text{O}_7\text{P}$ ) in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fludarabine Phosphate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous, solvent-free basis

#### IMPURITIES

##### CHLORIDE

**Standard stock solution:** 82.4  $\mu\text{g/mL}$  of sodium chloride in water

**Standard solution:** Transfer 2.0 mL of *Standard stock solution* to a test tube, add 13.0 mL of water, and mix.

**Sample solution:** Transfer 50.0 mg of Fludarabine Phosphate to a test tube, add 15 mL of water to dissolve, and heat gently if necessary.

**Analysis:** Add 1.0 mL of nitric acid to the *Standard solution* and *Sample solution*, and place each in separate, colorless test tubes containing 1.0 mL of silver nitrate TS.

**Acceptance criteria:** NMT 0.2%; the *Sample solution* shows less turbidity than the *Standard solution*.

##### LIMIT OF FREE PHOSPHATE

**Standard solution:** 7.16  $\mu\text{g/mL}$  of potassium dihydrogen phosphate in water. Transfer 2.0 mL of this solution to a test tube.

**Sample solution:** 10 mg of Fludarabine Phosphate in 2.0 mL of water in a test tube, heating gently

**Blank:** 2.0 mL of water in a test tube

**Reagent solution:** Mix 4 g of finely powdered ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in a 150-mL beaker. Add 70 mL of water, and grind the particles using a glass rod. A clear solution is obtained within a few min. Add 20 mL of nitric acid, adjust to room temperature, and dilute with water to 100 mL.

**Analysis:** To each of the test tubes containing the *Standard solution*, *Sample solution*, and *Blank*, add 2.0 mL of *Reagent solution*.

**Acceptance criteria:** NMT 0.1%; the color of the *Standard solution* must be more intense than that of the *Blank*. Viewed downward in diffuse daylight against a white background, the yellow coloration of the *Sample solution* must not be more intense than that of the *Standard solution*.

##### LIMIT OF SODIUM

**Standard stock solution:** 2.54 mg/mL of sodium chloride in water. Sodium chloride is previously dried at 105° for 2 h.

**Standard solution:** 1  $\mu\text{g/mL}$  of sodium chloride in water, from *Standard stock solution*

**Sample solution:** 0.5 mg/mL of Fludarabine Phosphate in water

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Flame photometry

**Analytical wavelength:** Sodium emission line at 589.0 nm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** NMT 0.2%; the emission response of the *Sample solution* is NMT that of the *Standard solution*.

- **HEAVY METALS, Method I** (231): NMT 20 ppm

##### ORGANIC IMPURITIES PROCEDURE 1: EARLY-ELUTING IMPURITIES

**Mobile phase, Standard solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 10 mg of Fludarabine Phosphate in 10 mL of 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.



**Sensitivity solution:** 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase*, from the *Standard solution*

**Sample solution:** 1 mg/mL of Fludarabine Phosphate in *Mobile phase*

**System suitability**

**Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each early-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_1) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of fludarabine phosphate from the *Sample solution*

$F_1$  = relative response factor for each individual impurity (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate	0.26	0.25	0.8
Iso-guanine	0.34	0.40	0.2
3',5'-Diphosphate analog	0.42	0.53	0.4
Any individual unspecified impurity	<1.0	1.0	0.1
Fludarabine phosphate	1.0	—	—

• **ORGANIC IMPURITIES PROCEDURE 2: LATE-ELUTING IMPURITIES**

**Solvent A:** 10 mM monobasic potassium phosphate

**Mobile phase:** Methanol and *Solvent A* (1:4)

**Standard solution and Chromatographic system:** Proceed as directed in the *Assay*.

**Sensitivity solution and Sample solution:** Prepare as directed in *Organic Impurities Procedure 1: Early-Eluting Impurities*.

**System suitability**

**Samples:** *Standard solution* and *Sensitivity solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_2) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of fludarabine phosphate from the *Sample solution*

$F_2$  = relative response factor for each individual impurity (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine	1.5	2.0	0.1
2-Fluoro-ara-adenine	1.9	1.7	0.2
2-Ethoxyphosphate analog	2.5	0.56	0.2
Any individual, unspecified impurity	>1.0	1.0	0.1
Total unspecified impurities <sup>a</sup>	—	—	0.5
Total impurities <sup>b</sup>	—	—	1.5

<sup>a</sup> The sum of all unspecified impurities found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

<sup>b</sup> The sum of all impurities found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

• **LIMIT OF ALCOHOL**

**Standard solution:** 0.50 mg/mL of alcohol in dimethylformamide

**Sample solution:** 50 mg/mL of Fludarabine Phosphate in dimethylformamide

**Blank:** Dimethylformamide

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC equipped with a headspace injector

**Detector:** Flame ionization

**Column:** 0.25-mm × 30-m; 1.4-µm coating of phase G43

**Carrier gas:** Helium

**Temperatures**

**Injection port:** 160°

**Detector:** 250°

**Column:** See *Table 3*.

**Table 3**

Initial Temperature (°)	Temperature Ramp (°)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	5	70	—
70	30	220	—

**Flow rate:** 27 cm/s

**Sample**

**Volume:** 2 mL/vial. [NOTE—Seal the vials using a flanged cap so that the cap can no longer be turned.]

**Conditioning temperature:** 80°

**Conditioning time:** 60 min

**Injection volume:** 1.0 mL

**System suitability**

**Samples:** *Standard solution* and *Blank*

[NOTE—The retention time for alcohol is about 3 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 4.0% for three injections, *Standard solution*

**Peak interference:** No peak at the retention time for alcohol, *Blank*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of alcohol in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of alcohol from the *Sample solution*  
 $r_S$  = peak area of alcohol from the *Standard solution*

$C_S$  = concentration of alcohol in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fludarabine Phosphate in the *Sample solution* (mg/mL)

[NOTE—Use the percentage obtained to calculate the Assay result on the solvent-free basis.]

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count is NMT  $10^3$  cfu/g.
- **OPTICAL ROTATION, Specific Rotation** <781S>  
**Sample solution:** 5 mg/mL in water  
**Acceptance criteria:**  $+10^\circ$  to  $+14^\circ$
- **WATER DETERMINATION, Method I** <921>: NMT 3.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.
- **USP REFERENCE STANDARDS** <11>  
USP Fludarabine Phosphate RS

**Fludarabine Phosphate Injection****DEFINITION**

Fludarabine Phosphate Injection is a sterile solution of Fludarabine Phosphate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of fludarabine phosphate ( $C_{10}H_{13}FN_5O_7P$ ).

[**CAUTION**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION** <197U>  
**Solution:** 27 µg/mL in 0.1 M hydrochloric acid
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Solution A:** 6.9 g/L of monobasic sodium phosphate monohydrate in water (50 mM). Adjust with 1.0 N sodium hydroxide to a pH of  $4.5 \pm 0.2$ .

**Mobile phase:** Methanol and *Solution A* (3:47)

**Standard solution:** 0.1 mg/mL of USP Fludarabine Phosphate RS in *Solution A*

**Sample solution:** Equivalent to 0.1 mg/mL of fludarabine phosphate from Injection diluted with *Solution A*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 1%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of Fludarabine Phosphate ( $C_{10}H_{13}FN_5O_7P$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of fludarabine phosphate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

**IMPURITIES****Organic Impurities**

- **PROCEDURE 1: EARLY-ELUTING IMPURITIES (IMPURITIES ELUTING BEFORE FLUDARABINE)**

**Solution A:** 10 mM monobasic potassium phosphate in water

**Mobile phase:** *Solution A* and methanol (47:3)

**System suitability solution:** 1 mg/mL of fludarabine phosphate in 0.1 N hydrochloric acid. Heat the solution at  $80^\circ$  in a water bath for 15 min.

**Standard solution:** 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*

**Quantitative limit solution:** 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase* from the *Standard solution*

**Sample solution:** Equivalent to 1 mg/mL of fludarabine phosphate from Injection diluted with *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm  $\times$  15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *Standard solution*, *System suitability solution*, and *Quantitative limit solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Quantitative limit solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each early-eluting impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of fludarabine phosphate from the *Sample solution*

F = relative response factor (see *Impurity Table 1*)

**Acceptance criteria** See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate <sup>a</sup>	0.26	0.25	1.0
Isoguanine <sup>b</sup>	0.34	0.40	0.2
3',5'-Diphosphate analog <sup>c</sup>	0.42	—	—
Fludarabine phosphate	1.0	—	—
Any individual degradation product	<1.0	1.0	0.2

<sup>a</sup> 6-Amino-9-β-D-arabinofuranosyl-2-oxo-1H-purine 5'-(dihydrogen phosphate).

<sup>b</sup> 6-Amino-1H-purin-2(9H)-one.

<sup>c</sup> 9-β-D-Arabinofuranosyl-2-fluoroadenine 3',5'-bis(dihydrogen phosphate). It is a process impurity and controlled in the drug substance monograph.

• **PROCEDURE 2: LATE-ELUTING IMPURITIES (IMPURITIES ELUTING AFTER FLUDARABINE)**

**Solution A, Standard solution, Quantitative limit solution, Sample solution, and Chromatographic system:** Proceed as directed in *Procedure 1: Early-Eluting Impurities*

**Mobile phase:** *Solution A* and methanol (4:1)

**System suitability**

**Samples:** *Standard solution* and *Quantitative limit solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Quantitative limit solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of fludarabine phosphate from the *Sample solution*

$F$  = relative response factor (see *Impurity Table 2*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 2*.

**Total impurities:** The sum of all fludarabine phosphate degradation products found in *Procedure 1* and *Procedure 2* is NMT 2.0%.

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine <sup>a</sup>	1.5	2.0	0.2
2-Fluoro-ara-adenine <sup>b</sup>	1.9	1.7	0.2

<sup>a</sup> 2-Fluoro-9H-purin-6-amine.

<sup>b</sup> 9-β-D-Arabinofuranosyl-2-fluoroadenine.

<sup>c</sup> 2-Ethoxy-9-β-D-arabinofuranosyladenine 5'-(dihydrogen phosphate). It is a process impurity and controlled in the drug substance monograph.

Impurity Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Ethoxyphosphate analog <sup>c</sup>	2.5	—	—
Any individual degradation product	>1.0	1.0	0.2

<sup>a</sup> 2-Fluoro-9H-purin-6-amine.

<sup>b</sup> 9-β-D-Arabinofuranosyl-2-fluoroadenine.

<sup>c</sup> 2-Ethoxy-9-β-D-arabinofuranosyladenine 5'-(dihydrogen phosphate). It is a process impurity and controlled in the drug substance monograph.

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
- **STERILITY TESTS (71):** Meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined, Membrane Filtration*
- **PH (791):** 6.0–7.1
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements
- **INJECTIONS (1):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, preferably of Type I glass, protected from light. Store in a refrigerator.
- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS  
USP Fludarabine Phosphate RS

## Fludarabine Phosphate for Injection

**DEFINITION**

Fludarabine Phosphate for Injection contains NLT 95.0% and NMT 105.0% of the labeled amount of fludarabine phosphate ( $C_{10}H_{13}FN_5O_7P$ ).

[**CAUTION**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION (197U)**  
**Sample solution:** 27 μg/mL in 0.1 M hydrochloric acid  
**Acceptance criteria:** Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

**Solution A:** 10 mM of monobasic potassium phosphate  
**Mobile phase:** Methanol and *Solution A* (6:94)

**Standard solution:** 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*

**Sample stock solution:** 1 mg/mL of fludarabine phosphate in *Mobile phase* prepared as follows. Inject 2.0 mL of *Mobile phase* into each of five vials of Fludarabine Phosphate for Injection. Transfer the contents of the vials into a 250-mL volumetric flask, using *Mobile phase* rinses. Dilute with *Mobile phase* to volume.

**Sample solution:** 0.02 mg/mL of fludarabine phosphate in *Mobile phase*, from *Sample stock solution*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 260 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 1.0 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of fludarabine phosphate (C<sub>10</sub>H<sub>13</sub>FN<sub>5</sub>O<sub>7</sub>P) in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of fludarabine phosphate in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–105.0%**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements

**IMPURITIES**

- **ORGANIC IMPURITIES PROCEDURE 1: EARLY-ELUTING IMPURITIES**

**Mobile phase, Standard solution, and Chromatographic system:** Proceed as directed in the *Assay*.**System suitability solution:** 10 mg of Fludarabine Phosphate in 10 mL of 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.**Sensitivity solution:** 0.5 μg/mL of USP Fludarabine Phosphate RS in *Mobile phase*, from the *Standard solution***Sample solution:** Use *Sample stock solution* as directed in the *Assay*.**System suitability****Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution***Suitability requirements****Resolution:** NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Sample:** *Sample solution*

Calculate the percentage of each early-eluting impurity in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F_1) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of fludarabine phosphate from the *Sample solution* $F_1$  = relative response factor for each individual impurity (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate	0.26	0.25	1.0
Isoguanine	0.34	0.40	0.2
3',5'-Diphosphate analog <sup>a</sup>	0.42	—	—
Any other individual degradation product	<1.0	1.0	0.2
Fludarabine phosphate	1.0	—	—

<sup>a</sup> This is a process impurity and controlled in the drug substance monograph.• **ORGANIC IMPURITIES PROCEDURE 2: LATE-ELUTING IMPURITIES****Solvent A:** 10 mM monobasic potassium phosphate**Mobile phase:** Methanol and *Solvent A* (1:4)**Standard solution and Chromatographic system:** Proceed as directed in the *Assay*.**Sensitivity solution and Sample solution:** Prepare as directed in *Organic Impurities Procedure 1: Early-Eluting Impurities*.**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Sample:** *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F_2) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of fludarabine phosphate from the *Sample solution* $F_2$  = relative response factor for each individual impurity (see *Table 2*)**Acceptance criteria:** See *Table 2*.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine	1.5	2.0	0.2
2-Fluoro-ara-adenine	1.9	1.7	0.2
2-Ethoxyphosphate analog <sup>a</sup>	2.5	—	—

<sup>a</sup> This is a process impurity and controlled in the drug substance monograph.<sup>b</sup> The sum of all degradation products found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual degradation product	>1.0	1.0	0.2
Total impurities <sup>b</sup>	—	—	2.0

<sup>a</sup> This is a process impurity and controlled in the drug substance monograph.

<sup>b</sup> The sum of all degradation products found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

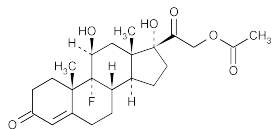
### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
- **PH (791):** 7.2–8.2
- **STERILITY TESTS (71):** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **WATER DETERMINATION, Method I (921):** NMT 5.0%
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections (1)*, *Constituted Solutions*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Injections (1)*, *Containers for Sterile Solids*, between 2° and 30°, or at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS  
USP Fludarabine Phosphate RS

## Fludrocortisone Acetate



$C_{23}H_{31}FO_6$  422.49

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate [514-36-3].

» Fludrocortisone Acetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{23}H_{31}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

**USP Reference standards (11)**—

USP Fludrocortisone Acetate RS

**Identification, Infrared Absorption (197M).**

**Specific rotation (781S):** between +126° and +138°.

*Test solution:* 5 mg per mL, in acetone.

**Loss on drying (731)**—Dry it in vacuum at 100° for 2 hours over magnesium perchlorate: it loses not more than 3.0% of its weight.

**Residue on ignition (281):** not more than 0.1%.

**Chromatographic purity**—Dissolve about 100 mg of it in a mixture of 5 mL of chloroform and 1 mL of acetone in a 10-mL volumetric flask, and dilute with chloroform to volume. Dilute 1 mL of this solution with chloroform to 100 mL. Apply 10  $\mu$ L, in 5- $\mu$ L increments, of the test solution and of its dilution to a line parallel to and about 2.5 cm

from the bottom of a thin-layer chromatographic plate (see *Chromatography (621)*) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the plate in a suitable chamber containing a mixture of chloroform, methanol, and water (85:14:1) until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine under short-wavelength UV light: no spot in the chromatogram of the more concentrated test solution, other than the principal spot, is larger or more intense than the spot from the diluted test solution (1.0%).

### Assay—

**Standard preparation**—Dissolve about 25 mg of USP Fludrocortisone Acetate RS, accurately weighed, in chloroform to make 250 mL, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add chloroform to volume, and mix.

**Assay preparation**—Prepare as directed under *Standard preparation*, using Fludrocortisone Acetate instead of the USP Reference Standard.

**Procedure**—Pipet 10 mL of the *Assay preparation* and the *Standard preparation*, respectively, into separate 25-mL volumetric flasks, and pipet 10 mL of chloroform into a third flask to provide a blank. Treat each flask as follows. Add 1.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Add 1.0 mL of a mixture of 1 volume of tetramethylammonium hydroxide TS and 4 volumes of methanol, mix, and allow to stand for 10 minutes. Dilute with a 1 in 100 solution of hydrochloric acid in methanol to volume. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* in 1-cm cells at about 525 nm, with a suitable spectrophotometer, against the reagent blank. Calculate the quantity, in mg, of  $C_{23}H_{31}FO_6$  in the portion of Fludrocortisone Acetate taken by the formula:

$$1.25C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fludrocortisone Acetate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Fludrocortisone Acetate Tablets

» Fludrocortisone Acetate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fludrocortisone acetate ( $C_{23}H_{31}FO_6$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards (11)**—

USP Fludrocortisone Acetate RS

USP Norethindrone RS

**Identification**—Transfer a portion of powdered Tablets, equivalent to about 1 mg of fludrocortisone acetate, to a glass-stoppered, 15-mL centrifuge tube, add 10 mL of acetone, and shake by mechanical means for 3 minutes. Centrifuge the mixture, and apply 20  $\mu$ L, in 5- $\mu$ L increments, of the clear solution and 20  $\mu$ L of a solution of USP Fludrocortisone Acetate RS in acetone, containing about 100  $\mu$ g per mL, at points along a line about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography (621)*) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the plate in a suitable chamber containing a mixture of chloroform, methanol, and water (85:14:1) until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine under short-wavelength UV light: the  $R_f$  value of the principal spot in the

chromatogram of the test solution corresponds to that obtained with the Standard solution.

**Dissolution** (711)—[NOTE—Use low-actinic glassware throughout this procedure for all solutions. Withdraw dissolution samples with glass syringes, and filter them through membrane filters that have been checked for absorptive loss.]

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 75 rpm.

*Time:* 30 minutes.

Determine the amount of  $C_{23}H_{31}FO_6$  dissolved, employing the following method.

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (45:55). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Transfer about 25 mg of USP Fludrocortisone Acetate RS, accurately weighed, to a 1000-mL volumetric flask. Add 50 mL of acetonitrile, and sonicate for 5 minutes to dissolve. Dilute with 0.01 N hydrochloric acid to volume to obtain a known concentration of fludrocortisone acetate similar to that expected in the solution under test.

*Chromatographic system* (see *Chromatography* (621))—The chromatograph is equipped with a 245-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 100  $\mu$ L) of a filtered portion of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of  $C_{23}H_{31}FO_6$  dissolved based on the peak responses obtained from the solution under test and the *Standard solution*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{23}H_{31}FO_6$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—

*Mobile solvent*—Prepare as directed in the Assay.

*Internal standard solution*—Prepare a solution of USP Nor-ethindrone RS in acetonitrile having a concentration of about 10  $\mu$ g per mL.

*Standard preparation*—Dissolve a suitable quantity of USP Fludrocortisone Acetate RS, accurately weighed, in *Internal standard solution* to obtain a solution having a known concentration of about 0.20 mg per mL. Add 5.0 mL of this solution to 10.0 mL of water contained in a low-actinic 50-mL volumetric flask. Dilute with *Internal standard solution* to volume to obtain a solution having a known concentration of about 20  $\mu$ g of fludrocortisone acetate per mL.

*Test preparation*—Add 1.0 mL of water to 1 Tablet in a 10-mL centrifuge tube, and mix on a vortex-type mixer for 1 minute or until disintegration is complete. Add 4.0 mL of *Internal standard solution*, mix on a vortex-type mixer for 1 minute, then shake by mechanical means for not less than 40 minutes. Centrifuge at 3600 rpm for 20 minutes, or until a clear supernatant is obtained. Use the clear supernatant.

*Procedure*—Proceed as directed for *Procedure* in the Assay. Calculate the quantity, in mg, of  $C_{23}H_{31}FO_6$  in the Tablet taken by the formula:

$$(T / D)C(R_U / R_S)$$

in which  $C$ ,  $R_U$ , and  $R_S$  are as defined in the Assay;  $T$  is the labeled quantity, in mg, of fludrocortisone acetate in the

Tablet; and  $D$  is the concentration, in  $\mu$ g per mL, of fludrocortisone acetate in the *Test preparation*, based on the labeled quantity per Tablet and the extent of dilution.

**Assay**—

*Mobile solvent*—Prepare a suitable, degassed acetonitrile solution, 40% to 45% (v/v), such that the resolution factor,  $R$ , between fludrocortisone acetate and the internal standard is not less than 2.5.

*Internal standard solution*—Prepare a solution of USP Nor-ethindrone RS in acetonitrile having a concentration of about 75  $\mu$ g per mL.

*Standard preparation*—Dissolve a suitable quantity of USP Fludrocortisone Acetate RS, accurately weighed, in *Internal standard solution* to obtain a solution having a known concentration of about 0.50 mg per mL. Pipet 5 mL of this solution into a 25-mL volumetric flask containing 5.0 mL of water. Dilute with *Internal standard solution* to volume to obtain a solution having a known concentration of about 0.1 mg of fludrocortisone acetate per mL.

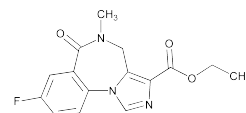
*Assay preparation*—Weigh and finely powder not fewer than 35 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2.5 mg of fludrocortisone acetate, to a low-actinic, glass-stoppered, 50-mL centrifuge tube. Add 5.0 mL of water, and mix for 1 minute. Add 20.0 mL of *Internal standard solution*, mix by mechanical means for 40 minutes, then centrifuge for 15 minutes or until a clear supernatant is obtained. Use the clear supernatant.

*Procedure*—Introduce equal volumes (about 20  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained with the *Standard preparation* is about 0.7 full scale. Typically, the apparatus is fitted with a 3.9-mm  $\times$  30-cm stainless steel column packed with packing L1, and equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of the *Standard preparation* is not more than 3.0% and the resolution factor,  $R$ , is not less than 2.5 between the two peaks. Measure the height of the peaks, at identical retention times, obtained with the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of fludrocortisone acetate ( $C_{23}H_{31}FO_6$ ) in the portion of Tablets taken by the formula:

$$25C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Fludrocortisone Acetate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak heights of the fludrocortisone acetate peak to the internal standard peak from the *Assay preparation* and the *Standard preparation*, respectively.

## Flumazenil



$C_{15}H_{14}FN_3O_3$  303.29

4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester.

Ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate [78755-81-4].

» Flumazenil contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{14}FN_3O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Flumazenil RS

USP Flumazenil Related Compound B RS

Ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4]benzodiazepine-3-carboxylate.  
 $C_{15}H_{15}N_3O_4$  301.30

USP Flumazenil Related Compound C RS

N,N-Dimethylformamide diethyl acetal.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak for flumazenil in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range**, *Class Ia* (741): between 198° and 202°.

**Bacterial endotoxins** (85)—It contains not more than 25.0 USP Endotoxin Units per mg of flumazenil.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Related compounds**—

TEST 1—

*Ninhydrin solution*—Dissolve 0.5 g of ninhydrin in 90 mL of alcohol, and add 10 mL of glacial acetic acid.

*Diluent*—Prepare a mixture of alcohol and chloroform (1:1).

*Adsorbent*: 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* (621)).

*Test solution*—Transfer about 250 mg of Flumazenil, accurately weighed, to a 5-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

*Standard solution 1*—Prepare a solution of USP Flumazenil RS and USP Flumazenil Related Compound C RS in *Diluent* having known concentrations of about 0.5 mg per mL and about 0.6 µL per mL, respectively.

*Standard solution 2*—Dilute 2.0 mL of *Standard solution 1* with *Diluent* to 10.0 mL.

*Application volume*: 10 µL.

*Developing solvent system*: a mixture of chloroform, glacial acetic acid, alcohol, and water (75:15:7.5:2.5).

*Procedure*—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate for 10 minutes in a current of cold air, and view under short-wavelength UV light. Spray the plate with *Ninhydrin solution*, and heat at 105° for 15 minutes. The  $R_f$  values of analytes are as follows.

Compound	$R_f$	Detection
Flumazenil	about 0.8	UV
Flumazenil related compound C	about 0.04	Ninhydrin

Any spot at an  $R_f$  value corresponding to flumazenil related compound C in the chromatogram obtained from the *Test solution* is not more intense than the corresponding spot in the chromatogram obtained from *Standard solution 2*: not more than 0.2% is found.

TEST 2—

*Diluted phosphoric acid*, pH 2.0, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Dilute the *Standard preparation* quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least three times the retention time of the flumazenil peak, and measure the areas for the major peaks. Calculate the percentage of any impurity in the portion of Flumazenil taken by the formula:

$$100(C_S / C_U)(r_i / r_S)(1/F)$$

in which  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of flumazenil in the *Standard solution* and the *Test solution*, respectively;  $r_i$  is the peak area for any impurity in the *Test solution*;  $r_S$  is the peak area for flumazenil in the *Standard solution*; and  $F$  is the relative response factor for each of the known impurities relative to flumazenil. [NOTE— $F$  values are given for all the impurities, along with the corresponding limits, in the *Table* below.]

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
Flumazenil related compound A	about 0.4	1.1	0.2
7-Fluoro-4-methyl-3,4-dihydro-2,5H-1,4-benzodiazepine-2,5-dione	about 0.5	1.5	0.2
Ethyl 5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4]benzodiazepine-3-carboxylate	about 0.7	1.3	0.2
Flumazenil related compound B	about 0.8	1.1	0.2
Flumazenil	1.0	—	—
Ethyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4]benzodiazepine-3-carboxylate	about 2.2	1.1	0.2
Any individual unknown impurity	—	1.0	0.1
Total	—	—	0.5

**Assay—**

*Diluted phosphoric acid, pH 2.0*—Adjust 800 mL of water with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

*Mobile phase*—Prepare a filtered and degassed mixture of *Diluted phosphoric acid, pH 2.0*, methanol, and tetrahydrofuran (80:13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve appropriate quantities of USP Flumazenil RS and USP Flumazenil Related Compound B RS in *Mobile phase*, and dilute, stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 6.4 µg per mL of each compound.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Flumazenil RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL of flumazenil.

*Assay preparation*—Transfer about 25.0 mg of Flumazenil, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph 5 µL of the *System suitability solution*, and record the peak responses: the relative retention times are about 0.8 for flumazenil related compound B and 1.0 for flumazenil; the resolution, *R*, between flumazenil related compound B and flumazenil is not less than 4.0; the column efficiency is not less than 1500 theoretical plates for the flumazenil peak; and the tailing factor is not more than 1.5 for the flumazenil peak. Chromatograph 5 µL of the *Standard preparation*, and record the peak responses: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the flumazenil peaks. Calculate the percentage of  $C_{15}H_{14}FN_3O_3$  in the portion of Flumazenil taken by the formula:

$$100(C_s/C_u)(r_u/r_s)$$

in which  $C_s$  and  $C_u$  are the concentrations, in mg per mL, of flumazenil in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flumazenil Injection

» Flumazenil Injection is a sterile solution of Flumazenil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flumazenil ( $C_{15}H_{14}FN_3O_3$ ).

**Packaging and storage**—Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature.

### USP Reference standards (11)—

USP Flumazenil RS

USP Flumazenil Related Compound A RS

8-Fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazol-[1,5-a][1,4]benzodiazepine-3-carboxylic acid.

$C_{13}H_{10}FN_3O_3$  275.24

USP Flumazenil Related Compound B RS

Ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4H-imidazol-[1,5-a][1,4]benzodiazepine-3-carboxylate.

$C_{15}H_{15}N_3O_4$  301.30

**Identification—**

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

*Adsorbent*: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator.

*Test solution*—Dilute, if necessary, a volume of injection with water to obtain a solution containing 0.1 mg of Flumazenil per mL.

*Standard solution*—Prepare a solution in methanol, and dilute, step wise if necessary, with water to obtain a solution containing about 0.1 mg of USP Flumazenil RS per mL in a mixture of water and methanol (9:1).

*Developing solvent system*: a mixture of ethyl acetate and methanol (9:1).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85): not more than 100 USP Endotoxin Units per mg of flumazenil.

**pH** (791): between 3.4 and 4.6.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Related compounds—**

*Diluent, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation* as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(V/D)(F)(C/L)(r_i/r_s)$$

in which *V* is the volume, in mL, of the *Test Solution*; *C* is the concentration, in mg per mL, of USP Flumazenil RS in the *Standard solution*; *L* is the dose, in mg, of Flumazenil per mL obtained in the *Assay*; *D* is the volume, in mL, of Injection taken to prepare the *Test solution*; *F* is the relative response factor as described in the table below;  $r_i$  is the peak response for each impurity in the *Test solution*; and  $r_s$  is the peak response of flumazenil obtained from the *Standard solution*: meets the requirements given in the table below.

Compound Name	Relative Response Factor	Limit (%)
Flumazenil related compound A	0.9	1.0
Unknown	1.0	0.5
Total unknown	—	1.0
Total	—	2.0

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Diluent*—Prepare a mixture of water, tetrahydrofuran, and methanol (75:20:5).

*0.02 M Phosphate buffer*—Add 0.02 M phosphoric acid to 0.02 M monobasic potassium phosphate to obtain a solution having a pH of  $2.7 \pm 0.05$ .

*Mobile phase*—Prepare a filtered and degassed mixture of 0.02 M *Phosphate buffer*, tetrahydrofuran, and methanol (75:20:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve appropriate quantities of USP Flumazenil RS, USP Flumazenil Related Compound A RS, and USP Flumazenil Related Compound B RS in *Diluent*,



and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 0.01 mg of each per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flumazenil RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Dilute a volume of Injection, if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL of flumazenil in a known volume, *V*.

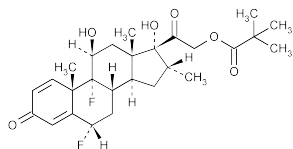
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L10. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.71 for flumazenil related compound A, about 0.85 for flumazenil related compound B, and 1.0 for flumazenil; the resolution, *R*, between flumazenil related compound B and flumazenil is not less than 1.8; and the tailing factor for flumazenil related compound A is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of flumazenil (C<sub>15</sub>H<sub>14</sub>FN<sub>3</sub>O<sub>3</sub>) in the volume of Injection taken by the formula:

$$VC(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Flumazenil RS in the *Standard preparation*; *V* is the volume, in mL, of the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flumethasone Pivalate



C<sub>27</sub>H<sub>36</sub>F<sub>2</sub>O<sub>6</sub> 494.57

Pregna-1,4-diene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-6,9-difluoro-11,17-dihydroxy-16-methyl-, (6α,11β,16α)-, 6α,9-Difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate [2002-29-1].

» Flumethasone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of C<sub>27</sub>H<sub>36</sub>F<sub>2</sub>O<sub>6</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—  
USP Flumethasone Pivalate RS

### Identification—

**A:** *Infrared Absorption* <197M>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 20 μg per mL.

*Medium:* methanol.

Absorptivities at 237 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** <781S>: between +71° and +82°.

*Test solution:* 10 mg per mL, in dioxane.

**Loss on drying** <731>—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Chromatographic purity**—Prepare a solution in dioxane containing 20 mg per mL. Apply 5 μL of this solution and 5 μL each of three dioxane solutions containing in each mL, respectively, 200 (1%), 400 (2%), and 600 (3%) μg of USP Flumethasone Pivalate RS to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of toluene and ethyl acetate (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2), heating at 100° for 30 minutes, and inspecting under long-wavelength UV light: the total content of any impurities detected, when compared to the Standard solutions, does not exceed 3.0%.

### Assay—

**Standard preparation**—Dissolve a suitable quantity of USP Flumethasone Pivalate RS, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution having a known concentration of about 20 μg per mL. Transfer 10.0 mL of this solution to a glass-stoppered, 20-mL conical flask.

**Assay preparation**—Transfer about 20 mg of Flumethasone Pivalate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a glass-stoppered, 20-mL conical flask.

**Procedure**—To each of the flasks containing the *Standard preparation* and the *Assay preparation*, and to a similar flask containing 10.0 mL of alcohol to provide the blank, add 1.0 mL of tetramethylammonium hydroxide TS. Mix, allow to stand for 20 minutes, accurately timed, add 1.0 mL of blue tetrazolium TS to each flask, and mix. Allow to stand for 40 minutes, add 1.0 mL of glacial acetic acid to each flask, mix, and concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of C<sub>27</sub>H<sub>36</sub>F<sub>2</sub>O<sub>6</sub> in the portion of Flumethasone Pivalate taken by the formula:

$$C(A_U / A_S)$$

in which *C* is the concentration, in μg per mL, of USP Flumethasone Pivalate RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Flumethasone Pivalate Cream

» Flumethasone Pivalate Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flumethasone pivalate ( $C_{27}H_{36}F_2O_6$ ) in a suitable cream base.

**Packaging and storage**—Preserve in collapsible tubes.

**USP Reference standards** (11)—

USP Flumethasone Pivalate RS

**Identification**—Place a quantity of Cream, equivalent to about 400  $\mu$ g of flumethasone pivalate, in a 50-mL centrifuge tube, and treat as directed in the Assay, collecting the extracts in a 50-mL centrifuge tube. Evaporate the acetonitrile on a water bath (about 75°) with the aid of a stream of nitrogen. Dissolve the residue in 2.0 mL of acetonitrile with the aid of heat, allow to cool, and centrifuge. Apply 50  $\mu$ L of this solution and 50  $\mu$ L of a solution of chloroform and methanol (1:1) of USP Flumethasone Pivalate RS containing 200  $\mu$ g per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in toluene until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Redevelop the plate in the same manner in a solvent system consisting of toluene and ethyl acetate (7:3), remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Solvent acetonitrile*—Saturate acetonitrile with isooctane.

*Solvent isooctane*—Saturate isooctane with acetonitrile.

*4-Aminoantipyrine solution*—Dissolve about 200 mg of 4-aminoantipyrine in a 1 in 100 solution of hydrochloric acid in methanol to make 50 mL of solution, and mix. Prepare this solution on the day of use.

*Standard preparation*—Dissolve a suitable quantity of USP Flumethasone Pivalate RS, accurately weighed, in *Solvent acetonitrile*, and dilute quantitatively and stepwise with *Solvent acetonitrile* to obtain a solution having a known concentration of about 40  $\mu$ g per mL.

*Assay preparation*—Transfer an accurately weighed portion of Cream, equivalent to about 400  $\mu$ g of flumethasone pivalate, to a tared glass-stoppered, 50-mL centrifuge tube. Place the tube in a vacuum desiccator, and dry the specimen in vacuum over silica gel until about 70% loss in weight is obtained.

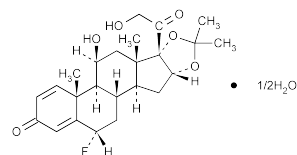
*Procedure*—[NOTE—Perform the extractions in glass-stoppered, 50-mL centrifuge tubes with separation being effected by centrifugation, the portion to be retained being withdrawn into a hypodermic syringe fitted with a blunt-end, 14-gauge, 15-cm needle.] Add 10.0 mL of *Solvent acetonitrile* to the Assay preparation, and transfer 10.0 mL of the Standard preparation to a separate, glass-stoppered, 50-mL centrifuge tube. Add 25 mL of *Solvent isooctane* to each tube, shake by mechanical means until the cream is dispersed, and then shake both mixtures for 5 minutes. Separate and withdraw each acetonitrile layer, and filter through cotton pledgets, previously saturated with *Solvent acetonitrile*, into separate 25-mL volumetric flasks. Repeat the extraction, using a 10-mL portion of *Solvent acetonitrile* for

each tube. Separate, withdraw, and filter each acetonitrile layer through the same respective filter, and combine the extracts with the main extracts. Dilute each with *Solvent acetonitrile* to volume, and mix. Transfer 10.0 mL of each solution to separate, glass-stoppered, 20-mL tubes, and to a third tube transfer 10.0 mL of *Solvent acetonitrile* to provide the blank. Evaporate the solvent on a water bath (about 75°) with the aid of a stream of nitrogen. Add 5.0 mL of *4-Aminoantipyrine solution* to each tube, insert the stopper, shake by mechanical means until the residue dissolves, and allow to stand for 1 hour. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 390 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of flumethasone pivalate ( $C_{27}H_{36}F_2O_6$ ) in the portion of Cream taken by the formula:

$$0.01C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Flumethasone Pivalate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

## Flunisolide



$C_{24}H_{31}FO_6 \cdot 1/2H_2O$  443.51

Pregna-1,4-diene-3,20-dione, 6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, hemihydrate, (6 $\alpha$ , 11 $\beta$ , 16 $\alpha$ )-.

6 $\alpha$ -Fluoro-11 $\beta$ , 16 $\alpha$ , 17, 21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone, hemihydrate [77326-96-6].

Anhydrous 434.51 [3385-03-3].

» Flunisolide contains not less than 97.0 percent and not more than 102.0 percent of  $C_{24}H_{31}FO_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Flunisolide RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* methanol.

**Specific rotation** (781S): between +103° and +111°.

*Test solution:* 10 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Water, Method I** (921)—The anhydrous form contains not more than 1.0%. The hemihydrate form contains between 1.8% and 2.5% (determined on a dried specimen).

**Residue on ignition** (281): not more than 0.1% from 250 mg.

**Chromatographic purity**—

*Standard solutions*—Prepare a solution of USP Flunisolide RS in acetone to contain 10 mg per mL (*Standard solution*

A). Dilute 1 mL of *Standard solution A* with acetone to 100 mL (*Standard solution B*).

**Test preparation**—Prepare a solution of Flunisolide in acetone to contain 10 mg per mL.

**Procedure**—Apply 10- $\mu$ L volumes of *Standard solution A*, *Standard solution B*, and the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a suitable chromatographic chamber previously equilibrated with a mixture of toluene and alcohol (90:10), seal the chamber, and develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to that obtained from *Standard solution A*. No secondary spot exhibits an intensity greater than that of the principal spot from *Standard solution B*.

#### Assay—

**Mobile phase**—Prepare a suitable degassed solution of water and acetonitrile (3:2) such that at an approximate flow rate of 1.6 mL per minute, the retention time of Flunisolide is about 6 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flunisolide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Using 20 mg of Flunisolide, accurately weighed, proceed as directed for *Standard preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  25-cm column that contains 5- to 10- $\mu$ m packing L7. The flow rate is about 1.6 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the column efficiency is not less than 2700 theoretical plates; the tailing factor for the flunisolide peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (between 15  $\mu$ L and 30  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{31}FO_6$  in the portion of Flunisolide taken by the formula:

$$(434.51 / 443.51)100C(r_U / r_S)$$

in which 434.51 and 443.51 are the molecular weights of  $C_{24}H_{31}FO_6$  and  $C_{24}H_{31}FO_6 \cdot \frac{1}{2}H_2O$ , respectively; C is the concentration, in mg per mL, of USP Flunisolide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flunisolide Nasal Solution

» Flunisolide Nasal Solution is an aqueous, buffered solution of Flunisolide. It is supplied in a form suitable for nasal administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{31}FO_6$ .

**Packaging and storage**—Preserve in tight containers, protected from light, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Flunisolide RS

**Identification**—Proceed as directed in the *Assay*, except to inject 50  $\mu$ L to 200  $\mu$ L of a mixture of the *Assay preparation* and the *Standard preparation* (1:1) onto the column, adjusting the response to obtain a response that is between 50% and 90% full scale: a single peak is observed in the chromatogram for the mixed solution.

**pH** <791>: between 4.5 and 6.0, a silver-silver chloride (internal element) electrode being used in conjunction with a fiber junction calomel electrode.

**Quantity delivered per spray**—Prime the spray pump by delivering 10 sprays into a fume hood. Accurately weigh the entire assembly, record the weight, and deliver 8 more sprays into the hood. Again weigh the assembly, and record the weight. Calculate the quantity, in  $\mu$ g, of  $C_{24}H_{31}FO_6$  delivered per spray taken by the formula:

$$[(W_1 - W_2) / 8][A / D]$$

in which  $W_1$  and  $W_2$  are the first and second weights, respectively, in g; A is the quantity, in  $\mu$ g per mL, of  $C_{24}H_{31}FO_6$  found in the *Assay*; and D is the density of Nasal Solution, in g per mL. The quantity delivered is between 17  $\mu$ g and 33  $\mu$ g per spray.

#### Assay—

**Mobile phase**—Prepare a suitable degassed solution of water, acetonitrile, and glacial acetic acid (69:30:1 to 64:35:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.

**Internal standard solution**—Dissolve norethindrone in acetonitrile to obtain a solution containing about 300  $\mu$ g per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flunisolide RS in a mixture of acetonitrile and *Mobile phase* (1:1) to obtain a solution having a known concentration of about 250  $\mu$ g per mL. Transfer 1.0 mL of this solution, and 1.0 mL of *Internal standard solution*, by means of "to contain" pipets, to a 50-mL volumetric flask. Rinse the pipets with *Mobile phase*, adding the rinsings to the flask, dilute with *Mobile phase* to volume, and mix. The final concentration of USP Flunisolide RS is about 5  $\mu$ g per mL.

**Assay preparation**—Transfer an accurately measured volume of Nasal Solution, equivalent to about 250  $\mu$ g of flunisolide, to a 50-mL volumetric flask, and add 1.0 mL of *Internal standard solution* by means of "to contain" pipets. Rinse the pipets with *Mobile phase*, adding the rinsing to the flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  25-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte and the internal standard is not less than 5.0; and the relative standard deviation for replicate injections is not more than 1.5%.

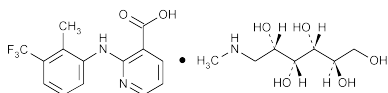
**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for flunisolide and 1.0 for norethindrone. Calculate the quantity, in mg, of  $C_{24}H_{31}FO_6$  in each mL of the Nasal Solution taken by the formula:

$$(434.51 / 443.51)(50C / V)(R_U / R_S)$$

in which 434.51 and 443.51 are the molecular weights of  $C_{24}H_{31}FO_6$  and  $C_{24}H_{31}FO_6 \cdot \frac{1}{2}H_2O$ , respectively; C is the concentration, in mg per mL, of USP Flunisolide RS in the *Standard preparation*; V is the volume, in mL, of Nasal Solution taken; and  $R_U$  and  $R_S$  are the peak response ratios of the

flunisolide peak and the norethindrone peak obtained from the Assay preparation and the Standard preparation, respectively.

## Flunixin Meglumine



$C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$  491.46

3-Pyridinecarboxylic acid, 2-[[2-methyl-3-(trifluoromethyl)phenyl]amino]-, compd. with 1-deoxy-1-(methylamino)-D-glucitol (1:1)

2-( $\alpha^3, \alpha^3, \alpha^3$ -Trifluoro-2,3-xylydino)nicotinic acid compound with 1-deoxy-1-(methylamino)-D-glucitol (1:1) [42461-84-7].

» Flunixin Meglumine contains not less than 99.0 percent and not more than 101.0 percent of  $C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$ .

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Flunixin Meglumine RS

**Labeling**—Label it to indicate that it is for veterinary use only.

### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

Solution: 1 in 30,000.

Medium: 0.1 N sodium hydroxide.

**Melting range** (741): between 137° and 140°.

**pH** (791): between 7.0 and 9.0, in a solution (1 in 20).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Specific rotation** (781S): between −9° and −12°.

Test solution: 120 mg per mL.

**Residue on ignition** (281): not more than 0.2%.

### Chromatographic purity—

**Test solution**—Prepare a solution of Flunixin Meglumine in methanol containing 40 mg per mL.

**Standard stock solution**—Prepare a solution of USP Flunixin Meglumine RS in methanol containing 40 mg per mL.

**Standard solution 1**—Transfer 50  $\mu$ L of the Standard stock solution to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

**Standard solution 2**—Dilute 20  $\mu$ L of the Standard stock solution with 10 mL of methanol, and mix.

**Procedure**—Separately apply 10- $\mu$ L portions of the Test solution, the Stock standard solution, Standard solution 1, and Standard solution 2 to a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and in a paper-lined chamber develop the chromatogram in a solvent system consisting of a mixture of toluene, ethyl acetate, glacial acetic acid, and water (65:30:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots in the chromatogram obtained from the Test solution with the intensity of the principal spot

in the chromatograms obtained from Standard solution 1 and from Standard solution 2: no individual secondary spot in the chromatogram obtained from the Test solution is more intense than the principal spot in the chromatogram obtained from Standard solution 2 (0.2%), and the sum of the intensities of all the secondary spots in the chromatogram obtained from the Test solution does not exceed the intensity of the principal spot in the chromatogram obtained from Standard solution 1 (0.5%).

**Assay**—Dissolve about 175 mg of Flunixin Meglumine, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see Titrimetry (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 24.573 mg of  $C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$ .

## Flunixin Meglumine Granules

» Flunixin Meglumine Granules contain an amount of flunixin meglumine ( $C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Granules to indicate that they are for veterinary use only.

**USP Reference standards** (11)—  
USP Flunixin Meglumine RS

### Identification—

**A:** The UV absorption spectrum of the solution employed for measurement of absorbance in the Assay exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Flunixin Meglumine RS, concomitantly measured.

**B:** Grind a quantity of Granules, equivalent to about 25 mg of flunixin, and transfer the powder to a 50-mL centrifuge tube. Add 20 mL of acetate buffer, prepared by dissolving 4.1 g of anhydrous sodium acetate in 500 mL of water, adding 2.9 mL of glacial acetic acid, and diluting with water to 1000 mL. Rotate the tube for 10 minutes. Extract with 25 mL of ethyl acetate, and use the upper phase as the test solution. Separately apply 10  $\mu$ L of the test solution and 10  $\mu$ L of a Standard solution of USP Flunixin Meglumine RS in methanol containing 1.5 mg per mL to a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of toluene, ethyl acetate, glacial acetic acid, and water (65:30:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

### Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

**Procedure**—Transfer a quantity of Granules, equivalent to about 12.5 mg of flunixin, to the dissolution flask. Determine the amount of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 252 nm on filtered portions of solution under test

in comparison with a Standard solution having a known concentration of about 23.6 µg per mL of USP Flunixin Meglumine RS in the same *Medium*. Each µg of flunixin meglumine is equivalent to 0.6028 µg of flunixin.

**Tolerances**—Not less than 75% (Q) of the labeled amount of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905)—meet the requirements.

#### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flunixin Meglumine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.82 mg per mL. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

**Assay preparation**—Dissolve an accurately weighed quantity of Granules in water by shaking for 30 minutes. Quantitatively dilute with water to obtain a solution containing about 0.5 mg of flunixin per mL, and mix. Centrifuge a portion of this solution. Transfer 4.0 mL of the supernatant layer to a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 283 nm using 0.1 N sodium hydroxide to set the instrument. Calculate the quantity, in mg, of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ) in the portion of Granules taken by the formula:

$$(296.25 / 491.46)(12,500C)(A_U / A_S)$$

in which 296.25 and 491.46 are the molecular weights of flunixin and flunixin meglumine, respectively; C is the concentration, in mg per mL, of USP Flunixin Meglumine RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*; respectively.

## Flunixin Meglumine Injection

» Flunixin Meglumine Injection is a sterile solution of Flunixin Meglumine in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ).

**Packaging and storage**—Preserve in multiple-dose containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Endotoxin RS

USP Flunixin Meglumine RS

**Labeling**—Label Injection to indicate that it is for veterinary use only.

**Bacterial endotoxins** (85)—It contains not more than 4.54 USP Endotoxin Units per mg of flunixin.

#### Identification—

**A:** The UV absorption spectrum of the *Assay preparation* obtained in the *Assay* exhibits maxima and minima at the same wavelengths as that of the *Standard preparation*, concomitantly measured.

**B:** Transfer a quantity of Injection, equivalent to about 50 mg of flunixin, to a 50-mL centrifuge tube. Add 10 mL of *Acetate buffer*, prepared as directed in the *Assay*, and extract with 25 mL of ethyl acetate. Use the upper phase as the test solution. Separately apply 10 µL of the test solution and 10 µL of a Standard solution of USP Flunixin Meglumine RS in methanol containing 3 mg per mL to a thin-layer chro-

matographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of toluene, ethyl acetate, glacial acetic acid, and water (75:25:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.8 and 9.0.

#### Assay—

**Acetate buffer**—Dissolve 4.1 g of anhydrous sodium acetate in 500 mL of water. Add 2.9 mL of glacial acetic acid, dilute with water to 1000 mL, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flunixin Meglumine RS in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.1 N sodium hydroxide to obtain a solution having a known concentration of about 1.65 mg per mL. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of flunixin, to a 50-mL centrifuge tube. Add 20 mL of *Acetate buffer*, and extract with three 25-mL portions of ethyl acetate. Combine the extracts, filter, and evaporate to dryness on a steam bath under a stream of nitrogen. Dissolve the residue in 0.1 N sodium hydroxide, and transfer to a 100-mL volumetric flask with the aid of 0.1 N sodium hydroxide. Dilute with 0.1 N sodium hydroxide to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 327 nm. Calculate the quantity, in mg, of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ) in each mL of Injection taken by the formula:

$$(296.25 / 491.46)(2500C / V)(A_U / A_S)$$

in which 296.25 and 491.46 are the molecular weights of flunixin and flunixin meglumine, respectively; C is the concentration, in mg per mL, of USP Flunixin Meglumine RS in the *Standard preparation*; V is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Flunixin Meglumine Paste

» Flunixin Meglumine Paste contains an amount of flunixin meglumine ( $C_{14}H_{11}F_3N_2O_2 \cdot C_7H_7NO_5$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flunixin ( $C_{14}H_{11}F_3N_2O_2$ ).

**Packaging and storage**—Preserve in a well-closed container.

#### USP Reference standards (11)—

USP Flunixin Meglumine RS

**Labeling**—Label the Paste to indicate that it is for veterinary use only.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve a quantity of sodium benzoate in water to obtain a solution containing 33 mg per mL.

**Diluent**—Prepare a mixture of methanol and water (7:3).

**Standard preparation**—Transfer about 83 mg of USP Flunixin Meglumine RS, accurately weighed, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution*, 20.0 mL of water, and 10.0 mL of methanol to the tube, and mix to dissolve. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer an accurately weighed quantity of Paste, equivalent to about 50 mg of flunixin, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 20.0 mL of water to the tube, and rotate for 20 minutes. Add 10.0 mL of methanol, and mix. Heat the tube in a water bath at 60° for 5 minutes, with occasional shaking. Continue rotating the tube until cool, and centrifuge. Transfer 10.0 mL of the clear supernatant to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

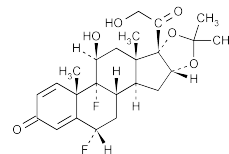
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for sodium benzoate and 1.0 for flunixin meglumine; the resolution, *R*, between sodium benzoate and flunixin meglumine is not less than 1.9; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flunixin (C<sub>14</sub>H<sub>11</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>) in the portion of Paste taken by the formula:

$$(296.25 / 491.46)(87.5C)(R_U / R_S)$$

in which 296.25 and 491.46 are the molecular weights of flunixin and flunixin meglumine, respectively; C is the concentration, in mg per mL, of USP Flunixin Meglumine RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak responses for flunixin and sodium benzoate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinolone Acetonide



C<sub>24</sub>H<sub>30</sub>F<sub>2</sub>O<sub>6</sub> (anhydrous) 452.49

Pregna-1,4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-, 6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone [67-73-2]. Dihydrate 488.53

» Fluocinolone Acetonide is anhydrous or contains two molecules of water of hydration. It contains not less than 97.0 percent and not more than 102.0 percent of C<sub>24</sub>H<sub>30</sub>F<sub>2</sub>O<sub>6</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate whether it is anhydrous or hydrous.

**USP Reference standards** (11)—

USP Fluocinolone Acetonide RS

**Identification**—

**A: Infrared Absorption** (197K)—If a difference appears, dissolve portions of both the test specimen and the USP Reference Standard in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

**B:** It responds to the *Thin-layer Chromatographic Identification Test* (201), the test solution and the Standard solution being prepared at a concentration of 5 mg per mL in acetone, chromatographic silica gel being used as the adsorbent, the solvent mixture being nitromethane, dichloromethane, and methanol (50:50:1), and UV light being used to locate the spots.

**Specific rotation** (781S): between +98° and +108°.

**Test solution:** 10 mg per mL, in methanol.

**Loss on drying** (731)—Dry it in vacuum at 105° for 3 hours: anhydrous Fluocinolone Acetonide loses not more than 1.0% of its weight, and hydrous Fluocinolone Acetonide loses not more than 8.5% of its weight.

**Assay**—

**Mobile phase**—Prepare a suitable, degassed solution of water, acetonitrile, and tetrahydrofuran (77:13:10).

**Standard preparation**—Dissolve about 20 mg of USP Fluocinolone Acetonide RS, accurately weighed, in 23 mL of a mixture of acetonitrile and tetrahydrofuran (13:10), dilute with water to 100.0 mL, and mix.

**Assay preparation**—Transfer about 20 mg of Fluocinolone Acetonide, accurately weighed, to a 100-mL volumetric flask, dissolve in 23 mL of a mixture of acetonitrile and tetrahydrofuran (13:10), dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.5-mm × 10-cm column that contains packing L1. Adjust the flow rate so that the retention time for fluocinolone acetonide is between 9 and 13 minutes. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{30}F_2O_6$  in the portion of Fluocinolone Acetonide taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinolone Acetonide Cream

» Fluocinolone Acetonide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{30}F_2O_6$ .

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

### USP Reference standards (11)—

USP Fluocinolone Acetonide RS

USP Norethindrone RS

**Identification**—Transfer a quantity of the Cream, equivalent to about 0.5 mg of fluocinolone acetonide, to a centrifuge tube, disperse it in 5 mL of water, add 10 mL of chloroform, shake, and centrifuge. Remove and discard the aqueous layer, add 10 mL of water to the tube, shake, and centrifuge. Dry about 2 mL of the chloroform extract over about 200 mg of anhydrous sodium sulfate: the dried extract responds to the *Thin-Layer Chromatographic Identification Test* (201), 50  $\mu$ L of the dried chloroform extract and 50  $\mu$ L of a Standard solution containing about 50  $\mu$ g per mL of USP Fluocinolone Acetonide RS being applied, and a mixture of chloroform and diethylamine (2:1) being used for development.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

### Assay—

**Internal standard solution**—Dissolve USP Norethindrone RS in acetonitrile to obtain a solution containing about 200  $\mu$ g per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 300  $\mu$ g per mL. Transfer 5.0 mL of this solution, 6.0 mL of *Internal standard solution*, and 15.0 mL of water to a 50-mL volumetric flask. Dilute with acetonitrile to volume, and mix. The *Standard preparation* contains 30  $\mu$ g of USP Fluocinolone Acetonide RS per mL.

**Mobile solvent**—Prepare a mixture of water and acetonitrile (5:3). Adjust the ratio as necessary to obtain suitable chromatographic performance.

**Assay preparation**—Dissolve an accurately weighed portion of Cream, equivalent to about 0.75 mg of fluocinolone acetonide, in about 10 mL of acetonitrile by heating on a steam bath. Transfer the mixture to a 25-mL volumetric flask with the aid of three 2-mL portions of acetonitrile. Add 3.0 mL of *Internal standard solution* and 5.0 mL of water, cool, and mix. Dilute with acetonitrile to volume, mix, and cool in an ice bath. Centrifuge or filter the mixture to obtain a clear solution.

**Apparatus**—Use a high-pressure liquid chromatograph (see *Chromatography* (621)) of the general type equipped

with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent*. Use a column that contains packing L1.

**Procedure**—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*. Three replicate injections of the *Standard preparation* show a resolution factor of not less than 2.0 between the peaks for norethindrone and fluocinolone acetonide and a relative standard deviation of not more than 1.5%. Calculate the quantity, in mg, of  $C_{24}H_{30}F_2O_6$  in the portion of Cream taken by the formula:

$$0.025C(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak areas of fluocinolone acetonide and norethindrone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinolone Acetonide Ointment

» Fluocinolone Acetonide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{30}F_2O_6$ .

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

### USP Reference standards (11)—

USP Fluocinolone Acetonide RS

USP Norethindrone RS

**Identification**—Evaporate 10.0 mL of the *Assay preparation* obtained in the *Assay* to dryness, and dissolve the residue in 1 mL of chloroform: it responds to the *Thin-layer Chromatographic Identification Test* (201), 50  $\mu$ L of the test solution and 50  $\mu$ L of the Standard solution, containing about 50  $\mu$ g per mL of USP Fluocinolone Acetonide RS, being applied and a mixture of chloroform and diethylamine (2:1) being used for development.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

### Assay—

**Internal standard solution**—Dissolve a suitable quantity of USP Norethindrone RS in methanol to obtain a solution containing about 850  $\mu$ g per mL.

**Diluted internal standard solution**—Transfer 5.0 mL of *Internal standard solution* to a 250-mL flask. Dilute with methanol to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 200  $\mu$ g per mL. Transfer 10.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. The concentration of USP Fluocinolone Acetonide RS in the *Standard preparation* is 20  $\mu$ g per mL.

**Mobile solvent**—Prepare a mixture of acetonitrile and water (1:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.

**Assay preparation**—Transfer an accurately weighed portion of Ointment, equivalent to about 0.7 mg of fluocinolone acetonide, to a 50-mL, round-bottom centrifuge tube. Add 35.0 mL of *Diluted internal standard solution*, emulsify using an ultrasonic probe, and centrifuge to bring the insol-

uble matter to the bottom. The clear supernatant is the *Assay preparation*.

**Apparatus**—Use a suitable high-pressure liquid chromatograph (see *Chromatography* <621>) of the general type equipped with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent*. Use a 50-cm × 4-mm column that contains packing L1 so as to provide a resolution factor, *R* (see *Chromatography* <621>), of at least 2.0 between peaks for norethindrone and fluocinolone acetonide. Three replicate injections of the *Standard preparation* show a relative standard deviation of not more than 1.5%.

**Procedure**—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*, adjusting the system as necessary to obtain peaks of between about 50% and 90% of full-scale. Calculate the quantity, in mg, of  $C_{24}H_{30}F_2O_6$  in the portion of Ointment taken by the formula:

$$0.035C(R_U / R_S)$$

in which *C* is the concentration, in µg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak areas of fluocinolone acetonide and the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinolone Acetonide Topical Solution

» Fluocinolone Acetonide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{30}F_2O_6$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Fluocinolone Acetonide RS

**Identification**—Transfer a quantity of Topical Solution, equivalent to about 0.5 mg of fluocinolone acetonide, to a separator, add 5 mL of water, and extract with 10 mL of chloroform. Withdraw the chloroform layer into a second separator, wash with 10 mL of water, and dry about 2 mL of the chloroform extract over about 200 mg of anhydrous sodium sulfate: the dried extract responds to the *Thin-Layer Chromatographic Identification Test* (201), 50 µL of the dried chloroform extract and 50 µL of the Standard solution being applied, and a mixture of chloroform and diethylamine (2:1) being used for development.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Assay**—

**Internal standard solution**—Dissolve norethindrone in acetonitrile to obtain a solution containing about 200 µg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 200 µg per mL. Transfer 5.0 mL of this solution, 4.0 mL of *Internal standard solution*, 10 mL of propylene glycol, and about 25 mL of acetonitrile to a 50-mL volumetric flask. Mix, cool to room temperature, dilute with acetonitrile to volume, and mix. The final concentration of USP Fluocinolone Acetonide RS is 20 µg per mL.

**Mobile solvent**—Prepare a mixture of water and acetonitrile (3:2). Adjust the ratio as necessary to obtain suitable chromatographic performance.

**Assay preparation**—Transfer an accurately measured volume of Topical Solution, equivalent to about 0.5 mg of fluocinolone acetonide, to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution* and 10 mL of acetonitrile. Mix, cool to room temperature, dilute with acetonitrile to volume, and mix.

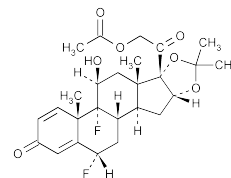
**Apparatus**—Use a suitable high-pressure liquid chromatograph (see *Chromatography* <621>) of the general type equipped with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent*. Use a column containing packing L1 so as to provide a resolution factor, *R*, of at least 2.0 between peaks for norethindrone and fluocinolone acetonide.

**Procedure**—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*, adjusting the system as necessary to obtain peaks of between about 50% and 90% full-scale. Calculate the quantity, in mg, of  $C_{24}H_{30}F_2O_6$  in each mL of the Topical Solution taken by the formula:

$$0.025(C / V)(R_U / R_S)$$

in which *C* is the concentration, in µg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation*; *V* is the volume, in mL, of Solution taken; and  $R_U$  and  $R_S$  are the ratios of the areas of the fluocinolone acetonide peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinonide



$C_{26}H_{32}F_2O_7$  494.52

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6,9-difluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α, 11β, 16α)-.

6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone, 21-acetate [356-12-7].

» Fluocinonide contains not less than 97.0 percent and not more than 103.0 percent of  $C_{26}H_{32}F_2O_7$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Fluocinonide RS

**Identification**—

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

*Medium:* methanol.

Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** <781S>: between +81° and +89°.

*Test solution:* 10 mg per mL, in chloroform.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.



**Residue on ignition** (281): negligible, from 100 mg.  
**Chromatographic purity**—

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay.

*Test preparation*—Transfer about 25 mg of fluocinonide, accurately weighed, to a 10-mL volumetric flask, add acetonitrile to volume, and mix.

*Procedure*—Inject 30  $\mu$ L of the *Test preparation* into the chromatograph, record the chromatogram, and measure the area responses of all peaks. Calculate the area percentage of each peak observed in the chromatogram. The largest secondary peak is not more than 1.0% of the total area, and no other secondary peak is more than 0.5% of the total area. The sum of the areas of all peaks, other than the main peak, does not constitute more than 2.0% of the total area.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 25 mg of USP Fluocinonide RS, accurately weighed, to a 100-mL volumetric flask, add acetonitrile to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.025 mg of USP Fluocinonide RS per mL.

*Assay preparation*—Using about 25 mg of Fluocinonide, accurately weighed, proceed as directed for *Standard preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{26}H_{32}F_2O_7$  in the portion of Fluocinonide taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fluocinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to the fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinonide Cream

» Fluocinonide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ( $C_{26}H_{32}F_2O_7$ ).

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

**USP Reference standards** (11)—  
 USP Fluocinonide RS

**Identification**—Weigh an amount of Cream, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 100-mL centrifuge tube containing 5 mL of water and 10 mL of methanol. Add 20 mL of cyclohexane, shake vigorously, centrifuge, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge until the lower phase is clear, and discard the upper

phase. The clear chloroform extract is the *Test solution*. Separately apply 10  $\mu$ L of the *Test solution* and 10  $\mu$ L of a Standard solution having a concentration of 0.5 mg per mL of USP Fluocinonide RS in chloroform to equidistant points about 2 cm from one end of a thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the applications to dry, and develop the chromatogram in a suitable chromatographic chamber using a mixture of chloroform and acetone (4:1). Air-dry, and view under short-wavelength UV light: the principal spot from the *Test solution* corresponds to that obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 200  $\mu$ g per mL. Transfer 10.0 mL of this solution and 10.0 mL of water to a 100-mL volumetric flask. Dilute with acetonitrile to volume, and mix. The final concentration of USP Fluocinonide RS is 20  $\mu$ g per mL.

*Assay preparation*—Transfer an accurately weighed quantity of Cream, containing about 2 mg of fluocinonide, to a 100-mL volumetric flask. Add about 60 mL of acetonitrile, and dissolve the cream by heating on a steam bath. Add 10.0 mL of water, and allow to cool. Dilute with acetonitrile to volume, and mix. Filter the mixture through a fine-sintered glass funnel, using vacuum, and use the filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Chromatograph equal volumes (about 20  $\mu$ L) of the *Assay preparation* and the *Standard preparation*, record the chromatograms, and measure the peak responses due to fluocinonide. Calculate the quantity, in mg, of fluocinonide ( $C_{26}H_{32}F_2O_7$ ) in the portion of Cream taken by the formula:

$$0.1 C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluocinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinonide Gel

» Fluocinonide Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ( $C_{26}H_{32}F_2O_7$ ).

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

**USP Reference standards** (11)—  
 USP Fluocinonide RS

**Identification**—Weigh an amount of Gel, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 50-mL centrifuge tube containing 20 mL of sodium chloride solution (1 in 10). Add 5 mL of chloroform and 15 mL of methanol, and shake vigorously. Centrifuge to clarify the chloroform layer, and remove the solid material present at the interphase. Discard the upper phase. Dry a portion of the chloroform layer over anhydrous sodium sulfate. Using the dried extract as the *Test preparation*, proceed as directed in the *Identification* test under *Fluocinonide Cream*, beginning with “Apply 10  $\mu$ L of the *Test solution*.”

**Minimum fill** (755): meets the requirements.

**Assay—**

*Mobile phase*—Prepare a mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 200  $\mu$ g per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix. The final concentration is 20  $\mu$ g per mL.

*Assay preparation*—Transfer an accurately weighed quantity of Gel, containing about 2 mg of fluocinonide, to a 100-mL volumetric flask. Add about 60 mL of acetonitrile, and dissolve the gel by heating on a steam bath. Cool to room temperature, dilute with acetonitrile to volume, and mix. Centrifuge a portion at about 2500 rpm for about 5 minutes. Filter a portion of the centrifugate through an acetonitrile-insoluble membrane filter. The filtrate is the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{26}H_{32}F_2O_7$  in the portion of Gel taken by the formula:

$$0.1C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluocinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinonide Ointment

» Fluocinonide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ( $C_{26}H_{32}F_2O_7$ ).

**Packaging and storage**—Preserve in collapsible tubes or tight containers.

**USP Reference standards** (11)—

USP Fluocinonide RS

**Identification**—Weigh an amount of Ointment, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 50-mL centrifuge tube containing 20 mL of cyclohexane. Gently disperse to form a suspension. Add 5 mL of water and 10 mL of methanol. Shake vigorously, allow the phases

to separate, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge, and transfer a portion of the chloroform layer to a small test tube containing about 200 mg of anhydrous sodium sulfate. Mix, and allow to stand until the extract is clear. Using the clear chloroform extract as the *Test preparation*, proceed as directed in the *Identification* test under *Fluocinonide Cream*, beginning with “Apply 10  $\mu$ L of the *Test solution*.”

**Minimum fill** (755): meets the requirements.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 400  $\mu$ g per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. The final concentration of USP Fluocinonide RS is about 40  $\mu$ g per mL.

*Assay preparation*—Transfer an accurately weighed quantity of Ointment, containing about 1.35 mg of fluocinonide, to a round-bottom, 50-mL centrifuge tube. Add 35.0 mL of methanol. Emulsify, using an ultrasonic probe, and centrifuge to bring the insoluble matter to the bottom. The clear supernatant is the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed under *Fluocinonide Cream*, except that the flow rate for the *Mobile phase* is about 1 mL per minute.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluocinonide ( $C_{26}H_{32}F_2O_7$ ) in the portion of Ointment taken by the formula:

$$0.035C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluocinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluocinonide Topical Solution

» Fluocinonide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{26}H_{32}F_2O_7$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Fluocinonide RS

**Identification**—Transfer an amount of Topical Solution, equivalent to about 2.5 mg of fluocinonide, to a glass-stoppered, 50-mL centrifuge tube containing 5 mL of water and 10 mL of methanol, add 20 mL of cyclohexane, shake vigorously, centrifuge, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge until the lower phase is clear, and discard the upper phase. The clear chloroform extract is the *test solution*. Proceed as directed in the *Identification* test under *Fluocinonide Cream* beginning with “Apply 10  $\mu$ L of the *Test solution*.”

**Minimum fill** (755): meets the requirements.

**Alcohol content—**

*Standard solution*—Dilute 20.0 mL of USP Alcohol with methanol to volume in a 200-mL volumetric flask.

**Internal standard solution**—Dilute 20.0 mL of isopropyl alcohol with methanol to volume in a 100-mL volumetric flask.

**Test preparation**—Using a “to contain” pipet, transfer 2 mL of Topical Solution to a 100-mL volumetric flask, rinsing the pipet 3 times with methanol and collecting the rinsings in the volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Standard preparation**—Pipet 6 mL of the *Standard solution* and 5 mL of the *Internal standard solution* into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m glass column that is packed with 80- to 100-mesh packing S3. The carrier gas is nitrogen or helium, flowing at a rate of about 40 mL per minute. The injection port and detector temperatures are maintained at about 225°. The column temperature is maintained at about 130°. Chromatograph the *Standard preparation*, record the chromatogram, and determine the peak response ratio as directed for *Procedure*. Adjust the carrier gas flow rate so that the resolution,  $R$ , of alcohol and isopropyl alcohol is not less than 1.5; the tailing factor of the alcohol peak is not more than 1.25; and the relative standard deviation for peak response ratios from replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (2 µL to 3 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of C<sub>2</sub>H<sub>5</sub>OH in the Topical Solution taken by the formula:

$$(0.3)(95.45)(R_U / R_S)$$

in which 95.45 is the percentage (v/v) of C<sub>2</sub>H<sub>5</sub>OH in USP Alcohol; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Test preparation* and the *Standard preparation*, respectively: between 28.4% and 39.0% of C<sub>2</sub>H<sub>5</sub>OH is present.

#### Assay—

**Mobile phase**—Use a mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution containing about 500 µg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 40 µg of USP Fluocinonide RS per mL.

**Assay preparation**—Using a “to contain” pipet, transfer a volume of Topical Solution, equivalent to about 1 mg of fluocinonide, to a 25-mL volumetric flask, rinsing the pipet with about 5 mL of *Mobile phase*, and adding the rinsings to the volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

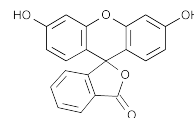
**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of C<sub>26</sub>H<sub>32</sub>F<sub>2</sub>O<sub>7</sub> in each mL of the Topical Solution taken by the formula:

$$0.025(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Fluocinonide RS in the *Standard preparation*;  $V$  is the volume, in mL, of Topical Solution taken; and  $r_U$  and  $r_S$  are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorescein



C<sub>20</sub>H<sub>12</sub>O<sub>5</sub> 332.31

Spiro[isobenzofuran-1(3H),9'-[9H]xanthen-3-one, 3',6'-dihydroxy-.

Fluorescein [2321-07-5].

» Fluorescein contains not less than 97.0 percent and not more than 102.0 percent of C<sub>20</sub>H<sub>12</sub>O<sub>5</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Diacetylfluorescein RS

C<sub>24</sub>H<sub>16</sub>O<sub>7</sub> 416.39

USP Fluorescein RS

**Identification, Infrared Absorption** <197K>: previously dried over silica gel for 16 hours.

**Water, Method I** <921>: not more than 1.0%.

**Zinc**—Suspend 100 mg in 10 mL of a saturated solution of sodium chloride, add 2 mL of 3 N hydrochloric acid, mix, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate: no turbidity is produced.

**Acriflavine**—Suspend 10 mg in 5 mL of water, swirl the mixture, and filter. To the filtrate add a few drops of sodium salicylate solution (1 in 10): no precipitate is formed.

#### Assay—

**Standard preparation**—Dissolve about 110 mg of USP Diacetylfluorescein RS, accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 1.1 µg of diacetylfluorescein per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.

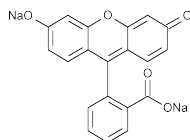
**Assay preparation**—Dissolve about 90 mg of Fluorescein, accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a concentration of 0.9 µg per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the fluorescence intensities,  $I$ , of the *Standard preparation* and the *Assay preparation* in a fluorometer at an excitation wavelength of 485 nm and an emission wavelength of 515 nm. Calculate the quantity, in mg, of  $C_{20}H_{12}O_5$  in the Fluorescein taken by the formula:

$$(332.31 / 416.39)(3333C)(I_U / I_S)$$

in which 332.31 and 416.39 are the molecular weights of fluorescein and diacetylfluorescein, respectively;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Diacetylfluorescein RS in the *Standard preparation*; and  $I_U$  and  $I_S$  are the fluorescence values observed for the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorescein Sodium



$C_{20}H_{10}Na_2O_5$  376.27

Spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, 3',6'-dihydroxy, disodium salt.

Fluorescein disodium salt [518-47-8].

» Fluorescein Sodium contains not less than 90.0 percent and not more than 102.0 percent of  $C_{20}H_{10}Na_2O_5$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diacetylfluorescein RS

$C_{24}H_{16}O_7$  416.39

**Identification**—

**A:** A solution of it is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid, and reappears when the solution is again made alkaline.

**B:** The residue remaining after the incineration of it responds to the tests for *Sodium* (191).

**C:** Place 1 drop of a solution (1 in 2000) upon a piece of filter paper: a yellow spot is produced and, when exposed while moist to the vapor of bromine for 1 minute and then to ammonia vapor, it becomes deep pink in color.

**Water**, *Method I* (921): not more than 17.0%.

**Zinc**—Dissolve 100 mg in 10 mL of a saturated solution of sodium chloride, add 2 mL of 3 N hydrochloric acid, shake well, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate: no turbidity is produced.

**Acriflavine**—Dissolve 10 mg in 5 mL of water, and add a few drops of sodium salicylate solution (1 in 10): no precipitate is formed.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diacetylfluorescein RS in 10 mL of alcohol contained in a 100-mL volumetric flask. [NOTE—110.7 mg of anhydrous USP Diacetylfluorescein RS is equivalent to 100.0 mg of fluorescein sodium.] Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Transfer a suitable aliquot to a volumetric flask, and dilute with water to volume

to obtain a solution having a concentration of 1  $\mu\text{g}$  of fluorescein sodium per mL. Transfer a 3-mL aliquot to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix. The concentration of fluorescein sodium in the *Standard preparation* is 0.03  $\mu\text{g}$  per mL.

**Assay preparation**—Dissolve about 100 mg of Fluorescein Sodium, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a concentration of 1  $\mu\text{g}$  per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the fluorescence intensities,  $I$ , of the *Standard preparation* and the *Assay preparation* in a fluorometer at an excitation wavelength of 485 nm and an emission wavelength at 515 nm. Calculate the quantity, in mg, of  $C_{20}H_{10}Na_2O_5$  in the Fluorescein Sodium taken by the formula:

$$3333C(I_U / I_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of fluorescein sodium in the *Standard preparation*; and  $I_U$  and  $I_S$  are the fluorescence values observed for the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorescein Injection

» Fluorescein Injection is a sterile solution, in Water for Injection, of Fluorescein prepared with the aid of Sodium Hydroxide. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ). It may contain Sodium Bicarbonate.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Diacetylfluorescein RS

$C_{24}H_{16}O_7$  416.39

**Identification**—It responds to *Identification tests A and C* under *Fluorescein Sodium*.

**Pyrogen**—It meets the requirements of the *Pyrogen Test* (151), the test dose being the equivalent of 250 mg of fluorescein sodium per kg.

**pH** (791): between 8.0 and 9.8.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Standard preparation**—Prepare as directed in the *Assay* under *Fluorescein Sodium*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of fluorescein sodium, and dilute quantitatively and stepwise with water to obtain a solution having a concentration of 1  $\mu\text{g}$  per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Fluorescein Sodium*. Calculate the quantity, in mg, of

fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) equivalent in the Injection taken by the formula:

$$3333C(I_U / I_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of fluorescein sodium in the *Standard preparation*; and  $I_U$  and  $I_S$  are the fluorescence values observed for the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorescein Sodium Ophthalmic Strips

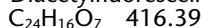
» Fluorescein Sodium Ophthalmic Strips contain not less than 100.0 percent and not more than 160.0 percent of the labeled amount of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ).

**Packaging and storage**—Package not more than 2 Strips in a single-unit container in such manner as to maintain sterility until the package is opened. Package individual packages in a second protective container.

**Labeling**—The label of the second protective container bears a statement that the contents may not be sterile if the individual package has been damaged or previously opened. The label states the amount of fluorescein sodium in each Strip.

### USP Reference standards (11)—

USP Diacetylfluorescein RS



**Identification**—Cut the colored tip from 1 Strip, place it in a small test tube containing 1 mL of water, and agitate for 1 minute: the resulting solution of fluorescein sodium responds to *Identification* tests A and C under *Fluorescein Sodium*.

**Sterility** (71): meets the requirements.

**Content uniformity**—The content of  $C_{20}H_{10}Na_2O_5$  in each of not less than 10 Strips, determined as directed in the *Assay*, is not less than 85.0% and not more than 175.0% of the labeled amount.

### Assay—

*Standard preparation*—Prepare as directed in the *Assay* under *Fluorescein Sodium*.

*Assay preparation*—Remove 1 Strip from its package, taking care not to allow any portion of the tip to adhere to the packaging material, transfer to a 100-mL volumetric flask, add 50 mL of water, shake the flask vigorously, and dilute with water to volume. Shake occasionally, and, after 1 hour, mix the contents of the flask. Transfer an aliquot ( $V$ ) of this solution, equivalent to about 100  $\mu\text{g}$  of fluorescein sodium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 3 mL of the resulting solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Fluorescein Sodium*. Calculate the quantity, in mg, of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) in the Strip taken by the formula:

$$(333)(C/V)(I_U / I_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of fluorescein sodium in the *Standard preparation*;  $V$  is the volume of the aliquot of solution taken for the *Assay preparation*; and  $I_U$  and  $I_S$  are the fluorescence intensities observed for the *Assay preparation* and the *Standard preparation*, respectively. Calculate the average content from the individual assays of not less than 10 Strips.

## Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution

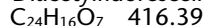
» Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Fluorescein Sodium and Benoxinate Hydrochloride. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) and benoxinate hydrochloride ( $C_{17}H_{28}N_2O_3 \cdot \text{HCl}$ ). It contains a suitable preservative.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Benoxinate Hydrochloride RS

USP Diacetylfluorescein RS



### Identification—

**A:** It responds to *Identification* test A under *Fluorescein Sodium*.

**B:** The relative retention times of the major peaks in the chromatogram of the *Assay* correspond to those in the chromatograms of the *Standard fluorescein sodium preparation* and the *Standard benoxinate hydrochloride preparation* as obtained in the *Assay*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.3 and 5.3.

### Assay—

*Mobile phase*—Dissolve 100 mg of sodium 1-pentanesulfonate in 40 mL of glacial acetic acid in a 2000-mL volumetric flask. Add 600 mL of acetonitrile and 10 mL of triethanolamine, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 3, and pass through a filter having a 0.5- $\mu\text{m}$  or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard fluorescein sodium preparation*—Transfer about 55 mg of USP Diacetylfluorescein RS, accurately weighed, to a 50-mL volumetric flask containing 5 mL of alcohol. Add 1 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 0.1 mg of fluorescein sodium per mL.

*Standard benoxinate hydrochloride preparation*—Quantitatively dissolve an accurately weighed quantity of USP Benoxinate Hydrochloride RS in *Mobile phase*, and if necessary dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL,  $J$  being the ratio of the labeled amount, in mg, of benoxinate hydrochloride to the labeled amount, in mg, of fluorescein sodium in each mL of Ophthalmic Solution.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of fluorescein sodium, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard fluorescein sodium preparation* and the *Standard benoxinate hydrochloride preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for each

analyte peak is not more than 2.0, and the relative standard deviation for replicate injections of each *Standard preparation* is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 25  $\mu$ L) of the *Standard fluorescein sodium preparation*, the *Standard benoxinate hydrochloride preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) in each mL of Ophthalmic Solution taken by the formula:

$$(376.28 / 416.39)(W / 10V)(r_U / r_S)$$

in which 376.28 and 416.39 are the molecular weights of fluorescein sodium and diacetylfluorescein, respectively; *W* is the quantity, in mg, of USP Diacetylfluorescein RS taken to prepare the *Standard fluorescein sodium preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the fluorescein peak responses obtained from the *Assay preparation* and the *Standard fluorescein sodium preparation*, respectively. Calculate the quantity, in mg, of benoxinate hydrochloride ( $C_{17}H_{28}N_2O_3 \cdot HCl$ ) in each mL of Ophthalmic Solution taken by the formula:

$$50(C / V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Benoxinate Hydrochloride RS in the *Standard benoxinate hydrochloride preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the benoxinate peak responses obtained from the *Assay preparation* and the *Standard benoxinate hydrochloride preparation*, respectively.

## Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution

» Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Fluorescein Sodium and Proparacaine Hydrochloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) and proparacaine hydrochloride ( $C_{16}H_{26}N_2O_3 \cdot HCl$ ). It contains a suitable preservative.

**Packaging and storage**—Preserve in tight, light-resistant containers, preferably of Type I amber glass, and store in a refrigerator.

**Labeling**—Label it to state that it is to be stored in a refrigerator before and after the container is opened.

**USP Reference standards** (11)—

USP Diacetylfluorescein RS

$C_{24}H_{16}O_7$  416.39

USP Proparacaine Hydrochloride RS

**Identification**—

**A:** It responds to *Identification test A* under *Fluorescein Sodium*.

**B:** It responds to the *Identification test* under *Proparacaine Hydrochloride Ophthalmic Solution*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 5.2.

**Assay for fluorescein sodium**—

**Standard preparation**—Prepare as directed in the *Assay* under *Fluorescein Sodium*.

**Assay preparation**—Using Ophthalmic Solution, prepare as directed in the *Assay* under *Fluorescein Sodium*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Fluorescein Sodium*. Calculate the quantity, in mg, of fluorescein sodium ( $C_{20}H_{10}Na_2O_5$ ) in the volume of Ophthalmic Solution taken by the formula:

$$3333C(I_U / I_S)$$

in which the terms are as defined therein.

**Assay for proparacaine hydrochloride**—

**Standard preparation**—Prepare as directed for *Standard preparation* in the *Assay* under *Proparacaine Hydrochloride Ophthalmic Solution*.

**Assay preparation**—Using Ophthalmic Solution, prepare as directed for *Assay preparation* under *Proparacaine Hydrochloride Ophthalmic Solution*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Proparacaine Hydrochloride Ophthalmic Solution*. Calculate the quantity, in mg, of proparacaine hydrochloride ( $C_{16}H_{26}N_2O_3 \cdot HCl$ ) in each mL of Ophthalmic Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which the terms are as defined therein.

## Fludeoxyglucose F 18 Injection

» Fludeoxyglucose F 18 Injection is a sterile, aqueous solution, suitable for intravenous administration, of 2-deoxy-2-[ $^{18}F$ ]fluoro-D-glucose in which a portion of the molecules are labeled with radioactive  $^{18}F$  (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823)). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{18}F$  expressed in MBq (mCi) per mL at the time indicated in the labeling. It may contain suitable preservatives and/or stabilizing agents.

**Specific activity:** no carrier added.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injection* (1): the time and date of calibration; the amount of  $^{18}F$  as fludeoxyglucose expressed as total MBq (mCi) per mL, at time of calibration; the expiration time and date; the name and quantity of any added preservative or stabilizer; and the statement "Caution—Radioactive Material." The labeling indicates, that in making dosage calculations, correction is to be made for radioactive decay. The radioactive half-life of  $^{18}F$  is 109.7 minutes. The label indicates "Do not use if cloudy or if it contains particulate matter."

**USP Reference standards** (11)—

USP Endotoxin RS

USP Fludeoxyglucose RS

USP Fludeoxyglucose Related Compound A RS  
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8.]hex-  
acosane.  
 $C_{18}H_{36}N_2O_6$   
USP Fludeoxyglucose Related Compound B RS  
 $C_6H_{11}ClO_5$  198.60

### Identification—

**A: Radionuclidic identity**—Its half-life, determined using a suitable detector system (see *Radioactivity* (821)), is between 105 and 115 minutes.

**B: Radiochemical identity**—The  $R_f$  value of Fludeoxyglucose F 18 in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the *Radiochemical purity* test.

**Bacterial endotoxins** (85) (see *Sterilization and Sterility Assurance* under *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823))—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum administered total dose, in mL, at the expiration time.

**pH** (791): between 4.5 and 7.5.

### Radiochemical purity—

**Standard solution**—Dissolve 10 mg of USP Fludeoxyglucose RS in 100 mL of acetonitrile and water (95:5). (The USP Fludeoxyglucose RS that is specified in this test is nonradioactive 2-deoxy-2-fluoro-D-glucose [molecular weight 182.15].)

**Test solution**—Use the Injection.

**Procedure**—Apply a volume of Injection, appropriately diluted such that it provides a count rate suitable for the radioactivity detection system being utilized, to an activated silica gel thin-layer chromatographic plate (see *Chromatography* (621)). Apply about 10  $\mu$ L of the *Standard solution* to the same chromatographic plate. Develop the chromatogram in a solvent system consisting of a mixture of acetonitrile and water (95:5) until the solvent has moved about three-fourths of the length of the plate. Remove the plate, and allow the chromatogram to dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Determine the location of the Fludeoxyglucose by spraying the developed chromatographic plate with 2 N sulfuric acid and heating the plate at 110° for 10 minutes: the  $R_f$  value of Fludeoxyglucose F 18 (determined by radiochromatogram scanning) corresponds to that of the *Standard solution* (about 0.4); the radioactivity of Fludeoxyglucose F 18 is not less than 90% of the total radioactivity.

**Radionuclidic purity**—Using a suitable gamma-ray spectrometer (see *Selection of a Counting Assembly* under *Radioactivity* (821)), count an appropriate aliquot of the Injection for a period of time sufficient to collect a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are not characteristic of  $^{18}F$  emissions. Not less than 99.5% of the observed gamma emissions should correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of  $^{18}F$ .

**Chemical purity**—[NOTE—The methods and limits described in this section relate to potential impurities associated with the acid-hydrolysis method of synthesis for the Injection. Specific examples include aminopolyether (Kryptofix®) and 2-chloro-2-deoxy-D-glucose. If methods of synthesis that may result in different impurities are used, the presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823)). Any ingredients with toxic potential must be within appropriate limits, and conformance with these limits is to be demonstrated by the use of one or more validated limit tests.]

**LIMIT OF AMINOPOLYETHER**—[NOTE—This test must be performed for Fludeoxyglucose F 18 produced by any route of synthesis that uses this reagent.]

**Absorbent:** 0.25-mm layer of chromatographic silica gel.<sup>1</sup>

**Test solution**—Use the Injection.

**Standard solution**—Dissolve an accurately weighed quantity of USP Fludeoxyglucose Related Compound A RS in saline TS to obtain a solution having a known concentration of 50  $\mu$ g per mL.

**Application volume:** about 1  $\mu$ L.

**Developing solvent system:** a mixture of methanol and 30% ammonium hydroxide (9:1).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Place the plate in a chamber containing iodine crystals. Develop the plate until a spot is visible on the chromatogram of the *Standard solution*: the size and intensity of the spot obtained from the *Test solution* does not exceed that obtained from the *Standard solution*.

**LIMIT OF 2-CHLORO-2-DEOXY-D-GLUCOSE**—[NOTE—This test is performed when the nucleophilic synthesis includes hydrolysis with hydrochloric acid or the use of anionic exchange resins in the chloride form to trap fluoride  $^{18}F$  released from the target prior to its use in the synthesis of Fludeoxyglucose F 18.]

**Mobile phase**—Dissolve about 16 g of 50% sodium hydroxide solution in 1000 mL of water, filter, and degas by sparging with helium.

**System suitability solution**—Dissolve accurately weighed quantities of USP Fludeoxyglucose RS and USP Fludeoxyglucose Related Compound B RS in *Mobile phase* to obtain a solution having known concentrations of 1.0 mg per mL and 0.1 mg per mL, respectively.

**Standard solution**—Dissolve an accurately weighed quantity of USP Fludeoxyglucose Related Compound B RS in water to obtain a solution having a known concentration of 0.1 mg per mL.

**Test solution**—Use the Injection.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a pulsed amperometric detector and a 4.0-mm  $\times$  25-cm column that contains 10- $\mu$ m packing L46. The flow rate is adjusted to about 0.5 mL per minute. Chromatograph the *Standard solution* and the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between fludeoxyglucose and fludeoxyglucose related compound B is not less than 1.5; and the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of 2-chloro-2-deoxy-D-glucose in each mL of the Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fludeoxyglucose Related Compound B RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the 2-chloro-2-deoxy-D-glucose peak areas obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1 mg per dose is found.

### Residual solvents—

**Standard solutions**—Prepare separate aqueous solutions of ether, acetonitrile, and dehydrated alcohol having known concentrations of 0.1%, 0.01%, and 0.1%, respectively.

<sup>1</sup> Available from Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015 as Machery Nagel SILG/UV 254 4  $\times$  8 cm, Alltech catalog No. 805021.

**Test solutions**—Use the Injection.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector, a splitless injector system, and a 0.53-mm × 30-m fused-silica column coated with a 0.25-μm, chemically cross-linked G16 stationary phase. The carrier gas is helium, flowing at a rate of 10 mL per minute. (Nitrogen may be used as a makeup gas.) The chromatograph is programmed as follows. Initially the temperature is maintained at 40° for 2 minutes, then the temperature is increased at a rate of 20° per minute to 130°, and maintained at 130° for 5.5 minutes. The injection port and detector temperatures are maintained at 250° and 300°, respectively. Inject the *Standard solutions*, and record the identity peak responses as directed for *Procedure*: the resolution,  $R$ , between any two components is not less than 1.0; and the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—Separately inject equal volumes (about 1 μL) of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of acetonitrile, ether, and alcohol in the Injection by the formula:

$$C(r_i / r_s)$$

in which  $C$  is the percentage of the relevant analyte in the *Standard solution*; and  $r_i$  and  $r_s$  are the peak responses of the relevant analyte obtained from the *Test solution*, if any, and the *Standard solution*, respectively: not more than 0.04% of acetonitrile is found; not more than 0.5% of ether is found; and not more than 0.5% of alcohol is found.

**Other requirements**—It meets the requirements under *Injections* <1>, except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility* <71>, the latter test being started within 24 hours of final manufacture, and except that it is not subject to the recommendation of *Container Content*.

**Assay for radioactivity**—Using a suitable calibrated system as directed under *Radioactivity* <821>, determine the radioactivity in MBq (or mCi) per mL, of the Injection.

## Fluorodopa F 18 Injection

» Fluorodopa F 18 Injection is a sterile aqueous solution, suitable for intravenous administration of 6-[<sup>18</sup>F]fluorodopa in which a portion of the molecules are labeled with radioactive <sup>18</sup>F (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* <823>). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>18</sup>F expressed in MBq (or mCi) per mL at the time indicated in the labeling. It may contain suitable preservatives and/or stabilizing agents.

### Specific activity—

**Mobile phase, Standard solution, Test solution, and Chromatographic system**—Proceed as directed in the test for *Radiochemical purity*.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the concentration of

L-fluorodopa found, in mg per mL, in the Injection by the formula:

$$C(r_u / r_s)$$

in which  $C$  is the concentration of the *Standard solution*; and  $r_u$  and  $r_s$  are the peak responses of the *Test solution* and the *Standard solution*, respectively. Determine the concentration of fluorodopa F 18, in mCi per mL, as directed in the *Assay for radioactivity*. Calculate the *Specific activity* by dividing the result from the *Assay* (in mCi per mL) by the concentration (in mg per mL): it is not less than 0.463 mCi per mg of L-fluorodopa ( $3.7 \times 10^3$  MBq [100 mCi] per mmol).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* <1>: the time and date of calibration; the amount of <sup>18</sup>F as fluorodopa expressed as total MBq (or mCi) per mL, at time of calibration; the expiration time and date; the name and quantity of any added preservative or stabilizer; and the statement "Caution—Radioactive Material". The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay. The radioactive half-life of <sup>18</sup>F is 109.7 minutes. The label also includes the statement "Do not use if cloudy or if it contains particulate matter."

### USP Reference standards <11>—

USP Endotoxin RS

USP L-Fluorodopa RS

### Radionuclidic identification (see *Radioactivity* <821>)—

**A:** Its half-life, determined using a suitable detector system, is between 105 and 115 minutes.

**B:** *Radiochemical identity*—The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Radiochemical purity*.

**Bacterial endotoxins** <85>: not more than 175/V USP Endotoxin Unit per mL of Injection, in which  $V$  is the maximum administered total dose, in mL, at the expiration time.

**pH** <791>: between 4.0 and 5.5.

### Radiochemical purity—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.1% acetic acid and methanol (97:3).

**Standard solution**—Dissolve an accurately weighed quantity of USP L-Fluorodopa RS in 10 mmol of pH 4.5 sodium acetate buffer, and dilute quantitatively, and stepwise if necessary, with the same buffer to obtain a solution having a known concentration of about 0.1 mg per mL.

**Test solution**—Use the Injection diluted with water such that it provides a count rate of about  $5 \times 10^5$  counts per minute.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 4.6-mm × 30-cm column that contains packing L1, a radioactivity detector, and a variable wavelength UV detector operating in the range of 260 to 290 nm. The flow rate is about 0.8 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Prepare a mixture of the *Test solution* and the *Standard solution* and inject about 50 μL into the chromatograph, record the chromatograms, and measure the areas for both the radioactive and nonradioactive peaks. The ratio and injected volume may be adjusted to obtain suitable detection system sensitivity. The radioactivity of the major peak is not less than 90% of the total radioactivity measured, and no individual radiochemical impurity is more than 2%. The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*. [NOTE—The typical re-



tention time for fluorodopa is about 6 minutes. Retention times are very sensitive to the pH of the solvent.]

**Radionuclidic purity**—Using a suitable gamma-ray spectrometer (see *Selection of a Counting Assembly* under *Radioactivity* (821)), count an appropriate aliquot of Injection for a period of time sufficient to obtain a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are not characteristic of F 18 emissions. Not less than 99.5% of the gamma emissions should correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of F 18, with no individual impurity peaks present above 0.1%.

**Chemical purity**—The methods and limits described in this section relate to potential impurities associated with commonly used methods of synthesis for Fluorodopa F 18 Injection. If methods of synthesis are used that may result in different impurities, the presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled and then potential for physiological or pharmacological effects must be considered.

**LIMIT OF ORGANOTIN** (to be determined if tin-containing starting materials or reagents are used in the synthesis)—

**Mobile phase**—Prepare a filtered and degassed 5  $\mu$ mol solution of morin in a mixture of toluene, acetic acid, methanol, and acetonitrile (91:5:2:2).

**Standard solution**—Prepare a mixture of 10 mmol each of dimethyltin dibromide and trimethyltin bromide in alcohol.

**Test solution**—Use the Injection.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a fluorescence detector (excitation at 420 nm and detection at 500 nm) and a 4.6-mm  $\times$  25-cm column that contains packing L32. The flow rate is about 1 mL per minute.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The volume of Injection in the *Standard solution* and the *Test solution* may be adjusted to obtain suitable detection system sensitivity. Calculate the concentration, in  $\mu$ g per mL, of dimethyltin and trimethyltin in the portion of Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of the relevant organotin compound in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5  $\mu$ g per mL of dimethyltin and trimethyltin is found.

**LIMIT OF MERCURY** (to be determined if mercury-containing starting materials or reagents are used in the synthesis)—  
[Caution—Because of the toxic nature of mercury vapor, great care must be taken to avoid inhaling it. A bypass has been included in the system, therefore, either to vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing media such as a solution containing equal volumes of 0.1 M potassium permanganate and dilute sulfuric acid (1 in 10).]

**Apparatus**—Use a flameless atomic absorption spectrophotometer for measuring radiation at 253.7 nm emitted by mercury vapor.

**Stannous chloride suspension**—Add 25 g of stannous chloride to 250 mL of 0.5 N sulfuric acid. This mixture is a suspension and is to be stirred continuously during use.

**Sodium chloride-hydroxylamine hydrochloride solution**—Dissolve 12 g of sodium chloride and 12 g of hydroxylamine hydrochloride in water, dilute with water to 100 mL, and mix.

**Mercury stock solution**—Dissolve 135.4 mg of mercuric chloride, accurately weighed, in 75 mL of water. Add 10 mL

of nitric acid, dilute with water to 100.0 mL, and mix. Each mL of this solution contains 1 mg of mercury.

**Mercury standard solution**—Before using, make successive dilutions of the *Mercury stock solution* with water to obtain a *Mercury standard solution* containing 0.1  $\mu$ g per mL.

**Calibration**—To six 300-mL glass-stoppered bottles, transfer, respectively, 0-, 0.5-, 1.0-, 2.0-, 5.0-, and 10.0-mL aliquots of the *Mercury standard solution* containing 0  $\mu$ g to 1.0  $\mu$ g of mercury. To each bottle add water to make 100 mL, mix, and add 5 mL of sulfuric acid and 2.5 mL of nitric acid. Add 15 mL of potassium permanganate solution (1 in 20). Allow to stand for 15 minutes. Add 8 mL of potassium persulfate solution (1 in 20), and heat in a water bath at 95° for 2 hours. Cool, and add 6 mL of *Sodium chloride-hydroxylamine hydrochloride solution* to reduce the excess permanganate. When the solution has been decolorized, wait for 30 seconds and add 5 mL of *Stannous chloride suspension*. Immediately attach the flask to the aeration apparatus to form a closed system. Allow the sample to stand without manual agitation. The circulating pump, previously adjusted to a rate of 1 L per minute, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 seconds. As soon as the recorder pen levels off, in about 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value. Close the bypass valve, remove the stopper and frit from the bottle, and continue the aeration. Plot a standard curve of the peak height versus micrograms of mercury.

**Test preparation**—Transfer 1.0 mL of Injection to a 300-mL glass-stoppered bottle, and proceed as directed under *Calibration*, beginning with "To each bottle add water". Measure the absorbance of the solution, and determine the quantity, in  $\mu$ g, of mercury in the *Test preparation* from the standard curve: not more than 0.5  $\mu$ g is found.

#### Enantiomeric purity—

**Mobile phase**—Prepare a filtered and degassed mixture of 100 mmol of monobasic potassium phosphate and 2 mmol of cupric sulfate (1:1). Adjust to a pH of 4.6. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Use the *Standard solution* as directed under *Radiochemical purity*.

**Test solution**—Use the Injection.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph as directed under *Radiochemical purity* is equipped with a 4.6-mm  $\times$  25-cm column that contains packing L32. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factors,  $k'$ , for the D- and L-isomers are not less than 2.1 and 7.3, respectively.

**Procedure**—Prepare a mixture of the *Test solution* and the *Standard solution*, and inject about 50  $\mu$ L into the chromatograph, record the chromatograms, and measure the areas for both the radioactive and nonradioactive peaks. The ratio and injected volume may be adjusted to obtain suitable detection system sensitivity. The radioactivity of the L-isomer is not less than 95%.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility* (71); the latter being started within 24 hours of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable calibrated system as directed under *Radioactivity* (821), determine the radioactivity, in MBq (or mCi) per mL, of Injection.

## Sodium Fluoride F 18 Injection

» Sodium Fluoride F 18 Injection is a sterile solution, suitable for intravenous administration, of sodium fluoride in Sodium Chloride Injection in which a portion of the molecules are labeled with radioactive  $^{18}\text{F}$  (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823)). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{18}\text{F}$  expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling.

**Specific activity:** no carrier added.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that are adequately shielded.

**Labeling**—Label container to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{18}\text{F}$  as fluoride anion expressed as MBq (or mCi) per mL, at time of calibration; total activity at time of calibration; the expiration time and date; and the statements "Caution—Radioactive Material" and "Do not use if cloudy or if it contains particulate matter." Label it to indicate that the radioactive half-life of  $^{18}\text{F}$  is 109.7 minutes, and that in making dosage calculations, correction is to be made for radioactive decay.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Sodium Fluoride RS

**Radionuclide identification** (see *Radioactivity* (821))—

**A:** Its half-life, determined using a suitable detector system, is between 105 and 115 minutes.

**B:** *Radiochemical identity*—The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Radiochemical purity*.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum administered total dose, in mL, at the expiration time. It also meets the requirements of *Quality Control under Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823).

**Sterility** (71): meets the requirements.

**pH** (791): between 4.5 and 8.0.

**Chemical purity**—This article may be synthesized by different methods and processes and may, therefore, may contain different impurities. The presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered.

**Radiochemical purity**—

*Mobile phase*—Prepare a filtered and degassed solution of 0.003 N sulfuric acid in water.

*Standard solution*—Dissolve an accurately weighed quantity of USP Sodium Fluoride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

*Test solution*—Use the Injection.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a flow-through gamma ray detector, a conductivity detector, and a 10-mm  $\times$  25-cm column that contains 10- $\mu\text{m}$  packing L31. The flow rate is about 0.8 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Pro-*

*cedure*: the relative standard deviation for replicate injections is not more than 5%.

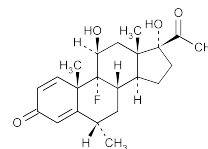
*Procedure*—Prepare a solution of the *Test solution* and the *Standard solution*, inject about 50  $\mu\text{L}$  of the combined solution into the chromatograph, record the chromatograms, and measure the areas for both the radioactive and nonradioactive peaks (the injection volume may be adjusted to obtain suitable detection system sensitivity): the radioactivity of the major peak is not less than 95% of the total radioactivity measured; and the retention time of the *Test solution* corresponds to the retention time (about 8 minutes) of the *Standard solution*.

**Radionuclide purity**—Using a suitable gamma-ray spectrometer (see *Selection of a Counting Assembly under Radioactivity* (821)), count an appropriate aliquot of Injection for a period of time sufficient to obtain a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are not characteristic of  $^{18}\text{F}$  emission: not less than 99.5% of the gamma emissions correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of  $^{18}\text{F}$ .

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility* (71), the latter test started within 24 hours of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable calibrated system as directed under *Radioactivity* (821), determine the radioactivity, in MBq (or MCi) per mL, of Injection.

## Fluorometholone



$\text{C}_{22}\text{H}_{29}\text{FO}_4$  376.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-6-methyl-, (6 $\alpha$ ,11 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17-dihydroxy-6 $\alpha$ -methylpregna-1,4-diene-3,20-dione [426-13-1].

» Fluorometholone contains not less than 97.0 percent and not more than 103.0 percent of  $\text{C}_{22}\text{H}_{29}\text{FO}_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Fluorometholone RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10  $\mu\text{g}$  per mL.

*Medium:* methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Prepare a solution in methanol containing 500  $\mu\text{g}$  per mL. Apply 100  $\mu\text{L}$  of this solution and 100  $\mu\text{L}$  of a methanol solution of USP Fluorometholone RS containing 500  $\mu\text{g}$  per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 250- $\mu\text{m}$  layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a

mixture of methylene chloride and acetone (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the specimen solution corresponds to that obtained from the Standard solution.

**Specific rotation** (781S): between +52° and +60°.

*Test solution:* 10 mg per mL, in pyridine.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

#### Assay—

*Mobile phase*—Prepare a suitable filtered solution of methanol and water (60:40) such that the retention time of fluorometholone is about 3 minutes.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fluorometholone RS in *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL.

*Assay preparation*—Transfer about 20 mg of Fluorometholone, accurately weighed, to a 200-mL volumetric flask, add *Mobile phase* to volume, and mix.

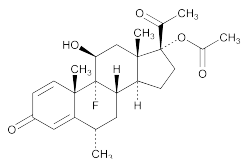
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, using a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Fluorometholone taken by the formula:

$$0.2C(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Fluorometholone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorometholone Acetate



$C_{24}H_{31}FO_5$  418.50

Pregna-1,4-diene-3,20-dione, 17-(acetyloxy)-9-fluoro-11-hydroxy-6-methyl-, (6 $\alpha$ ,11 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17-dihydroxy-6 $\alpha$ -methylpregna-1,4-diene-3,20-dione, 17 acetate [3801-06-7].

» Fluorometholone Acetate contains not less than 98.0 percent and not more than 101.0 percent of  $C_{24}H_{31}FO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Fluorometholone RS

USP Fluorometholone Acetate RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U).

*Solution:* 10 µg per mL.

*Medium:* methanol.

**Specific rotation** (781S): between +25.0° and +31.0°.

*Test solution:* 20 mg per mL, in chloroform.

**Loss on drying** (731)—Dry in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

#### Related compounds—

*Mobile phase*, *System suitability solution*, and *Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Fluorometholone RS in methanol, and dilute quantitatively and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.03 mg per mL.

*Test solution*—Use the *Assay preparation*.

*Blank*—Use acetonitrile.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Blank*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. [NOTE—Allow the elution to continue for about two and a half times the elution time of the fluorometholone acetate peak before making the next injection.]

Calculate the percentage of fluorometholone or fluorometholone diacetate in the portion of Fluorometholone Acetate taken by the formula:

$$100 \times 50 \times (1/F)(C/W)(r_U / r_S)$$

in which  $F$  is the relative response factor (see values in the accompanying *Table*);  $C$  is the concentration, in mg per mL, of USP Fluorometholone RS in the *Standard solution*;  $W$  is the weight, in mg, taken to prepare the *Test solution*;  $r_U$  is the fluorometholone or fluorometholone diacetate peak height response obtained from the *Test solution*; and  $r_S$  is the fluorometholone peak height response obtained from the *Standard solution*.

Calculate the percentage of all other fluorometholone acetate impurities in the portion of Fluorometholone Acetate taken by the formula:

$$100(1/F)(r_i / r_T)$$

in which  $F$  is the relative response factor (see values in the accompanying *Table*);  $r_i$  is the peak area for each impurity (except fluorometholone and fluorometholone diacetate) obtained from the *Test solution*; and  $r_T$  is the sum of the peak areas of all impurity peaks plus the fluorometholone acetate peak obtained from the *Test solution*.

Impurity	Relative Retention Time	Relative Response Factor	Limit (%)
Fluorometholone	0.6	1.0 <sup>a</sup>	1.0
Compound SOX <sup>1</sup>	0.89	1.0	0.5
Fluorometholone Acetate	1.0	—	—
Fluorometholone Diacetate	1.39	0.45 <sup>a</sup>	1.0
Fluorometholone Acetate, epoxy analog <sup>2</sup>	1.58	1.0	0.5
Fluorometholone Acetate, Delta 9(11) <sup>3</sup>	1.82	1.0	0.2
Fluorometholone Acetate 7, 9(11) Diene <sup>4</sup>	1.77	1.8	0.3
Individual unknown	—	1.0	0.1
Total impurities	—	—	1.5

<sup>1</sup>19b,11b-Epoxy-17a-hydroxy-6a-methylpregna-1,4-dien-3,20-dione.

<sup>2</sup>17a-Acetoxy-9b,11b-epoxy-6a-methylpregna-1,4-dien-3,20-dione.

<sup>3</sup>17a-Acetoxy-6a-methylpregna-1,4,9(11)-trien-3,20-dione.

<sup>4</sup>17a-Acetoxy-6a-methylpregna-1,4,7,9(11)-tetraen-3,20-dione.

<sup>a</sup>Relative to fluorometholone.

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluorometholone Acetate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 1.0 mg per mL.

**System suitability solution**—Prepare a solution of USP Fluorometholone RS by dissolving a quantity in methanol and diluting in acetonitrile to a final concentration of about 1 mg per mL. Mix equal volumes of this solution and the *Standard preparation*, and dilute with acetonitrile to a final concentration of about 0.03 mg per mL each for fluorometholone and fluorometholone acetate.

**Assay preparation**—Transfer about 50 mg of Fluorometholone Acetate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between fluorometholone and fluorometholone acetate is not less than 10. The column efficiency for fluorometholone acetate is not less than 10,000 theoretical plates, and the tailing factor for fluorometholone acetate is not more than 2.0. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>24</sub>H<sub>31</sub>FO<sub>5</sub> in the portion of Fluorometholone Acetate taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Fluorometholone Acetate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorometholone Cream

» Fluorometholone Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone (C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub>).

**Packaging and storage**—Preserve in collapsible tubes.

**USP Reference standards** (11)—

USP Fluorometholone RS

**Identification**—The retention ratios of the main peak to the internal standard peak obtained with the *Standard preparation* and the *Assay preparation* as directed in the *Assay* do not differ by more than 2.0%.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

### Assay—

**Internal standard solution**—Dissolve fluoxymesterone in acetonitrile to obtain a solution containing about 100 μg per mL.

**Mobile solvent**—Prepare a solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).

**Standard preparation**—Dissolve a suitable quantity of USP Fluorometholone RS, accurately weighed, in *Internal standard solution* to obtain a solution having a known concentration of about 50 μg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 1 mg of fluorometholone, into a suitable container, add 20.0 mL of *Internal standard solution*, and mix.

**Procedure**—Treat 20.0 mL each of the *Standard preparation* and the *Assay preparation* in the following manner. To each add 10.0 mL of hexane, and shake for about 15 minutes, then allow the layers to separate, and centrifuge, if necessary. Using a suitable microsyringe or sampling valve, inject equal volumes of lower (acetonitrile) layers obtained from the *Standard preparation* and the *Assay preparation* into a suitable high-pressure liquid chromatograph (see *Chromatography* (621)) of the general type equipped with a detector for monitoring UV light absorption at about 254 nm, equipped with a suitable recorder, capable of providing column pressure up to about 1000 psi. The instrument contains a 4-mm × 30-cm stainless steel column that contains packing L3. In a suitable chromatogram, the resolution factor, *R* (see *Chromatography* (621)), is not less than 2.4 be-

tween peaks for fluorometholone and the internal standard, and the lowest and highest peak area ratios ( $R_S$ ) of three replicate injections of the *Standard preparation* agree within 2.0%. Calculate the quantity, in mg, of fluorometholone ( $C_{22}H_{29}FO_4$ ) in the portion of Cream taken by the formula:

$$20C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Fluorometholone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak area ratios of the fluorometholone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorometholone Ophthalmic Suspension

» Fluorometholone Ophthalmic Suspension is a sterile suspension of Fluorometholone in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone ( $C_{22}H_{29}FO_4$ ). It may contain suitable stabilizers, buffers, and antimicrobial agents.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Fluorometholone RS

**Identification**—Mix 1 mL of the well-shaken Ophthalmic Suspension with 2 mL of a mixture of methanol and water (3:2) until a solution is obtained. Apply 20- $\mu$ L portions of this solution and of a Standard solution of USP Fluorometholone RS in the same solvent containing 500  $\mu$ g per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture and previously activated by heating at 80° for 5 minutes. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of methylene chloride and acetone (4:1) until the solvent front has moved not less than 15 cm. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value and intensity of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

**Sterility** (71): meets the requirements.

**pH** (791): between 6.0 and 7.5.

**Assay**—

*Mobile phase*—Prepare a suitable filtered solution of methanol and water (60:40) such that the retention time of fluorometholone is about 3 minutes.

*Standard preparation*—Using a suitable quantity of USP Fluorometholone RS, accurately weighed, prepare a solution in methanol containing 0.5 mg per mL. Pipet 10 mL of this solution and 5 mL of water into a 50-mL volumetric flask. Dilute with methanol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 100  $\mu$ g per mL.

*Assay preparation*—Pipet a volume of well-shaken Ophthalmic Suspension, equivalent to about 5 mg of fluorometholone, into a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a 5- $\mu$ m membrane filter, and use the clear filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record

the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, using a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluorometholone ( $C_{22}H_{29}FO_4$ ) in the portion of Ophthalmic Suspension taken by the formula:

$$0.05C(R_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluorometholone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorouracil



$C_4H_3FN_2O_2$  130.08  
2,4(1*H*,3*H*)-Pyrimidinedione, 5-fluoro-.  
5-Fluorouracil [51-21-8].

» Fluorouracil contains not less than 98.0 percent and not more than 102.0 percent of  $C_4H_3FN_2O_2$ , calculated on the dried basis. [Caution—Great care should be taken to prevent inhaling particles of Fluorouracil and exposing the skin to it.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Fluorouracil RS

**Identification**—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* pH 4.7 acetate buffer (prepared from 8.4 g of sodium acetate and 3.35 mL of glacial acetic acid mixed with water to make 1000 mL).

Absorptivities at 266 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** To 5 mL of a solution (1 in 100) add 1 mL of bromine water TS: the bromine color is discharged.

**Loss on drying** (731)—Dry it in vacuum over phosphorus pentoxide at 80° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Content of fluorine**—[NOTE—All laboratory utensils used in this procedure should be scrupulously clean and free from even trace amounts of fluoride. The use of plasticware, wherever possible, in the preparation and storage of solutions and for measurement of potentials is recommended.]

*Isopropyl alcohol solution*—Dilute 295 mL of isopropyl alcohol with water to 500 mL.

*Buffer solution*—To 55 g of sodium chloride in a 1-L volumetric flask add 500 mg of sodium citrate, 255 g of sodium acetate, and 300 mL of water. Shake to dissolve, and add 115 mL of glacial acetic acid. Cool to room temperature, add 300 mL of isopropyl alcohol, dilute with water to vol-

ume, and mix. The pH of the resulting solution is between 5.0 and 5.5.

**Reagent blank**—Pipet 15 mL of 1,2-dimethoxyethane into a flat-bottom, glass-joint, 500-mL flask, and proceed as directed for *Test stock solution*, beginning with “add the contents of a 15-mL vial of sodium biphenyl solution.”

**Modified calomel reference electrode**—Mix 70 mL of a freshly prepared saturated potassium chloride solution with 30 mL of isopropyl alcohol, fill the electrode with the clear supernatant, and allow the electrode to soak in the remainder of the solution for a minimum of 2 hours before using. Store the electrode immersed in the potassium chloride-isopropyl alcohol solution when not in use.

**Standard stock solution**—Accurately weigh 2.211 g of sodium fluoride, previously dried at 150° for 4 hours, transfer to a 1-L volumetric flask, and dissolve in about 200 mL of water. Add 1 mL of sodium hydroxide solution (1 in 25), dilute with water to volume, and mix. Store this solution in plastic containers. One mL is equivalent to 1 mg of fluoride.

**Standard curve**—Dilute 10.0 mL of *Standard stock solution* with water to 100 mL. Into each of four 100-mL volumetric flasks, pipet 0.8, 1.0, 1.2, and 1.6 mL, respectively, of the resulting solution. To each flask add 15 mL of *Reagent blank*, dilute with *Buffer solution* to volume, and mix. Use these dilutions, containing, respectively, 0.8, 1.0, 1.2, and 1.6 µg per mL, to construct the standard curve as follows. Determine the potentials of each solution as directed for *Procedure*. Plot the results of fluorine concentration, as the abscissa, in mg per 100 mL versus the potential, as the ordinate, on semilogarithmic graph paper, for each of the standards. Draw the best straight line through the plotted points.

**Test stock solution**—Place 200 mg of Fluorouracil, accurately weighed, in a 250-mL volumetric flask, add about 150 mL of 1,2-dimethoxyethane, shake by mechanical means to dissolve, dilute with the same solvent to volume, and mix. Pipet 15 mL of this solution into a flat-bottom, glass-joint, 500-mL flask, add the contents of a 15-mL vial of sodium biphenyl solution through a long-stem funnel to prevent splattering, swirl the flask gently, and cover with a watch crystal. Allow to stand at room temperature for 20 minutes, then cautiously add 50.0 mL of isopropyl alcohol while swirling the flask. Add 10.0 mL of 30 percent hydrogen peroxide and 4.0 mL of 1 N sodium hydroxide, and connect the flask to a water-cooled reflux condenser that has previously been cleaned with water and isopropyl alcohol and dried. Place the flask on a hot plate, set at about 245°, and reflux for 1 hour. Cool to room temperature, rinse the condenser with 15 mL of *Isopropyl alcohol solution*, transfer the contents of the flask to a 250-mL volumetric flask using *Isopropyl alcohol solution* as a rinse, dilute with the same solvent to volume, and mix.

**Test solution**—Pipet 15 mL of the *Test stock solution* into a 100-mL volumetric flask, and dilute with *Buffer solution* to volume.

**Procedure**—Measure the potential, in mV, of the *Test solution*, with a suitable pH meter having a minimum reproducibility of ±0.2 mV, and equipped with a fluoride-specific ion electrode and a glass-sleeved *Modified calomel reference electrode*. When taking a measurement, immerse the electrodes into the solution, which has been transferred to a 150-mL plastic beaker, insert a suitable plastic-coated stirring bar, place the beaker on a magnetic stirrer, taking adequate precautions to prevent heat transfer, and stir for 2 minutes before reading. Dry the electrodes between measurements, taking care not to scratch the crystal surface of the specific ion electrode. Determine the quantity of fluorine, in mg per 100 mL of the *Test solution*, from the *Standard curve*. Multiply the quantity by the factor 138.9 to express the result as a percentage: not less than 13.9% and not more than 15.0% of fluorine, calculated on the dried basis, is found.

#### Assay—

**Mobile phase**—Use degassed and filtered water.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluorouracil RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 10 µg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of about 20 mg of Fluorouracil to a 200-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Quantitatively dilute a known volume of this solution with water to obtain a solution having a concentration of about 10 µg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>4</sub>H<sub>3</sub>FN<sub>2</sub>O<sub>2</sub> in the portion of Fluorouracil taken by the formula:

$$2C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Fluorouracil RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the fluorouracil peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorouracil Cream

» Fluorouracil Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorouracil (C<sub>4</sub>H<sub>3</sub>FN<sub>2</sub>O<sub>2</sub>). It may contain Sodium Hydroxide to adjust the pH.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards <11>—

USP Fluorouracil RS

**Identification**—Prepare a test solution by placing a quantity of Cream, equivalent to about 5 mg of fluorouracil, in a glass-stoppered conical flask, add 50 mL of alcohol, and shake until dissolved. Dissolve 5 mg of USP Fluorouracil RS in 50 mL of alcohol to obtain a Standard solution. In 20-µL increments, apply 100 µL each of the Standard solution and the test solution to a line about 3 cm from the bottom edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, which previously has been dried and activated at 105° for 5 minutes. Develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (75:25:1), allowing the solvent front to move about 15 cm beyond the initial spotting line. Remove the plate, air-dry for 15 minutes, and examine under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Microbial enumeration tests <61> and Tests for specified microorganisms <62>**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Fluorouracil*.

*Assay preparation*—Transfer an accurately weighed portion of Cream, equivalent to about 10 mg of fluorouracil, to a 100-mL volumetric flask, add 20 mL of methanol, and mix on a vortex mixer to dissolve. Dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluorouracil ( $C_4H_3FN_2O_2$ ) in the portion of Cream taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluorouracil RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the fluorouracil peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorouracil Injection

» Fluorouracil Injection is a sterile solution of Fluorouracil in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains, in each mL, not less than 45 mg and not more than 55 mg of fluorouracil ( $C_4H_3FN_2O_2$ ).

**NOTE**—If a precipitate is formed as a result of exposure to low temperatures, redissolve it by heating to 60° with vigorous shaking, and allow to cool to body temperature prior to use.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, and store at controlled room temperature. Avoid freezing and exposure to light.

**Labeling**—Label it to indicate the expiration date, which is not more than 24 months after date of manufacture.

**USP Reference standards** (11)—

USP Fluorouracil RS

USP Endotoxin RS

**Identification—**

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the Assay.

**B:** Carefully acidify a portion of Injection, equivalent to about 100 mg of fluorouracil, with glacial acetic acid. Stir and slightly chill the solution to precipitate the fluorouracil, collect the precipitate, wash with 1 mL of water, and then dry in vacuum over phosphorus pentoxide at 80° for 4 hours: the residue so obtained responds to *Identification test A* under *Fluorouracil*.

**C:** It responds to *Identification test C* under *Fluorouracil*.

**Bacterial endotoxins** (85)—It contains not more than 0.33 USP Endotoxin Unit per mg of fluorouracil.

**pH** (791): between 8.6 and 9.4.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Fluorouracil*.

*Assay preparation*—Transfer a suitable volume of the Injection, equivalent to 50 mg of fluorouracil, to a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute quantitatively a known volume of this solution with water to obtain a solution having a concentration of 10  $\mu$ g per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantity, in mg, of  $C_4H_3FN_2O_2$  in the volume of Injection taken by the formula:

$$5(C / V)(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluorouracil RS in the *Standard preparation*; V is the volume, in mL, of the Injection taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the fluorouracil peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluorouracil Topical Solution

» Fluorouracil Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_4H_3FN_2O_2$ .

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Fluorouracil RS

**Identification**—It responds to the *Identification test* under *Fluorouracil Cream*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Assay—**

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Fluorouracil*.

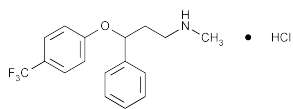
*Assay preparation*—Transfer an accurately weighed portion of Solution, equivalent to about 20 mg of fluorouracil, to a 100-mL volumetric flask, dilute with water to volume, and mix. Quantitatively dilute a volume of this solution with water to obtain a concentration of 10  $\mu$ g per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantity, in mg, of fluorouracil ( $C_4H_3FN_2O_2$ ) in the portion of Solution taken by the formula:

$$2C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Fluorouracil RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the fluorouracil responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxetine Hydrochloride



$C_{17}H_{18}F_3NO \cdot HCl$  345.79

Benzenepropanamine, *N*-methyl- $\gamma$ -[4-(trifluoromethyl)phenoxy]-, hydrochloride, ( $\pm$ ).  
( $\pm$ )-*N*-Methyl-3-phenyl-3-[( $\alpha,\alpha,\alpha$ -trifluoro-*p*-tolyl)oxy]propylamine, hydrochloride [59333-67-4].

» Fluoxetine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{17}H_{18}F_3NO \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Fluoxetine Hydrochloride RS

USP Fluoxetine Related Compound A RS

*N*-Methyl-3-phenyl-3-[( $\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)oxy]propylamine hydrochloride.

$C_{17}H_{18}F_3NO \cdot HCl$  345.79

USP Fluoxetine Related Compound B RS

*N*-Methyl-3-phenylpropylamine.

$C_{10}H_{15}N$  149.24

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** A solution meets the requirements of the tests for *Chloride* (191).

**Water**, *Method I* (921): not more than 0.5%.

**Heavy metals**, *Method II* (231): 0.003%.

**Related compounds**—

**Mobile phase**—Proceed as directed in the *Assay*.

**Test solution 1**—Transfer about 56 mg of Fluoxetine Hydrochloride, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Test solution 2**—Transfer 2 mL of *Test solution 1*, accurately measured, to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability solution**—Dissolve about 22 mg of USP Fluoxetine Hydrochloride RS in 10 mL of 1 N sulfuric acid, and heat to 85° for 3 hours. Cool, transfer 0.4 mL of this solution to a 25-mL volumetric flask, and add about 28 mg of USP Fluoxetine Hydrochloride RS, 1 mg of USP Fluoxetine Related Compound A RS, and 1 mg of USP Fluoxetine Related Compound B RS. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m base-deactivated packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.24 for  $\alpha$ -[2-(methylamino)ethyl]benzenemethanol (if present), 0.27 for fluoxetine related compound B, 0.94 for fluoxetine related compound A, 1.0 for fluoxetine, and 2.17 for 4-trifluoromethylphenol; and the ratio of the height of the fluoxetine related compound A peak to the depth of the valley between the fluoxetine and fluoxetine related compound A peaks (measured from the fluoxetine related compound A peak height) is not more than 1.1.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of *Test solution 1* and *Test solution 2* into the chromatograph, record the chromatograms for not less than twice the elution time for fluoxetine, and measure the peak responses. Calculate the percentage of fluoxetine related compound A in the portion of Fluoxetine Hydrochloride taken by the formula:

$$100r_A / (r_A + r_U)$$

in which  $r_A$  is the peak response of fluoxetine related compound A obtained from *Test solution 2*; and  $r_U$  is the peak response of fluoxetine obtained from *Test solution 2*.

Calculate the percentage of each of the other impurities in the portion of Fluoxetine Hydrochloride taken by the formula:

$$100r_i / (r_s + 5r_U)$$

in which  $r_i$  is the peak response for each impurity obtained from *Test solution 1*;  $r_s$  is the sum of the responses of all the peaks, excluding fluoxetine, obtained from *Test solution 1*; and  $r_U$  is as defined above: not more than 0.15% of fluoxetine related compound A is found; not more than 0.25% of  $\alpha$ -[2-(methylamino)ethyl]benzenemethanol is found; not more than 0.25% of fluoxetine related compound B is found; and not more than 0.1% of any other individual impurity is found. The sum of all impurities found is not more than 0.5%.

**Assay**—

**Triethylamine buffer**—Transfer about 10 mL of triethylamine, accurately measured, to a suitable container, add about 980 mL of water, and adjust with phosphoric acid to a pH of 6.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Triethylamine buffer*, stabilizer-free tetrahydrofuran, and methanol (6:3:1). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.11 mg per mL.

**Assay preparation**—Transfer about 11 mg of Fluoxetine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m base-deactivated packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{17}H_{18}F_3NO \cdot HCl$  in the portion of Fluoxetine Hydrochloride taken by the formula:

$$100C(r_U / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Fluoxetine Capsules

» Fluoxetine Capsules contain an amount of Fluoxetine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxetine ( $C_{17}H_{18}F_3NO$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Fluoxetine Hydrochloride RS

### Identification—

**A: Infrared Absorption** (197K)—

**Test specimen**—Transfer a quantity of Capsule contents, equivalent to about 10 mg of fluoxetine, to a suitable container, dissolve in 10 mL of methanol, and filter. Rinse the container and filter with 5 mL of methanol, and evaporate with the aid of a current of air and mild heat to dryness.

### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_{17}H_{18}F_3NO$  dissolved by employing the following method.

**Diethylamine phosphate suspension**—Transfer 250 mL of acetonitrile to a suitable container, add 1.0 mL of diethylamine, mix, and adjust with phosphoric acid to a pH of 3.5. [NOTE—Diethylamine phosphate will precipitate; therefore, keep well-mixed.]

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and diethylamine (600:400:4), and adjust with phosphoric acid to a pH of 3.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Prepare a solution of USP Fluoxetine Hydrochloride RS having a concentration similar to that of the *Test solution*, and filter. Transfer 5.0 mL of this solution to a suitable container, add 2.0 mL of *Diethylamine phosphate suspension*, and mix.

**Test solution**—Filter 20 mL of the solution under test. Transfer 5.0 mL of this solution to a suitable container, add 2.0 mL of *Diethylamine phosphate suspension*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 226-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L10. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluoxetine ( $C_{17}H_{18}F_3NO$ ) dissolved by the formula:

$$(309.33/345.79)900C(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively; C is the concentration, in  $\mu$ g per mL, of USP Fluoxetine Hydrochloride RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{18}F_3NO$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Chromatographic purity—

**Triethylamine buffer**—Proceed as directed in the *Assay* under *Fluoxetine Hydrochloride*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Triethylamine buffer* and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg per mL.

**Test solution**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the combined contents, equivalent to about 20 mg of fluoxetine, to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L10. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution* for at least 22 minutes, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1100 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Capsules taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for each impurity; and  $r_S$  is the sum of the responses of all the peaks: not more than 0.25% of any individual impurity is found, and not more than 0.80% of total impurities is found.

### Assay—

**Triethylamine buffer, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Fluoxetine Hydrochloride*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of fluoxetine, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluoxetine ( $C_{17}H_{18}F_3NO$ ) in the portion of Capsules taken by the formula:

$$(309.33 / 345.79)100C(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxetine Delayed-Release Capsules

» Fluoxetine Delayed-Release Capsules contain an amount of Fluoxetine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxetine ( $C_{17}H_{18}F_3NO$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Fluoxetine Hydrochloride RS

USP Fluoxetine Related Compound C RS

*N*-Methyl-*N*-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-succinamic acid.

$C_{21}H_{22}F_3NO_4$  409.40

### Identification, Infrared Absorption (197F)—

**Test specimen**—Transfer the contents of 3 Capsules to a suitable container, and grind to a fine powder. Transfer a portion of the powder, equivalent to about 40 mg of fluoxetine, to a suitable container, and dissolve in 25 mL of 0.1 N hydrochloric acid. Filter, and transfer 10 mL of the solution so obtained to a separatory funnel, add 20 mL of methylene chloride, and mix. Allow the phases to separate, and transfer the organic layer to a small glass container. Evaporate to dryness with the aid of a current of air and mild heat. Redissolve the residue with a few drops of methylene chloride, and transfer to a potassium bromide plate. Dry or evaporate to a thin film with the aid of a stream of nitrogen.

### Drug release (711)—

**Apparatus 3:** 12 dips per minute (dpm), using a polypropylene 40-mesh screen on the top and bottom of the reciprocating cylinder.

#### ACID STAGE—

**Medium:** 0.1 N hydrochloric acid; 250 mL, deaerated. Operate the apparatus for 2 hours at 12 dpm, withdraw an aliquot of the *Medium*, and allow the apparatus to proceed to the *Buffer stage*.

**Standard solution**—Prepare a solution of USP Fluoxetine Hydrochloride RS in *Acid stage Medium* having a known concentration of about 0.036 mg per mL.

**Test solution**—Use portions of the solution under test passed through a filter having a 0.45- $\mu$ m porosity.

**Procedure**—Determine the amount of  $C_{17}H_{18}F_3NO$  dissolved from the minimum (most negative) of the first derivative of UV absorbances at about 278 nm in comparison with the *Standard solution*.

**Tolerances**—Not more than 10% of the labeled amount of  $C_{17}H_{18}F_3NO$  is dissolved in 2 hours.

#### BUFFER STAGE—

**Medium:** pH 6.8 phosphate buffer (prepared by mixing 3 L of 0.1 N hydrochloric acid and 1 L of 0.2 M tribasic sodium phosphate, and adjusting, if necessary, with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ ); 250 mL, deaerated. Operate the apparatus for 45 minutes at 12 dpm, and withdraw an aliquot of the *Medium*.

**Standard solution**—Prepare a solution of USP Fluoxetine Hydrochloride RS in *Buffer stage Medium*, having a known concentration of about 0.36 mg per mL.

**Test solution**—Use portions of the solution under test passed through a filter having a 0.45- $\mu$ m porosity.

**Procedure**—Determine the amount of  $C_{17}H_{18}F_3NO$  dissolved from the difference between the maximum UV absorbance at about 264 nm and the absorbance at 290 nm in comparison with the *Standard solution*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{18}F_3NO$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Chromatographic purity—

**Ion-pair solution**—Dissolve about 6.5 g of sodium 1-octanesulfonate and 2.9 g of anhydrous sodium acetate in 1 L of water, and adjust with glacial acetic acid to a pH of 5.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Ion-pair solution* and acetonitrile (58:42). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Degraded fluoxetine solution**—Dissolve a quantity of USP Fluoxetine Hydrochloride RS in 1.0 N sulfuric acid to obtain a solution containing about 2.2 mg per mL. Heat to 85° for 3 hours, and cool to room temperature.

**Fluoxetine related compound solution**—Dissolve a quantity of USP Fluoxetine Related Compound C RS in *Mobile phase* to obtain a solution containing about 0.5 mg per mL.

**System suitability solution**—Transfer about 13.5 mg of USP Fluoxetine Hydrochloride RS to a 100-mL volumetric flask, add 2 mL of *Degraded fluoxetine solution* and 2 mL of *Fluoxetine related compound solution*, and dissolve in and dilute with *Mobile phase* to volume. Transfer 10.0 mL of this solution to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Detector sensitivity solution**—Transfer 2 mL of the *System suitability solution* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Test solution**—Weigh and finely powder not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of fluoxetine, to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Filter before injection.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  15-cm column that contains 3.5- $\mu$ m packing L7. The column temperature is maintained at 30°. The flow rate is about 1 mL per minute. Inject the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.49 for  $\alpha, \alpha, \alpha$ -trifluoro-*p*-cresol, 0.70 for fluoxetine related compound C, and 1.0 for fluoxetine; the resolution,  $R$ , between  $\alpha, \alpha, \alpha$ -trifluoro-*p*-cresol and fluoxetine related compound C is not less than 2.0; and the resolution,  $R$ , between fluoxetine related compound C and fluoxetine is not less than 6.0. Chromatograph the *Detector sensitivity solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio for the fluoxetine peak is not less than 10.

**Procedure**—Inject a volume (about 50  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram for at least three times the retention time of the fluoxetine peak, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Capsules taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the sum of the responses of all the peaks: not more than 0.2% of any individual impurity is found, and not more than 0.7% of total impurities is found.

### Assay—

**Ion-pair solution**—Dissolve about 2.9 mL of glacial acetic acid and about 7.1 g of sodium 1-pentanesulfonate in 1 L of water. Adjust with 5 N sodium hydroxide to a pH of 5.0.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Ion-pair solution* (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve suitable quantities of USP Fluoxetine Hydrochloride RS and  $\alpha, \alpha, \alpha$ -trifluoro-*p*-cresol in *Mobile phase* to obtain a solution containing about 110  $\mu$ g per mL and 20  $\mu$ g per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.11 mg per mL.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of fluoxetine, to a 500-mL volumetric flask, shake by mechanical means for about 10 minutes, and then sonicate for about 5 minutes. Cool the solution to room temperature, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 10.0-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Filter the solution before injection.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 7.5-cm column that contains 3.5-μm packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 38°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for α,α,α-trifluoro-*p*-cresol and 1.0 for fluoxetine; the resolution, *R*, between α,α,α-trifluoro-*p*-cresol and fluoxetine is not less than 4.0; the tailing factor for the fluoxetine peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluoxetine (C<sub>17</sub>H<sub>18</sub>F<sub>3</sub>NO) in the portion of Capsules taken by the formula:

$$1000(309.33/345.79)C(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxetine Oral Solution

» Fluoxetine Oral Solution contains an amount of Fluoxetine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxetine (C<sub>17</sub>H<sub>18</sub>F<sub>3</sub>NO). It may contain one or more preservatives.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Fluoxetine Hydrochloride RS

**Identification, Infrared Absorption** <197S>—

**Solution**—Transfer a volume of Oral Solution, equivalent to about 20 mg of fluoxetine, to a separatory funnel, add 5.0 mL of water and 0.5 mL of 1 N sodium hydroxide, extract with 5 mL of chloroform, and discard the aqueous layer. Evaporate the remaining layer to dryness, and dissolve the residue in 0.4 mL of chloroform.

**Uniformity of dosage units** <905>—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** <791>: between 2.5 and 4.5.

**Chromatographic purity**—

**Ion-pair solution**—Transfer about 4.3 g of sodium 1-octanesulfonate and 13.8 g of monobasic sodium phosphate to a suitable container, dissolve in 1 L of water, and adjust with phosphoric acid to a pH of 3.0.

**Diluent**—Prepare a mixture of *Ion-pair solution*, methanol, and acetonitrile (6:3:1).

**Solution A**—Prepare a filtered and degassed mixture of *Ion-pair solution*, methanol, and acetonitrile (53:26:21).

**Solution B**—Prepare a filtered and degassed mixture of *Ion-pair solution*, acetonitrile, and methanol (43:35:22).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve a quantity of USP Fluoxetine Hydrochloride RS in 1 N sulfuric acid to obtain a solution having a known concentration of about 2.0 mg per mL, and heat at 85° for 1 hour. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add about 10 mg of USP Fluoxetine Hydrochloride RS, dissolve in and dilute with *Diluent* to volume, and mix.

**Test solution**—Transfer an accurately measured volume of Oral Solution, equivalent to about 19 mg of fluoxetine, to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Diluted test solution**—Transfer 1.0 mL of the *Test solution* to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–13	100	0	isocratic
13–15	100→0	0→100	linear gradient
15–29	0	100	isocratic
29–30	0→100	100→0	linear gradient
30–end	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the retention time of any peak, except the peak for fluoxetine, is less than 13 minutes.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the volume of Oral Solution taken by the formula:

$$100r_i / (\Sigma r_i + 25r_s)$$

in which *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response for fluoxetine obtained from the *Diluted test solution*: not more than 0.4% of any individual impurity is found; and not more than 0.8% of total impurities is found.

**Assay**—

**Triethylamine buffer**—Transfer about 10 mL of triethylamine, accurately measured, to a suitable container, add about 980 mL of water, and adjust with phosphoric acid to a pH of 6.0.

**Mobile phase**—Prepare a filtered and degassed mixture of Triethylamine buffer and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 45 µg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 4.0 mg of fluoxetine, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the fluoxetine peak. Calculate the quantity, in mg, of fluoxetine ( $C_{17}H_{18}F_3NO$ ) in the volume of Oral Solution taken by the formula:

$$(309.33/345.79)(0.1C)(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights for fluoxetine and fluoxetine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxetine Tablets

» Fluoxetine Tablets contain an amount of Fluoxetine Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxetine ( $C_{17}H_{18}F_3NO$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** <11>—

USP Fluoxetine Hydrochloride RS  
USP Fluoxetine Related Compound B RS  
N-Methyl-3-phenylpropylamine.  
 $C_{10}H_{15}N$  149.24

**Identification, Infrared Absorption** <197K>—

**Test specimen**—Transfer 1 Tablet to a suitable container, dissolve in 10 mL of chloroform, and filter. Rinse the container with 5 mL of chloroform, and filter. Evaporate the combined filtrate in a hood with the aid of a current of air and mild heat to dryness.

**Dissolution** <711>—

**Medium:** 0.1 N hydrochloric acid; 1000 mL.

**Apparatus 1:** 100 rpm.

**Time:** 15 minutes.

Determine the amount of  $C_{17}H_{18}F_3NO$  dissolved by employing the following method.

**Ion-pair solution, Mobile phase, and System suitability solution**—Prepare as directed in the Assay.

**Standard solution**—Prepare a solution of USP Fluoxetine Hydrochloride RS in *Medium* having a known concentration similar to that of the solution under test.

**Test solution**—Filter 20 mL of the solution under test.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 7.5-cm column that contains 3.5-µm packing L7. The column temperature is maintained at 38°. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between fluoxetine and  $\alpha, \alpha$ ,  $\alpha$ -trifluoro-*p*-cresol is not less than 2.0; the tailing factor for the fluoxetine peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of  $C_{17}H_{18}F_3NO$  dissolved by the formula:

$$(309.33/345.79)1000C(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{18}F_3NO$  is dissolved in 15 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Chromatographic purity**—

**Ion-pair solution**—Dissolve 6.5 g of sodium 1-octanesulfonate in 1000 mL of water, add 2.9 mL of phosphoric acid, and adjust with a sodium hydroxide solution (1 in 5) to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of *Ion-pair solution* and acetonitrile (57:43). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Resolution solution**—Transfer 1 mg of USP Fluoxetine Related Compound B RS and about 13.5 mg of USP Fluoxetine Hydrochloride RS to a 100-mL volumetric flask. Add 2 mL of a solution prepared by dissolving about 22 mg of USP Fluoxetine Hydrochloride RS in 10 mL of 1 N sulfuric acid, heating at about 85° for 3 hours, and cooling to room temperature. Dilute with *Mobile phase* to volume, and mix. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Detector sensitivity solution**—Prepare a solution of *Resolution solution* in *Mobile phase* (1 in 100).

**Standard solution**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.0135 mg per mL.

**Test solution**—Place 10 Tablets in a volumetric flask of suitable size. Dissolve in and dilute with *Mobile phase* to volume to obtain a solution having a concentration of about 2 mg of fluoxetine per mL. Pass a portion of the solution through a suitable filter, and use the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains 3.5-µm packing L7. The column temperature is maintained at 30°. The flow rate is about 1.0 mL per minute. Chromatograph in the following order the *Mobile phase*, the *Detector sensitivity solution*, and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.19 for  $\alpha$ -[2-(methylamino)ethyl]benzenemethanol, 0.26 for fluoxetine related compound B, and 1.0 for fluoxetine; the resolution,  $R$ , between  $\alpha$ -[2-(methylamino)ethyl]benzenemethanol and fluoxetine related com-

pound B is not less than 4.5; and the signal-to-noise ratio for the *Detector sensitivity solution* is not less than 10.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time equal to three times the retention time of the main peak, and measure the areas for all the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(309.33/345.79)(C_S / C_U)(r_i / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively;  $C_S$  is the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of fluoxetine in the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the peak response for fluoxetine obtained from the *Standard solution*: not more than 0.25% of any individual impurity is found; and not more than 0.80% of total impurities is found.

#### Assay—

**Ion-pair solution**—Dissolve 7.1 g of sodium 1-pentane-sulfonate in 1000 mL of water, add 2.9 mL of glacial acetic acid, and adjust with a sodium hydroxide solution (1 in 5) to a pH of 5.0.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Ion-pair solution* (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Transfer about 10 mg of  $\alpha, \alpha$ ,  $\alpha$ -trifluoro-*p*-cresol, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask containing about 11 mg of USP Fluoxetine Hydrochloride RS, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluoxetine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer 10 Tablets to a 1000-mL volumetric flask. Add about 500 mL of *Mobile phase*, and shake to disintegrate the Tablets. Dilute with *Mobile phase* to volume, and sonicate for 10 minutes. Transfer a portion of this solution to a volumetric flask of suitable size, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.1 mg of fluoxetine per mL. Pass through a suitable filter, and use the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm  $\times$  7.5-cm column that contains 3.5- $\mu$ m packing L7. The column temperature is maintained at 38°. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between fluoxetine and  $\alpha, \alpha, \alpha$ -trifluoro-*p*-cresol is not less than 4.0; the tailing factor for the fluoxetine peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

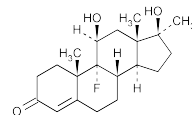
**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluoxetine ( $C_{17}H_{18}F_3NO$ ) in the portion of Tablets taken by the formula:

$$(309.33/345.79)(1000CD)(r_U / r_S)$$

in which 309.33 and 345.79 are the molecular weights of fluoxetine and fluoxetine hydrochloride, respectively;  $C$  is

the concentration, in mg per mL, of USP Fluoxetine Hydrochloride RS in the *Standard preparation*;  $D$  is the dilution factor, if performed, used in preparing the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxymesterone



$C_{20}H_{29}FO_3$  336.44

Androst-4-en-3-one, 9-fluoro-11,17-dihydroxy-17-methyl-, (11 $\beta$ ,17 $\beta$ )-.  
9-Fluoro-11 $\beta$ ,17 $\beta$ -dihydroxy-17-methylandrost-4-en-3-one [76-43-7].

» Fluoxymesterone contains not less than 97.0 percent and not more than 102.0 percent of  $C_{20}H_{29}FO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light.

#### USP Reference standards <11>—

USP Fluoxymesterone RS

#### Identification—

**A: Infrared Absorption** <197K>—If a difference appears, dissolve portions of both the test specimen and the USP Reference Standard in dehydrated alcohol, evaporate the solutions to dryness, and repeat the test on the residues.

**B: Ultraviolet Absorption** <197U>—

**Solution:** 10  $\mu$ g per mL.

**Medium:** alcohol.

Absorptivities at 242 nm do not differ by more than 2.5%.

**Specific rotation** <781S>: between +104° and +112°.

**Test solution:** 10 mg per mL, in alcohol.

**Loss on drying** <731>—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

#### Chromatographic purity—

**Solution A**—Prepare a filtered and degassed mixture of methanol and water (55:45).

**Solution B**—Use filtered and degassed methanol.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

**Blank solution**—Use *Solution B*.

**System suitability solution**—Dilute a volume of the *Test solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 5  $\mu$ g of fluoxymesterone per mL.

**Test solution**—Prepare a solution of Fluoxymesterone in *Solution B* containing about 0.5 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate is 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–20	100→60	0→40	linear gradient
20–40	60→0	40→100	linear gradient
40–45.0	0	100	isocratic
45.0–45.1	0→100	100→0	linear gradient
45.1–60	100	0	isocratic

Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 15,000 theoretical plates. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio for the fluoxymesterone peak is not less than 100.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Blank solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for any peaks that do not appear in the *Blank solution* that have an area equal to or greater than 0.1% of the fluoxymesterone peak. Calculate the percentage of each impurity in the portion of Fluoxymesterone taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

#### Assay—

**Internal standard solution**—Dissolve methylprednisolone in a mixture of chloroform and methanol (95:5) to obtain a solution containing about 200  $\mu$ g per mL.

**Mobile phase**—Prepare a solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluoxymesterone RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Dissolve about 25 mg of Fluoxymesterone, accurately weighed, in 100.0 mL of *Internal standard solution* to obtain a solution having a concentration of about 0.25 mg per mL.

**Procedure**—Inject equal volumes of the *Assay preparation* and the *Standard preparation* into a suitable high-pressure liquid chromatograph (see *Chromatography* <621>) of the general type equipped with a detector for monitoring UV light at 254 nm, equipped with a suitable recorder, and capable of providing column pressure up to about 2000 psi. The instrument contains a 4-mm  $\times$  30-cm stainless steel column that contains packing L3. In a suitable chromatogram, the resolution,  $R$ , between fluoxymesterone and the internal standard is not less than 3.0; and the relative standard deviation of the peak response ratios of four replicate injections of the *Standard preparation* is not more than 2.0%. Calculate the quantity, in mg, of  $C_{20}H_{29}FO_3$  in the portion of Fluoxymesterone taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Fluoxymesterone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of fluoxymesterone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluoxymesterone Tablets

» Fluoxymesterone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxymesterone ( $C_{20}H_{29}FO_3$ ).

**Packaging and storage**—Preserve in well-closed containers, protected from light.

#### USP Reference standards <11>—

USP Fluoxymesterone RS

USP Norethindrone RS

**Identification**—Triturate a quantity of powdered Tablets, equivalent to about 20 mg of fluoxymesterone, with 20 mL of hot chloroform, and decant the supernatant through a filter. Repeat the extraction with two 20-mL portions of hot chloroform. Evaporate the combined chloroform solutions on a water bath to dryness, digest the residue with 5 mL of acetone, decant the supernatant, add to it 20 mL of water, and filter off the precipitate. Dissolve the precipitate in 5 mL of acetone, add 20 mL of water, and filter: the precipitate, after being dried at 105° for 3 hours, meets the requirements for *Identification test A* under *Fluoxymesterone*.

#### Dissolution <711>—

**Medium**: 0.01 N hydrochloric acid; 900 mL.

**Apparatus 2**: 75 rpm.

**Time**: 60 minutes.

Determine the amount of  $C_{20}H_{29}FO_3$  dissolved by employing the following method.

**Mobile phase**—Prepare a degassed and filtered solution of water and acetonitrile (58:42). Make adjustments if necessary (see *Chromatography* <621>).

**Internal standard solution**—Dissolve a quantity of USP Norethindrone RS in alcohol to obtain a solution having a final concentration of about 46  $\mu$ g per mL.

**Standard solution**—Transfer about 28 mg of USP Fluoxymesterone RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix.

Pipet 5 mL of the resulting solution into a 250-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix. Pipet 5 mL of this solution and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

**Test solution**—Pipet a filtered 20-mL aliquot of the solution under test and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph replicate injections of the *Standard solution*, and measure the peak responses as directed for *Procedure*: the relative retention times are 0.5 for fluoxymesterone and 1.0 for norethindrone; the resolution,  $R$ , between fluoxymesterone and norethindrone is not less than 2; and the relative standard deviation is not more than 2.0%.

**Procedure**—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the amount of  $C_{20}H_{29}FO_3$  dissolved by comparison with the *Standard solution*, similarly chromatographed.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{20}H_{29}FO_3$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

## PROCEDURE FOR CONTENT UNIFORMITY—

*Internal standard solution, Mobile phase, and Standard preparation*—Prepare as directed in the Assay under Fluoxymesterone.

*Test preparation*—Transfer 1 Tablet to a suitable container, add 2 mL of water, and sonicate for 30 minutes or until the Tablet completely disintegrates. Add an accurately measured volume of *Internal standard solution* (5.0 mL for each mg of fluoxymesterone in the Tablet), and shake the mixture for 15 minutes. Filter a portion of the chloroform layer, and use the clear filtrate.

*Procedure*—Proceed as directed in the Assay under Fluoxymesterone, using the *Test preparation* in place of the Assay preparation. Calculate the quantity, in mg, of fluoxymesterone ( $C_{20}H_{29}FO_3$ ) in the Tablet taken by the formula:

$$(TC/D)(R_U / R_S)$$

in which  $T$  is the labeled quantity, in mg, of fluoxymesterone in the Tablet;  $D$  is the concentration, in mg per mL, of fluoxymesterone in the *Test preparation*, based on the labeled quantity per Tablet and the extent of dilution; and the other terms are as defined therein.

**Assay—**

*Internal standard solution, Mobile phase, and Standard preparation*—Prepare as directed in the Assay under Fluoxymesterone.

*Assay preparation*—Accurately weigh 20 Tablets, and grind to a fine powder in a mortar and pestle. Accurately weigh a portion of the powder, equivalent to about 5 mg of fluoxymesterone, and transfer to a suitable container. Add 20.0 mL of *Internal standard solution*, sonicate for 10 minutes, and shake for 15 minutes. Filter a portion of the liquid, and analyze the clear filtrate as directed for *Procedure*.

*Procedure*—Proceed as directed in the Assay under Fluoxymesterone. Calculate the quantity, in mg, of fluoxymesterone ( $C_{20}H_{29}FO_3$ ) in the portion of Tablets taken by the formula:

$$20C(R_U / R_S)$$

in which the terms are as defined therein.

## Fluphenazine Decanoate

» Fluphenazine Decanoate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{32}H_{44}F_3N_3O_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Fluphenazine Decanoate Dihydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification—**

**A:** Place about 50 mg of Fluphenazine Decanoate and about 50 mg of USP Fluphenazine Decanoate Dihydrochloride RS in separate, glass-stoppered, small centrifuge tubes, and treat each tube as follows. Add 1.5 mL of sodium hydroxide solution (1 in 250), and mix. Add 2 mL of carbon disulfide, shake vigorously for 2 minutes, and centrifuge. Dry the lower, clear layer by filtering through 2 g of anhydrous sodium sulfate: the IR absorption spectrum of the test preparation, determined in a 0.1-mm cell, exhibits maxima only

at the same wavelengths as that of the Standard preparation, similarly measured.

**B:** Use a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Impregnate the plate with a 1 in 20 solution of tetradecane in hexane, and allow to air-dry. Prepare a test solution in alcohol containing 20 mg per mL. Apply 1  $\mu$ L each of the test solution and of a Standard solution of USP Fluphenazine Decanoate Dihydrochloride RS in alcohol, similarly prepared. Allow the spots to dry. Apply to the spot from the Standard solution, 1.0  $\mu$ L of 0.1 N sodium hydroxide and allow it to dry. Develop the plate in an equilibrated chamber using a mixture of methanol and water (9:1) as the mobile phase. Examine the plate under short-wavelength UV light at 254 nm: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Eluant:* a mixture of acetone, cyclohexane, and ammonium hydroxide (16:6:1).

*Visualization:* 1; then spray the plate with 50% sulfuric acid.

*Interpretation:* no individual ordinary impurity observed exceeds 1.0% and the total of any ordinary impurities observed does not exceed 2.0%.

**Assay**—Dissolve about 500 mg of Fluphenazine Decanoate, accurately weighed, in 50 mL of glacial acetic acid, add one drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.59 mg of  $C_{32}H_{44}F_3N_3O_2S$ .

## Fluphenazine Decanoate Injection

» Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of fluphenazine decanoate ( $C_{32}H_{44}F_3N_3O_2S$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Fluphenazine Decanoate Dihydrochloride RS

USP Fluphenazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification—**

**A:** To a volume of Injection, equivalent to about 50 mg of fluphenazine decanoate, add 2 mL of methanol and 3 mL of palladium chloride solution (1 in 1000): a rust-red color is produced. Add an excess of the palladium chloride solution: the color is intensified to a brownish red.

**B:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Chromatographic purity**—From the chromatograms obtained in the *Assay*, calculate the percentage of fluphenazine in the portion of *Injection* taken by the formula:

$$3.43(W/V)(r_U/r_S)$$

in which *W* is the weight, in mg, of USP Fluphenazine Hydrochloride RS used to prepare the *Standard preparation*; *V* is the amount, in mg, of fluphenazine decanoate in the portion of *Injection* taken to prepare the *Assay preparation*, based on the labeled concentration; and *r<sub>U</sub>* and *r<sub>S</sub>* are the fluphenazine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. The fluphenazine content is not more than 4% of the labeled amount of fluphenazine decanoate. [NOTE—Use peak heights where peak responses are indicated.] Calculate the percentage of each impurity eluting after fluphenazine decanoate in the portion of *Injection* taken by the formula:

$$100(r_i/r_S)$$

in which *r<sub>i</sub>* is the peak response for each peak eluting after fluphenazine decanoate; and *r<sub>S</sub>* is the sum of the responses for fluphenazine decanoate and all of the peaks eluting after fluphenazine decanoate: the sum of the responses for all of the peaks eluting after fluphenazine decanoate is not more than 2%.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, acetonitrile, and 0.05 M ammonium acetate (2:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Accurately weigh about 25 mg of USP Fluphenazine Hydrochloride RS, and transfer it to a 250-mL volumetric flask. Dissolve in isopropyl alcohol, dilute with isopropyl alcohol to volume, and mix (*Solution A*). Accurately weigh about 25 mg of Fluphenazine Decanoate Dihydrochloride RS. Transfer it to a 50-mL volumetric flask, and add 10 mL of *Solution A*, quantitatively measured. Add 20 mL of isopropyl alcohol and dissolve sonicating, if necessary. Dilute with isopropyl alcohol to volume, and mix. Quantitatively dilute the resulting solution with acetonitrile (1:5) to obtain a solution having a known concentration of about 0.1 mg of fluphenazine decanoate per mL.

**Assay preparation**—Transfer an accurately measured quantity of the *Injection*, equivalent to about 25 mg of fluphenazine decanoate, to a glass-stoppered, 50-mL volumetric flask. Add about 20 mL of isopropyl alcohol, insert the stopper, and shake vigorously for at least 1 minute. Add 20 mL more of isopropyl alcohol, and repeat the vigorous shaking. Dilute with isopropyl alcohol to volume, and mix. Quantitatively dilute the resulting solution with acetonitrile (1:5) to obtain a solution having a concentration of fluphenazine decanoate of about 0.1 mg per mL.

**System suitability preparation**—Weigh suitable quantities of USP Fluphenazine Decanoate Dihydrochloride RS and USP Fluphenazine Hydrochloride RS (5:4), and dissolve in a mixture of methanol and acetonitrile (1:1). Dilute the solution quantitatively and stepwise with the same solvent mixture to obtain a solution having a concentration of anhydrous USP Fluphenazine Decanoate Dihydrochloride RS of about 0.1 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a

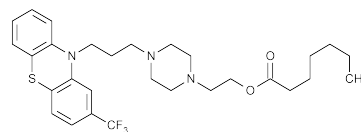
4.6-mm × 25-cm column containing 10-μm packing L1 preceded by a 4.6-mm × 5-cm pre-column containing similar packing of larger particle size (30–40 μm). The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between fluphenazine and fluphenazine decanoate in the *System suitability preparation* is not less than 3.0; and the relative standard deviation of the fluphenazine decanoate peak for replicate injections of the *Standard preparation* does not exceed 2.0. The relative retention times for fluphenazine and fluphenazine decanoate are 0.6 and 1, respectively.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, continuing the chromatography, in each case, for a time equal to 2.5 times the elution time of fluphenazine decanoate. Record the chromatograms, and measure responses for the major peaks and for the peaks from all substances eluting after fluphenazine decanoate. Calculate the quantity, in mg, of fluphenazine decanoate (C<sub>32</sub>H<sub>44</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S) in each mL of the *Injection* taken by the formula:

$$0.890(W_S/W_U)(r_U/r_S)$$

in which *W<sub>S</sub>* is the weight, in mg, of USP Fluphenazine Decanoate Dihydrochloride RS corrected for its moisture content, used to prepare the *Standard preparation*; *W<sub>U</sub>* is the volume, in mL, of *Injection* taken, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of fluphenazine decanoate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluphenazine Enanthate



C<sub>29</sub>H<sub>38</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S 549.69

Heptanoic acid, 2-[4-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl ester.

2-[4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl heptanoate [2746-81-8].

» Fluphenazine Enanthate contains not less than 97.0 percent and not more than 103.0 percent of C<sub>29</sub>H<sub>38</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Fluphenazine Enanthate Dihydrochloride RS

C<sub>29</sub>H<sub>38</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S · 2HCl 622.63

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.



**Identification—**

**A:** Place about 50 mg of Fluphenazine Enanthate and about 50 mg of USP Fluphenazine Enanthate Dihydrochloride RS in separate, glass-stoppered, small centrifuge tubes, and treat each tube as follows. Add 1.5 mL of sodium hydroxide solution (1 in 250), and mix. Add 2 mL of carbon disulfide, shake vigorously for 2 minutes, and centrifuge. Dry the lower, clear layer by filtering it through 2 g of anhydrous sodium sulfate: the IR absorption spectrum of the test preparation, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of the Standard preparation, similarly measured.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* methanolic hydrochloric acid (8.5 in 1000).

Absorptivities at 258 nm, calculated on the dried basis, do not differ by more than 2.5%.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.003%.

**Ordinary impurities** (466)—

*Test solution:* alcohol.

*Standard solution:* alcohol.

*Eluant:* a mixture of alcohol, glacial acetic acid, and water (3:1:1).

*Visualization:* 1.

**Assay**—Dissolve about 500 mg of Fluphenazine Enanthate, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.49 mg of  $C_{29}H_{38}F_3N_3O_2S$ .

## Fluphenazine Enanthate Injection

» Fluphenazine Enanthate Injection is a sterile solution of Fluphenazine Enanthate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine enanthate ( $C_{29}H_{38}F_3N_3O_2S$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, protected from light.

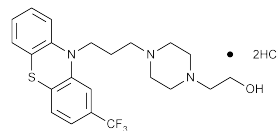
**NOTE**—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification**—To a volume of Injection, equivalent to about 50 mg of fluphenazine enanthate, add 2 mL of methanol and 3 mL of palladium chloride solution (1 in 1000): a rust-red color is produced. Add an excess of the palladium chloride solution: the color is intensified to a brownish red.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Dissolve an accurately measured volume of Injection, equivalent to about 150 mg of fluphenazine enanthate, in 75 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.49 mg of fluphenazine enanthate ( $C_{29}H_{38}F_3N_3O_2S$ ).

## Fluphenazine Hydrochloride



$C_{22}H_{26}F_3N_3OS \cdot 2HCl$  510.44

1-Piperazineethanol, 4-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-, dihydrochloride.

4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride [146-56-5].

» Fluphenazine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of  $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Fluphenazine Hydrochloride RS

**NOTE**—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* methanol.

Absorptivities at 259 nm, calculated on the dried basis, do not differ by more than 2.5%.

**C:** A solution of Fluphenazine Hydrochloride responds to the tests for *Chloride* (191).

**Loss on drying** (731)—Dry it at 65° for 3 hours: it loses not more than 1% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** (231): 0.003%.

**Ordinary impurities** (466)—

*Test solution:* 0.1 M methanolic sodium hydroxide.

*Standard solution:* 0.1 M methanolic sodium hydroxide.

*Eluant:* a mixture of acetone, cyclohexane, and diethylamine (40:15:1).

*Visualization:* 1.

**Assay—**

*Diluent solution*—Prepare a filtered and degassed mixture of 0.05 M monobasic potassium phosphate (adjusted with phosphoric acid to a pH of 2.5), acetonitrile, and methanol (40:30:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Mobile phase*—Prepare a mixture containing 0.2% triethylamine in *Diluent solution*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fluphenazine Hydrochloride RS in *Diluent solution*, and dilute quantitatively, and stepwise if necessary, with *Diluent solution* to obtain a solution having a known concentration of about 0.06 mg per mL.

*Assay preparation*—Transfer about 120 mg of Fluphenazine Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent solution* to volume, and mix. Pipet 5.0 mL of this solution into a 100-mL volumetric flask, dilute with *Diluent solution* to volume, and mix. Filter, discarding the first 5 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 12.5-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{26}F_3N_3OS \cdot 2HCl$  in the portion of Fluphenazine Hydrochloride taken by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fluphenazine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluphenazine Hydrochloride Elixir

» Fluphenazine Hydrochloride Elixir contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** <11>—  
USP Fluphenazine Hydrochloride RS

**NOTE**—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification**—Transfer a volume of Elixir, equivalent to about 10 mg of fluphenazine hydrochloride, to a separator, and to a second separator transfer 10 mg of USP Fluphenazine Hydrochloride RS. To each separator add 20 mL of 6 N sodium hydroxide, and extract each mixture with 20 mL of isoctane. Evaporate the isoctane solutions to dryness, and proceed as directed in the *Identification* test under *Fluphenazine Hydrochloride Tablets*, beginning with “dissolve the residues in 0.5-mL portions.”

**Uniformity of dosage units** <905>—

FOR ELIXIR PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** <698>—

FOR ELIXIR PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** <791>: between 5.3 and 5.8.

**Alcohol content** <611>: not less than 90.0% and not more than 110.0% of the labeled amount, the labeled amount being not more than 15.0% of  $C_2H_5OH$ .

**Assay**—

*Diluent solution, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Fluphenazine Hydrochloride*.

*Assay preparation*—Using a “to contain” pipet transfer a volume of Elixir, equivalent to about 6 mg of fluphenazine hydrochloride, to a 100-mL volumetric flask. Rinse the pipet with *Diluent solution* to complete the transfer, dilute with

*Diluent solution* to volume, and mix. Filter, discarding the first 5 mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ) in each mL of the Elixir taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fluphenazine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Elixir taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluphenazine Hydrochloride Injection

» Fluphenazine Hydrochloride Injection is a sterile solution of Fluphenazine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Fluphenazine Hydrochloride RS

**NOTE**—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification**—Transfer a volume of Injection, equivalent to about 10 mg of fluphenazine hydrochloride, to a separator, and to a second separator transfer 10 mg of USP Fluphenazine Hydrochloride RS. To each separator add 20 mL of 6 N sodium hydroxide, and extract each mixture with 20 mL of isoctane. Evaporate the isoctane solutions to dryness, and proceed as directed in the *Identification* test under *Fluphenazine Hydrochloride Tablets*, beginning with “dissolve the residues in 0.5-mL portions.”

**Bacterial endotoxins** <85>—It contains not more than 166.7 USP Endotoxin Units per mg of fluphenazine hydrochloride.

**pH** <791>: between 4.8 and 5.2.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Proceed with Injection as directed in the *Assay* under *Fluphenazine Hydrochloride Elixir*.

## Fluphenazine Hydrochloride Oral Solution

» Fluphenazine Hydrochloride Oral Solution is an aqueous solution of Fluphenazine Hydrochloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Label it to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration.

**USP Reference standards** (11)—  
USP Fluphenazine Hydrochloride RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification**—Transfer a volume of Oral Solution, equivalent to about 10 mg of fluphenazine hydrochloride, to a separator, and to a second separator transfer 10 mg of USP Fluphenazine Hydrochloride RS. To each separator add 20 mL of sodium hydroxide solution (1 in 4), and extract each mixture with 20 mL of isooctane. Evaporate the isooctane solutions to dryness, and proceed as directed in the *Identification* test under *Fluphenazine Hydrochloride Tablets*, beginning with “dissolve the residues in 0.5-mL portions.”

**pH** (791): between 4.0 and 5.0.

**Alcohol content** (611): not less than 90.0% and not more than 110.0% of the labeled amount, the labeled amount being not more than 15.0% of  $C_2H_5OH$ .

**Assay**—Proceed with Oral Solution as directed in the *Assay* under *Fluphenazine Hydrochloride Elixir*.

## Fluphenazine Hydrochloride Tablets

» Fluphenazine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Fluphenazine Hydrochloride RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Identification**—Transfer a portion of finely powdered Tablets, equivalent to about 10 mg of fluphenazine hydrochloride, to a separator, and to a second separator transfer 10 mg of USP Fluphenazine Hydrochloride RS. Add 5 mL of water and 20 mL of dilute hydrochloric acid (1 in 120) to each separator, shake for 10 minutes, and to each mixture add 20 mL of chloroform-saturated sodium carbonate solution (1 in 10). Extract each mixture with five 20-mL portions of chloroform, shaking gently to avoid emulsion formation, and pass the extracts through separate chloroform-washed cotton filters into separate 150-mL beakers. Evaporate the extracts on a steam bath to dryness, and dissolve the residues in 0.5-mL portions of a mixture of 4 volumes of methanol and 1 volume of water. Apply 10  $\mu$ L of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone, cyclohexane, and diethylamine (40:15:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by

lightly spraying with a solution of sulfuric acid in methanol (2 in 5): the  $R_f$  value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

**Dissolution** (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{22}H_{26}F_3N_3OS \cdot 2HCl$  dissolved by employing the procedure set forth in the *Assay*, with the following differences: in the *Mobile phase*, use 0.3% triethylamine; in the *Assay preparation*, dilute the amount of sample to be withdrawn with an equal volume of *Mobile phase*; in the *Standard preparation*, use a concentration and composition similar to that of the *Assay preparation*; in the *Chromatographic system*, use a flow rate of about 2.0 mL per minute; in the *Procedure*, inject a volume of about 100  $\mu$ L.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{22}H_{26}F_3N_3OS \cdot 2HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Diluent solution, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Fluphenazine Hydrochloride*.

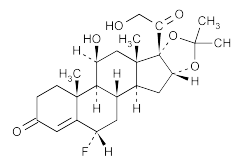
*Assay preparation*—Transfer 6 Tablets to a suitable volumetric flask, add *Diluent solution*, shake for 1 hour, and sonicate for 10 minutes or until a fine suspension is obtained. Dilute quantitatively, and stepwise if necessary, with *Diluent solution* to obtain a final concentration of 0.06 mg of fluphenazine hydrochloride per mL. Filter, discarding the first 5 mL of the filtrate.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluphenazine hydrochloride ( $C_{22}H_{26}F_3N_3OS \cdot 2HCl$ ) in the portion of Tablets taken by the formula:

$$100CT(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Fluphenazine Hydrochloride RS in the *Standard preparation*; T is the labeled quantity, in mg, of fluphenazine hydrochloride in the Tablet; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurandrenolide



$C_{24}H_{33}FO_6$  436.51

Pregn-4-ene-3,20-dione, 6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-,  $6\alpha,11\beta,16\alpha$ -.

6 $\alpha$ -Fluoro-11 $\beta$ ,16 $\alpha$ ,17,21-tetrahydroxypregn-4-ene-3,20-dione, cyclic 16,17-acetal with acetone [1524-88-5].

» Flurandrenolide contains not less than 97.0 percent and not more than 102.0 percent of  $C_{24}H_{33}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers in a cold place, protected from light.

**USP Reference standards** (11)—  
USP Flurandrenolide RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 20 µg per mL.

*Medium:* methanol.

Absorptivities at 237 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +145° and +153°.

*Test solution:* 10 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it in vacuum at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Application volume:* 10 µL.

*Eluent:* a mixture of toluene and isopropyl alcohol (90:10), in a nonequilibrated chamber.

*Visualization:* 1.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve Prednisone in *Mobile phase*, with the aid of sonication, to obtain a solution containing about 1 mg per mL.

**Standard preparation**—Transfer about 5 mg of USP Flurandrenolide RS, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, sonicate to aid solution, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Flurandrenolide RS per mL.

**Assay preparation**—Transfer about 5 mg of Flurandrenolide, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, sonicate to aid solution, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the order of elution is prednisone followed by flurandrenolide, the resolution; *R*<sub>1</sub> between the analyte and internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for prednisone and 1.0 for flurandrenolide. Calculate the quantity, in mg, of C<sub>24</sub>H<sub>33</sub>FO<sub>6</sub> in the portion of Flurandrenolide taken by the formula:

$$10C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*; and *R*<sub>U</sub> and *R*<sub>S</sub> are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurandrenolide Cream

» Flurandrenolide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide (C<sub>24</sub>H<sub>33</sub>FO<sub>6</sub>).

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** (11)—  
USP Flurandrenolide RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Extract a quantity of weighed Cream, equivalent to about 500 µg of flurandrenolide, as directed for the *Assay preparation*. Omit the addition of the internal standard, and evaporate the chloroform extracts on a steam bath under a stream of nitrogen to about 3 mL. Transfer the chloroform solution to a 10-mL flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 2 mL of chloroform.

*Application volume:* 4.0 µL.

**Developing solvent system:** a mixture of ethyl acetate and ethyl ether (70:30).

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Methanolic sodium chloride**—Transfer 100 mL of sodium chloride solution (1 in 10) to a 500-mL volumetric flask. Dilute with methanol to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 10 mg of testosterone to a 100-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Transfer about 16 mg of USP Flurandrenolide RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 3.0 mL of this solution to a 10-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a solution having a known concentration of about 48 µg of USP Flurandrenolide RS per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 500 µg of flurandrenolide, to a 125-mL separator. Add 50 mL of hexanes and 25 mL of *Methanolic sodium chloride*, and shake until the Cream is thoroughly dispersed. Allow the phases to separate, and drain the lower aqueous phase into a second 125-mL separator containing 15 mL of hexanes. Shake vigorously, allow the phases to separate, and drain the lower aqueous phase into a 250-mL separator containing 75 mL of water. Serially extract the hexane phases remaining in the two 125-mL separators with two additional 25-mL portions of *Methanolic sodium chloride*, adding each aqueous phase to the 250-mL separator. Discard the hexane phases. Extract the combined aqueous phases with four 25-mL portions of chloroform. Filter each chloroform extract through 10 g of anhydrous sodium sulfate into a 125-mL conical beaker. Rinse the sodium sulfate with water-washed chloroform, and add the wash to the beaker. Add 4.0 mL of *Internal standard solution* to the beaker containing the chloroform extract. Evaporate the solution on a steam bath under a stream of nitrogen nearly to dryness. Remove the beaker from the steam bath, and evaporate the remaining solution with the aid of nitrogen to dryness. Add 10 mL of *Mobile phase* to

the beaker, and place it in an ultrasonic bath to dissolve the residue. Pass the solution through a suitable filter having a 0.5- $\mu$ m porosity and a prefilter above the membrane filter to prevent clogging.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 2 for testosterone and 1.0 for flurandrenolide; the resolution,  $R$ , between the analyte and internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flurandrenolide ( $C_{24}H_{33}FO_6$ ) in the portion of Cream taken by the formula:

$$10C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurandrenolide Lotion

» Flurandrenolide Lotion contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ( $C_{24}H_{33}FO_6$ ).

**Packaging and storage**—Preserve in tight containers, protected from heat, light, and freezing.

**USP Reference standards** <11>—  
USP Flurandrenolide RS

**Identification**—It responds to the *Identification* test under *Flurandrenolide Cream*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

**pH** <791>: between 3.5 and 6.0, determined in a 1 in 10 dilution of the Lotion in water containing 0.30 mL of saturated potassium chloride solution per 100 mL.

**Assay**—

*Methanolic sodium chloride, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Flurandrenolide Cream*.

*Assay preparation*—Transfer an accurately weighed portion of Lotion, calculated from the density to contain about 500  $\mu$ g of flurandrenolide, to a separator. (Determine the density by taring a 100-mL volumetric flask containing 50.0 mL of water, adding approximately 25 g of well-shaken Lotion, and again weighing, then carefully adjusting the contents of the volumetric flask with water from a buret to volume, and finally calculating the density taken by the formula:

$$A / B$$

in which  $A$  is the weight, in g, of the Lotion taken; and  $B$  is 50.0 mL minus the volume, in mL, of water necessary to

adjust the contents of the volumetric flask to volume.) Proceed as directed for *Assay preparation* in the *Assay* under *Flurandrenolide Cream*, beginning with "Add 50 mL of hexane and 25 mL of *Methanolic sodium chloride*."

**Procedure**—Proceed as directed in the *Assay* under *Flurandrenolide Cream*. Calculate the quantity, in mg, of flurandrenolide ( $C_{24}H_{33}FO_6$ ) in each mL of the Lotion taken by the formula:

$$10C(D / W)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*;  $D$  is the density of the Lotion;  $W$  is the weight, in g, of Lotion taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurandrenolide Ointment

» Flurandrenolide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ( $C_{24}H_{33}FO_6$ ).

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** <11>—  
USP Flurandrenolide RS

**Identification**—It meets the requirements for *Thin-layer chromatographic identification test* under *Flurandrenolide Cream*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** <755>: meets the requirements.

**Assay**—Proceed as directed in the *Assay* under *Flurandrenolide Cream*, using Ointment in place of Cream.

## Flurandrenolide Tape

» Flurandrenolide Tape is a nonporous, pliable, adhesive-type tape having Flurandrenolide impregnated in the adhesive material, the adhesive material on one side being transported on a removable, protective slit-paper liner. Flurandrenolide Tape contains not less than 80.0 percent and not more than 125.0 percent of the labeled amount of  $C_{24}H_{33}FO_6$ .

**Packaging and storage**—Preserve at controlled room temperature.

**USP Reference standards** <11>—  
USP Flurandrenolide RS

**Identification**—Extract a portion of Tape, equivalent to about 200  $\mu$ g of flurandrenolide, as directed for the *Assay preparation* in the *Assay*. Omit the addition of the internal standard, and evaporate the chloroform extracts on a steam bath under a stream of nitrogen to about 3 mL. Transfer the chloroform solution to a 10-mL flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of equal volumes of chloroform and methanol, warming gently to effect solution: it meets

the requirements of the *Thin-Layer Chromatographic Identification Test* (201), 5  $\mu$ L each of the test solution and Standard solution being applied, and the solvent mixture consisting of ethyl acetate and ether (70:30).

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### Assay—

*Methanolic sodium chloride, Mobile phase, and Chromatographic system*—Prepare as directed in the Assay under *Flurandrenolide Cream*.

*Flurandrenolide standard solution*—Dissolve about 7 mg of USP Flurandrenolide RS, accurately weighed, in 50 mL of methanol in a 100-mL volumetric flask. Dilute with methanol to volume, and mix.

*Internal standard solution*—Dissolve about 4 mg of Testosterone in 50 mL of methanol in a 100-mL volumetric flask. Dilute with methanol to volume, and mix.

*Standard preparation*—Pipet 3.0 mL of *Flurandrenolide standard solution* and 4.0 mL of *Internal standard solution* into a 10-mL volumetric flask. Dilute with water to volume, and mix.

*Assay preparation*—Accurately measure and cut a portion of Tape, equivalent to about 200  $\mu$ g of flurandrenolide. Remove and discard the paper liner from the portion of Tape. Touch the flattened end of a glass rod to the adhesive side of the Tape, and carefully transfer the tape to the bottom of a 600-mL beaker containing 15 mL of anhydrous methanol, taking care that the adhesive side of the tape does not adhere to the wall of the beaker. Remove the glass rod from the tape, and wash it with 5 mL of anhydrous methanol, adding the wash to the beaker. Place the beaker containing the Tape and the methanol in an ultrasonic bath for 3 minutes, rotating the beaker in such manner that the methanol is in contact with all portions of the Tape. Transfer the methanol to a 250-mL separator. Extract the Tape, using sonication, with two additional 20-mL portions of anhydrous methanol, adding each portion to the separator. To the combined methanol extract add 15 mL of sodium chloride solution (1 in 10) and 50 mL of hexane, and shake vigorously. Allow the phases to separate, and drain the lower aqueous phase into a second separator containing 15 mL of hexane. Shake vigorously, allow the phases to separate, and drain the lower phase into a third 250-mL separator containing 100 mL of water. Serially extract the hexane phases remaining in the two separators with one 25-mL portion of *Methanolic sodium chloride*, adding the extract to the third separator. Discard the hexane phases. Extract the combined aqueous phases with four 25-mL portions of chloroform. Filter each chloroform extract through 10 g of anhydrous sodium sulfate into a 125-mL conical beaker. Rinse the sodium sulfate with water-washed chloroform, and add the wash to the beaker. Add 4.0 mL of *Internal standard solution* to the beaker. Evaporate the solution on a steam bath under a stream of nitrogen to near dryness. Remove the beaker from the steam bath and evaporate the remaining solution with the aid of nitrogen to dryness. Add 10 mL of *Mobile solvent* to the beaker, and place it in an ultrasonic bath to dissolve the residue. Pass the solution through a suitable porosity filter having a 0.5- $\mu$ m filter with a prefilter above the membrane filter to prevent clogging.

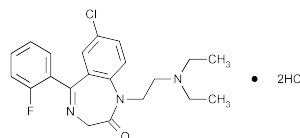
*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2 for testosterone and 1.0 for flurandrenolide.

Calculate the quantity, in  $\mu$ g, of  $C_{24}H_{33}FO_6$  in the portion of Tape taken by the formula:

$$10C(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Flurandrenolide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the Assay preparation and the *Standard preparation*, respectively.

## Flurazepam Hydrochloride



$C_{21}H_{23}ClFN_3O \cdot 2HCl$  460.80

2H-1,4-Benzodiazepin-2-one, 7-chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-, dihydrochloride.

7-Chloro-1-[2-(diethylamino)ethyl]-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride [1172-18-5].

» Flurazepam Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $C_{21}H_{23}ClFN_3O \cdot 2HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Flurazepam Hydrochloride RS

USP Flurazepam Related Compound C RS

5-Chloro-2-(2-diethylaminoethyl(amino)-2'-fluorobenzophenone hydrochloride.

$C_{19}H_{22}ClFN_2O \cdot HCl$  385.31

USP Flurazepam Related Compound F RS

7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one.

$C_{15}H_{10}ClFN_2O$  288.71

#### Identification—

**A: Infrared Absorption** (197K). [NOTE—Do not grind excessively, as decomposition may occur.]

**B: Ultraviolet Absorption** (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* sulfuric acid in methanol (1 in 36).

Absorptivities at 239 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**C:** Prepare a solution of it in methanol containing 3 mg per mL. Apply 10  $\mu$ L of this solution and 10  $\mu$ L of a methanol solution of USP Flurazepam Hydrochloride RS containing 3 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate and ammonium hydroxide (200:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot in the chromatogram of the test solution corresponds to that obtained from the solution of the Reference Standard.

**D:** To 2 mL of a solution (1 in 20) add 1 mL of 2 N nitric acid: the solution responds to the tests for *Chloride* (191), 5 drops of silver nitrate TS being used.

**Water, Method Ia** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Limit of fluoride ion**—[NOTE—Use plasticware throughout the procedure.]

**pH 5.25 Buffer**—Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix: the pH of this solution is between 5.0 and 5.5.

**Standard stock solution**—Transfer 221 mg of sodium fluoride to a 100-mL volumetric flask, add about 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ions. Store in a tightly closed, plastic container.

**Standard preparations**—Dilute portions of the *Standard stock solution* quantitatively and stepwise with *pH 5.25 Buffer* to obtain 100-mL solutions having concentrations of 1, 3, 5, and 10 µg per mL.

**Test preparation**—Transfer 1.0 g of Flurazepam Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *pH 5.25 Buffer* to volume, and mix.

**Procedure**—Concomitantly measure the potential (see *Titrimetry* (541)), in mV, of the *Standard preparations* and of the *Test preparation*, with a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved calomel-fluoride specific-ion electrode system. [NOTE—When taking measurements, immerse the electrodes in the solution, which has been transferred to a 150-mL beaker containing a polytetrafluoroethylene-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and record the potential. Rinse and dry the electrodes between measurements, being careful to avoid damaging the crystal of the specific-ion electrode.] Plot the logarithm of the fluoride-ion concentrations, in µg per mL, of the *Standard preparations* versus the potential in mV. From the measured potential of the *Test preparation* and the standard curve determine the concentration, in µg per mL, of fluoride ion in the *Test preparation*: not more than 0.05% is found.

#### Related compounds—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 1% ammonium acetate (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve accurately weighed quantities of USP Flurazepam Related Compound C RS and USP Flurazepam Related Compound F RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 2 µg per mL for each component. [NOTE—Prepare fresh daily.]

**Test solution**—Transfer about 50 mg of Flurazepam Hydrochloride, accurately weighed, to a 25-mL volumetric flask, add methanol to volume, and mix. [NOTE—Prepare this solution just prior to use.]

**System suitability solution**—Dissolve accurately weighed quantities of USP Flurazepam Hydrochloride RS and 2-amino-5-chlorobenzophenone in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration in each mL of about 150 µg of USP Flurazepam Hydrochloride RS and about 60 µg of 2-amino-5-chlorobenzophenone.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between 2-amino-5-chlorobenzophenone and flurazepam is not less than 2. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percentage, of flurazepam related compound C and flurazepam related compound F in the portion of Flurazepam Hydrochloride taken by the formula:

$$2.5(C/W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Flurazepam Related Compound C RS or USP Flurazepam Related Compound F RS in the *Standard solution*; *W* is the weight, in mg, of Flurazepam Hydrochloride taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for the related compounds obtained from the *Test solution* and the *Standard solution*, respectively. The limit is not more than 0.1% of flurazepam related compound C and not more than 0.1% of flurazepam related compound F.

**Assay**—Transfer about 600 mg of Flurazepam Hydrochloride, accurately weighed, to a 250-mL beaker, dissolve in 80 mL of glacial acetic acid, and add 20 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.04 mg of C<sub>21</sub>H<sub>23</sub>ClFN<sub>3</sub>O · 2HCl.

## Flurazepam Hydrochloride Capsules

» Flurazepam Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurazepam hydrochloride (C<sub>21</sub>H<sub>23</sub>ClFN<sub>3</sub>O · 2HCl).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Flurazepam Hydrochloride RS

#### Identification—

**A:** Dissolve a portion of Capsules, equivalent to about 30 mg of flurazepam hydrochloride, in 10 mL of methanol, filter, and proceed as directed for *Identification* test C under *Flurazepam Hydrochloride*.

**B:** The retention time of the major peak for flurazepam hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** Capsules meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

#### Dissolution (711)—

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 20 minutes.

**Standard solution**—Prepare a solution of USP Flurazepam Hydrochloride RS having an accurately known concentration similar to the concentration of the solution under test. Pipet

5 mL of this solution to a 10-mL volumetric flask, and dilute with 1% ammonium acetate to volume.

**Test solution**—Pipet 5 mL of a filtered portion of the solution under test into a 10-mL volumetric flask, and dilute with 1% ammonium acetate to volume.

**Procedure**—Determine the amount of  $C_{21}H_{23}ClFN_3O \cdot 2HCl$  dissolved, using the *Chromatographic system* as set forth in the *Related compounds* test under *Flurazepam Hydrochloride*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{23}ClFN_3O \cdot 2HCl$  is dissolved in 20 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase, System suitability solution, and Chromatographic system**—Prepare as directed for *Related compounds* under *Flurazepam Hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flurazepam Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL. Prepare fresh daily.

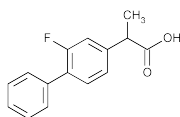
**Assay preparation**—[NOTE—Prepare fresh daily.] Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the Capsule contents, equivalent to about 30 mg of flurazepam hydrochloride, to a 200-mL volumetric flask. Add 40 mL of methanol, and shake by mechanical means for 10 minutes. Add 10 mL of 1% ammonium acetate, and shake by mechanical means for 5 minutes. Dilute with *Mobile phase* to volume, mix and sonicate for 2 minutes, and filter.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flurazepam hydrochloride ( $C_{21}H_{23}ClFN_3O \cdot 2HCl$ ) in the portion of Capsules taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flurazepam Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurbiprofen



$C_{15}H_{13}FO_2$  244.26  
[1,1'-Biphenyl]-4-acetic acid, 2-fluoro- $\alpha$ -methyl-, ( $\pm$ )-.  
( $\pm$ )-2-(2-Fluoro-4-biphenyl)propionic acid [5104-49-4].

» Flurbiprofen contains not less than 99.0 percent and not more than 100.5 percent of  $C_{15}H_{13}FO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Flurbiprofen RS  
USP Flurbiprofen Related Compound A RS  
2-(4-Biphenyl)propionic acid.  
 $C_{15}H_{14}O_2$  226.28

#### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

**Solution:** 10  $\mu$ g per mL.

**Medium:** 0.1 N sodium hydroxide.

Absorbance maximum at 247 nm is about 0.8.

**Melting range** (741): between 114° and 117°.

**Loss on drying** (731)—Dry it in vacuum at 60° to constant weight: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.001%.

#### Related compounds—

**Diluent**—Prepare a mixture of water and acetonitrile (11:9).

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (12:7:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Flurbiprofen Related Compound A RS in *Diluent* to obtain a solution having a concentration of about 50  $\mu$ g per mL.

**System suitability solution**—Pipet 2.0 mL of *Standard stock solution* into a 10-mL volumetric flask, add about 20 mg of USP Flurbiprofen RS, dilute with *Diluent* to volume, and mix.

**Standard solution**—Transfer 2.0 mL of *Standard stock solution* to a 10.0-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Test solution**—Prepare a solution of Flurbiprofen in *Diluent* containing 2.0 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  15-cm column that contains 4- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for flurbiprofen related compound A and 1.0 for flurbiprofen; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of flurbiprofen related compound A in the portion of Flurbiprofen taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in  $\mu$ g per mL, of USP Flurbiprofen Related Compound A RS in the *Standard solution*;  $C_U$  is the concentration, in  $\mu$ g per mL, of Flurbiprofen in the *Test solution*; and  $r_U$  and  $r_S$  are the peak responses for flurbiprofen related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% of flurbiprofen related compound A is found. Calculate the percentage of each impurity in the portion of Flurbiprofen taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the sum of the responses of all the peaks obtained from the *Test solution*: the sum of all impurities is not more than 1.0%.



**Assay**—Dissolve about 0.5 g of Flurbiprofen, accurately weighed, in 100 mL of alcohol, previously neutralized with 0.1 N sodium hydroxide VS to the phenolphthalein endpoint, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to the first appearance of a faint pink color that persists for not less than 30 seconds. Each mL of 0.1 N sodium hydroxide is equivalent to 24.43 mg of  $C_{15}H_{13}FO_2$ .

## Flurbiprofen Tablets

» Flurbiprofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurbiprofen ( $C_{15}H_{13}FO_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Flurbiprofen RS

**Identification**—

**A:** Place a number of Tablets, equivalent to about 100 mg of flurbiprofen, in a flask, add 10 mL of 0.1 N hydrochloric acid, and sonicate until the Tablets disintegrate. Extract with two 15-mL portions of ether, combining the ether extracts in a flask containing about 1 g of anhydrous sodium sulfate. Decant the ether, and evaporate to dryness: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Flurbiprofen RS.

**B:** The retention time of the flurbiprofen peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

**pH 7.2 Phosphate buffer**—Dissolve 245 g of monobasic potassium phosphate and 50 g of sodium hydroxide in water to make 2000 mL of solution. Dilute 333 mL of this stock solution to 6000 mL with water. If necessary, adjust with 5 N sodium hydroxide or with phosphoric acid to a pH of  $7.20 \pm 0.05$ .

**Medium:** pH 7.2 phosphate buffer; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{15}H_{13}FO_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 247 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Flurbiprofen RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{15}H_{13}FO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements, the following procedure being used where the test for *Content Uniformity* is required.

**Procedure for content uniformity**—Proceed as directed in the *Assay*, except in preparing the *Assay preparation* to use 1 Tablet and to use 10.0 mL of *Internal standard solution* for each 25 mg of flurbiprofen in the Tablet, based on the labeled amount.

**Assay**—

**Mobile phase**—Dissolve 1.4 g of monobasic sodium phosphate in 570 mL of water, add 430 mL of acetonitrile, and adjust with phosphoric acid to a pH of 3.0. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve acetophenone in *Mobile phase* to obtain a solution having a concentration of about 0.8  $\mu$ L per mL.

**Standard preparation**—Accurately weigh about 30 mg of USP Flurbiprofen RS. Add 10.0 mL of *Internal standard solution*, and swirl to dissolve. This stock solution contains about 3 mg of USP Flurbiprofen RS per mL. Dilute a portion of this stock solution with 20 volumes of *Mobile phase*, and mix.

**Assay preparation**—Place 3 Tablets in a stoppered container. Based on the labeled amount, in mg, of flurbiprofen in each Tablet, add 25.0 mL of *Internal standard solution* for each 75 mg of flurbiprofen in the 3 Tablets. Shake by mechanical means for about 15 minutes, and centrifuge. Dilute a portion of this solution with 20 volumes of *Mobile phase*, and mix.

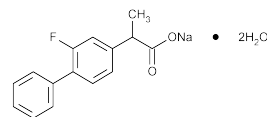
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  25-cm column containing packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative retention times are about 0.4 for acetophenone and 1.0 for flurbiprofen; the resolution, *R*, between the acetophenone and flurbiprofen is not less than 8; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flurbiprofen ( $C_{15}H_{13}FO_2$ ) in the portion of Tablets taken by the formula:

$$(WV / 10)(R_U / R_S)$$

in which *W* is the quantity, in mg, of USP Flurbiprofen RS used to prepare the *Standard preparation*; *V* is the volume, in mL, of *Internal standard solution* used to prepare the *Assay preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the flurbiprofen peak response to the acetophenone peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flurbiprofen Sodium



$C_{15}H_{12}FNaO_2 \cdot 2H_2O$  302.27

[1,1'-Biphenyl]-4-acetic acid, 2-fluoro- $\alpha$ -methyl, sodium salt dihydrate, ( $\pm$ )-.

Sodium ( $\pm$ )-2-(2-fluoro-4-biphenyl)propionate dihydrate. Anhydrous 266.25

» Flurbiprofen Sodium contains not less than 97.0 percent and not more than 103.0 percent of  $C_{15}H_{12}FNaO_2 \cdot 2H_2O$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Flurbiprofen RS

USP Flurbiprofen Sodium RS

USP Flurbiprofen Related Compound A RS  
2-(4-Biphenyl)propionic acid.

$C_{15}H_{14}O_2$  226.28

**Identification—****A:** Infrared Absorption (197M)—

Test specimen: previously dried.

**B:** Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: pH 6.0 buffer consisting of 2.42 g of monobasic sodium phosphate and 0.66 g of dibasic sodium phosphate dissolved in water to make 1000 mL.

Absorptivities at 246 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** The residue obtained by igniting it meets the requirements of the tests for Sodium (191).**Specific rotation** (781S): between  $-0.45^\circ$  and  $+0.45^\circ$ .

Test solution: 50 mg per mL, in methanol.

**Loss on drying** (731)—Dry about 0.3 g of it in vacuum at a pressure not exceeding 1 mm of mercury over phosphorus pentoxide in a suitable drying tube at  $60^\circ$  for 18 hours: it loses not less than 11.3% and not more than 12.5% of its weight.**Heavy metals, Method II** (231): 0.001%.**Limit of flurbiprofen related compound A—***Diluent, Mobile phase, and System suitability preparation—*Proceed as directed in the Assay.*Standard solution—*Use *Standard flurbiprofen related compound A preparation*, prepared as directed in the Assay.*Test solution—*Use the *Assay preparation*.*Chromatographic system—*Proceed as directed in the Assay, except to chromatograph the *Standard solution* instead of the *Standard preparation*.*Procedure—*Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of flurbiprofen related compound A in the portion of Flurbiprofen Sodium taken by the formula:

$$200(C/W)(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Flurbiprofen Related Compound A RS in the *Standard solution*; W is the weight, in mg, of the portion of Flurbiprofen Sodium taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the peak areas for flurbiprofen related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.5% is found.**Assay—***Diluent—*Mix 500 mL of methanol and 250 mL of water.*Mobile phase—*Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (50:49:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).*Standard flurbiprofen related compound A preparation—*Dissolve an accurately weighed quantity of USP Flurbiprofen Related Compound A RS in methanol to obtain a stock solution having a known concentration of about 150 µg per mL. Transfer 1.0 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.*Standard preparation—*Dissolve an accurately weighed quantity of USP Flurbiprofen RS in methanol to obtain a stock solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.*System suitability preparation—*Transfer 5 mL of the stock solution used to prepare the *Standard preparation* and 2 mL of the stock solution used to prepare the *Standard flurbiprofen related compound A preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.*Assay preparation—*Transfer about 100 mg of Flurbiprofen Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 25-cm column that contains 10-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between flurbiprofen related compound A and flurbiprofen is not less than 1.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.0%.*Procedure—*Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of  $C_{15}H_{12}FNaO_2 \cdot 2H_2O$  in the portion of Flurbiprofen Sodium taken by the formula:

$$200(302.27/244.27)(C/W)(r_U / r_S)$$

in which 302.27 and 244.27 are the molecular weights of flurbiprofen sodium dihydrate and anhydrous flurbiprofen, respectively; C is the concentration, in µg per mL, of USP Flurbiprofen RS in the *Standard preparation*; W is the weight, in mg, of the portion of Flurbiprofen Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the flurbiprofen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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**Flurbiprofen Sodium Ophthalmic Solution**


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» Flurbiprofen Sodium Ophthalmic Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurbiprofen sodium ( $C_{15}H_{12}FNaO_2 \cdot 2H_2O$ ).**Packaging and storage—**Preserve in tight containers.**USP Reference standards** (11)—

USP Flurbiprofen RS

USP Flurbiprofen Related Compound A RS

2-(4-Biphenyl)propionic acid.

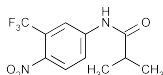
 $C_{15}H_{14}O_2$  226.28**Identification—**The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.**pH** (791): between 6.0 and 7.0.**Antimicrobial effectiveness** (51): meets the requirements.**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.**Assay—***Diluent, Mobile phase, Standard flurbiprofen related compound A preparation, Standard preparation, and System suitability preparation—*Proceed as directed in the Assay under *Flurbiprofen Sodium*.*Assay preparation—*Use the undiluted Ophthalmic Solution.*Chromatographic system—*Proceed as directed in the Assay under *Flurbiprofen Sodium*, using a 4.0-mm × 5-cm guard column that contains 5-µm packing L1.*Procedure—*Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the areas for the major peaks. Calculate the quantity of flurbiprofen sodium ( $C_{15}H_{12}FNaO_2 \cdot 2H_2O$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$(302.27 / 244.27)C(r_U / r_S)$$

in which 302.27 and 244.27 are the molecular weights of flurbiprofen sodium dihydrate and anhydrous flurbiprofen, respectively;  $C$  is the concentration, in mg per mL, of USP Flurbiprofen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flutamide



$C_{11}H_{11}F_3N_2O_3$  276.21

Propanamide, 2-methyl-N-[4-nitro-3-(trifluoromethyl)-phenyl]-.

$\alpha, \alpha, \alpha$ -Trifluoro-2-methyl-4'-nitro-*m*-propionotoluidide [13311-84-7].

» Flutamide contains not less than 98.0 percent and not more than 101.0 percent of  $C_{11}H_{11}F_3N_2O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Flutamide RS

USP *o*-Flutamide RS

2-Methyl-N-[6-nitro-3-(trifluoromethyl)phenyl]propanamide

$C_{11}H_{11}F_3N_2O_3$  276.22

### Identification—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 110° and 114°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 10 ppm.

### Related compounds—

*Mobile phase* and *System suitability solution*—Prepare as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Detector sensitivity solution*—Transfer 1.0 mL of the *Standard solution* into a 100-mL volumetric flask, dilute with a mixture of water and acetonitrile (4:1) to volume, and mix. Dilute quantitatively, and stepwise if necessary, with a mixture of water and acetonitrile (4:1) to obtain a solution having a known concentration of about 0.1 µg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 25 ± 5°. The flow rate is about 1.0 mL per minute. Chromatograph the *System*

*suitability solution*, and record the peak area responses as directed for *Procedure*: the relative retention times are about 1.4 for *o*-flutamide and 1.0 for flutamide; and the resolution,  $R$ , between flutamide and *o*-flutamide is not less than 6.0. Chromatograph the *Detector sensitivity solution*, and record the peak area responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10.0% for flutamide.

*Procedure*—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak area responses. Calculate the percentage of each impurity in the portion of Flutamide taken by the formula:

$$100(1/F)(r_i / r_s)$$

in which  $F$  is the relative response factor of the impurities according to the table below;  $r_i$  is the peak area response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: the impurities meet the requirements tabulated below.

Compound name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
4-Nitro-3-trifluoromethyl-acetanilide	0.42	1.06	0.2
4-Nitro-3-trifluoromethyl-aniline	0.45	1.10	0.15
3-trifluoromethylaniline	0.63	1.10	0.2
4-Nitro-3-trifluoromethyl-propionanilide	0.66	1.02	0.3
3-trifluoromethyl-isobutyranilide	0.80	1.95	0.2
<i>o</i> -Flutamide	1.40	1.78	0.2
Flutamide	1.0	1.0	—
Unknown	—	1.0	0.05
Total unknown	—	—	0.1
Total impurities	—	—	0.4

### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Flutamide RS in 50 mL of acetonitrile, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.2 mg per mL.

*System suitability solution*—Transfer about 50 mg of USP *o*-Flutamide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 10 mL of acetonitrile, dilute with water to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Standard preparation* into a 100-mL volumetric flask, dilute with a mixture of water and acetonitrile (4:1) to volume, and mix.

*Assay preparation*—Transfer about 50 mg of Flutamide, previously dried and accurately weighed, to a 250-mL volumetric flask. Add 50 mL of acetonitrile, and sonicate until the Flutamide dissolves. Add 150 mL of water, mix, and allow to warm to room temperature. Dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 25 ± 5°. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak area responses as directed for *Procedure*: the relative retention times are about 1.4 for *o*-flutamide and 1.0 for flutamide; and the resolution,  $R$ , between flutamide and *o*-flutamide is not less than

6.0. Chromatograph the *Standard preparation*, and record the peak area responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{11}H_{11}F_3N_2O_3$  in the portion of Flutamide taken by the formula:

$$250C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flutamide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Flutamide Capsules

» Flutamide Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of flutamide ( $C_{11}H_{11}F_3N_2O_3$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—  
USP Flutamide RS

### Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

B: Remove the contents of 20 Capsules, and grind the contents to a fine powder. Dissolve a portion of the powder in a mixture of chloroform and methanol (5:1) to obtain a solution containing 3 mg of flutamide per mL. The test solution so obtained responds to the *Thin-Layer Chromatographic Identification Test* (201), a mixture of chloroform and ethyl acetate (3:1) being used as the developing solvent and 20  $\mu$ L each of the test solution and the Standard solution being applied to the thin-layer chromatographic plate.

### Dissolution (711)—

*Medium*: 2% sodium lauryl sulfate solution; 1000 mL.

*Apparatus 2*: 75 rpm.

*Time*: 60 minutes.

*Procedure*—Determine the amount of  $C_{11}H_{11}F_3N_2O_3$  dissolved from UV absorbances at the wavelength of maximum absorbances at the wavelength of maximum absorbance at about 306 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Flutamide RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{11}H_{11}F_3N_2O_3$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Chromatographic purity—

*Mobile phase*—Prepare as directed in the *Assay*.

*Standard solution*—Prepare as directed in the *Assay* for *Standard preparation*.

*Test solution*—Use the *Assay preparation*.

*Detector sensitivity solution*—Transfer an accurately measured volume of the *Standard solution* into a volumetric flask, and dilute quantitatively, and stepwise if necessary, with a mixture of water and acetonitrile (4:1) to obtain a solution having a known concentration of about 0.2 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The column temperature is maintained at  $25 \pm 5^\circ$ . The flow rate is about 1.0 mL per minute. Chromatograph the *Detector sensitivity solution*, and record the peak area responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10% for flutamide.

*Procedure*—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak area responses. Calculate the percentage of each impurity in the portion of Capsules taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak area response for each impurity, excluding those where peak area responses are less than those obtained from the *Detector sensitivity solution*; and  $r_S$  is the sum of the responses of all the peaks: not more than 0.2% for any impurity having a relative retention time of about 0.45 is found; not more than 0.1% of any other impurity is found; and not more than 0.3% of total impurities is found.

### Assay—

*Diluent*—Prepare a mixture of acetonitrile and water (1:1).

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Flutamide RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 20.0 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume to obtain a final concentration of 0.2 mg per mL.

*Assay preparation*—Remove the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to 125 mg of flutamide, into a 250-mL volumetric flask. Add 180 mL of *Diluent*. Shake the flask for 15 minutes. Dilute with *Diluent* to volume, and mix. Allow the insoluble material to settle. Transfer 20.0 mL of supernatant into a 50-mL volumetric flask, dilute with water to volume, mix, and pass through a polytetrafluoroethylene membrane filter having a 0.45- $\mu$ m porosity.

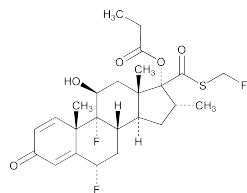
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The column temperature is maintained at  $25 \pm 5^\circ$ . The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak area response as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area response for the flutamide peak. Calculate the quantity, in mg, of flutamide ( $C_{11}H_{11}F_3N_2O_3$ ) in the portion of Capsules taken by the formula:

$$625C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flutamide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluticasone Propionate



$C_{25}H_{31}F_3O_5S$  500.57  
 Androsta-1,4-diene-17-carbothioic acid, 6,9-difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxopropoxy)-, (6 $\alpha$ ,11 $\beta$ ,16 $\alpha$ ,17 $\alpha$ )-S-(fluoromethyl) ester;  
 S-Fluoromethyl 6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate [80474-14-2].

### DEFINITION

Fluticasone Propionate contains NLT 98.0% and NMT 101.0% of  $C_{25}H_{31}F_3O_5S$ , calculated on the anhydrous, solvent-free basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197M)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 1.15 g/L of monobasic ammonium phosphate, adjusted with phosphoric acid to a pH of  $3.5 \pm 0.05$

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (50:15:35)

**System suitability solution:** 0.05 mg/mL of USP Fluticasone Propionate Resolution Mixture RS in *Mobile phase*

**Standard solution:** 0.04 mg/mL of USP Fluticasone Propionate RS in *Mobile phase*

**Sample solution:** 0.04 mg/mL of Fluticasone Propionate in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for fluticasone propionate and fluticasone propionate related compound D are about 1.0 and 1.10, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fluticasone propionate ( $C_{25}H_{31}F_3O_5S$ ) in the portion of Fluticasone Propionate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of fluticasone propionate from the *Sample solution*

$r_S$  = peak response of fluticasone propionate from the *Standard solution*

$C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fluticasone Propionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.0% on the anhydrous, solvent-free basis

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A:** 0.5 mL of phosphoric acid in 1000 mL of acetonitrile

**Solution B:** 0.5 mL of phosphoric acid in 1000 mL of methanol

**Solution C:** 0.5 mL of phosphoric acid in 1000 mL of water

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	42	3	55
40	53	3	44
60	87	3	10
70	87	3	10
75	42	3	55

**System suitability solution:** Dissolve 2.0 mg of USP Fluticasone Propionate System Suitability Mixture RS in 5 mL of *Solution A* using sonication. Add 5 mL of *Solution C*.

**Sample solution:** 2.0 mg/mL prepared as follows: Dissolve 2.0 mg of Fluticasone Propionate in 5 mL of *Solution A* using sonication. Add 5 mL of *Solution C*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 0.6 between fluticasone propionate related compound B and fluticasone propionate related compound C; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Fluticasone Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all the peaks

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluticasone propionate related compound A	0.5	0.2
Fluticasone propionate related compound B	0.75	0.1
Fluticasone propionate related compound C	0.8	0.1
Fluticasone propionate related compound D	0.95	0.3
Fluticasone propionate	1.0	—
Fluticasone propionate related compound E	1.3	0.3
Any individual unspecified impurity	—	0.1
Total impurities*	—	1.0

\* Include all impurity peaks greater than or equal to 0.05%.

#### • LIMIT OF ACETONE

**Internal standard solution:** 0.05% (v/v) solution of tetrahydrofuran in dimethylformamide

**Standard solution:** 0.05% (v/v) of acetone in *Internal standard solution*

**Sample solution:** 50 mg/mL of Fluticasone Propionate in the *Internal standard solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 25-m; 2-μm film of phase G16

**Carrier gas:** Nitrogen or helium

**Temperature**

**Detector:** 250°

**Splitless injector:** 150°

**Column:** See Table 3.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	0	60	3.5
60	30	180	3.0

**Flow rate:** 5.5 mL/min

**Injection size:** 0.1 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 5.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetone (w/w) in the portion of Fluticasone Propionate taken:

$$\text{Result} = (R_U/R_S) \times D \times (C_S/C_U)$$

$R_U$  = peak response ratio of acetone to tetrahydrofuran from the *Sample solution*

$R_S$  = peak response ratio of acetone to tetrahydrofuran from the *Standard solution*

$D$  = density of acetone at 20° (g/mL)

$C_S$  = concentration of acetone in the *Standard solution* (%v/v)

$C_U$  = concentration of Fluticasone Propionate in the *Sample solution* (g/mL)

Acceptance criteria: NMT 1.0% (w/w)

#### SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* (781S): +32° to +36° at 20°, calculated on the anhydrous, solvent-free basis  
**Sample solution:** 0.5% (w/v) of Fluticasone Propionate in dichloromethane (0.5 g in 100 mL)

- **WATER DETERMINATION**, *Method I* (921): NMT 0.2% (w/w)

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at a temperature not exceeding 30°.

- **LABELING:** Fluticasone Propionate in the form of microcrystals is so labeled.

- **USP REFERENCE STANDARDS** (11)

USP Fluticasone Propionate RS

USP Fluticasone Propionate Resolution Mixture RS

USP Fluticasone Propionate System Suitability Mixture RS

It is a mixture of USP Fluticasone Propionate RS and fluticasone propionate related compounds B, C, and D.

*Fluticasone propionate related compound A:* 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrost-1,4-diene-17β-carbonylsulfenic acid.

*Fluticasone propionate related compound B:* 6α,9α-Difluoro-11β-hydroxy-16α-methyl-2',3,4'-trioxo-17α-spiro(androst-1,4-diene-17,5'-(1,3)oxathiolane).

*Fluticasone propionate related compound C:* S-Fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-androst-1,4-diene-17β-carbothioate.

*Fluticasone propionate related compound D:* S-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy-androst-1,4-diene-17β-carbothioate.

*Fluticasone propionate related compound E:* 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxo-androst-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio) carbonyl-11β-hydroxy-16α-methyl-3-oxo-androst-1,4-dien-17α-yl ester.

## Fluticasone Propionate Cream

#### DEFINITION

Fluticasone Propionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate (C<sub>25</sub>H<sub>31</sub>F<sub>3</sub>O<sub>5</sub>S).

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.4 mg/mL of USP Fluticasone Propionate RS in acetonitrile

**Test solution:** Transfer a quantity of Cream, equivalent to 1000 μg of fluticasone propionate, to a 125-mL separatory funnel. Add 25 mL of acetonitrile and 25 mL of hexane to the separatory funnel. Stopper and shake the funnel until the Cream is completely dispersed. Shake the separatory funnel for an additional 3 min, and allow the phases to separate. Filter the lower layer through a 20-mL syringe containing a cotton plug into a 50-mL volumetric flask. Repeat the extraction with one 7-mL aliquot of acetonitrile, and filter the lower layer into the volumetric flask. Wash the cotton plug with 2 mL of acetonitrile, and collect the washings into the volumetric flask. Dilute the sample extract with acetonitrile to volume. Transfer 12 mL of the sample extract to a glass tube suitable for evaporation, and evaporate to dryness at about 40°. Dissolve the residue in 0.6 mL of acetonitrile. [NOTE—The *Test solution* may be cloudy because of the presence of undissolved excipients.]

**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Adsorbent:** 0.2-mm layer of chromatographic silica gel mixture on a high-performance thin-layer chromatographic plate, 5- $\mu$ m particle size**Application volume:** 40  $\mu$ L**Developing solvent system:** Dichloromethane, ethyl acetate, and glacial acetic acid (30:8:1)**Analysis****Samples:** *Standard solution* and *Test solution*

Separately apply the *Standard solution* and the *Test solution* to the plate. On the same plate, apply 20  $\mu$ L of the *Standard solution*, allow the application to dry, and apply 20  $\mu$ L of the *Test solution* on top of the dried 20- $\mu$ L *Standard solution* spot. Allow each of the applications to dry thoroughly. Place the plate in a tank equilibrated with the developing solvent, and allow the developing solvent to travel about 8 cm from the point of application. Remove the plate and allow to air-dry. Examine the plate under ultraviolet light at 254 nm.

**Acceptance criteria:** The  $R_f$  value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*. [NOTE—If the excipients in the Cream interfere with the appearance of the principal spot obtained for the *Test solution*, use the *Standard solution* and the *Test solution* overspot to confirm identity.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

[NOTE—Protect the *Standard solution* and the *Sample solution* from direct light by using a light-protective volumetric flask and autosampler vials.]

**Buffer:** 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of  $3.50 \pm 0.03$ .

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (46:14:40)

**Diluent:** Alcohol and water (65:35)

**System suitability stock solution:** 0.5 mg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in methanol. [NOTE—USP Fluticasone Propionate Nasal Spray Resolution Mixture RS is a mixture of fluticasone propionate and fluticasone propionate related compound D.]

**System suitability solution:** 10  $\mu$ g/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent* from *System suitability stock solution*

**Standard stock solution:** 0.5 mg/mL USP Fluticasone Propionate RS. Dissolve the standard first in a volume of methanol, equivalent to 80% of the final volume, and dilute with water to volume.

**Standard solution:** 20  $\mu$ g/mL USP Fluticasone Propionate RS in *Diluent*, from *Standard stock solution*

**Sample solution:** Equivalent to 20  $\mu$ g/mL of fluticasone propionate. Transfer a quantity of Cream, equivalent to 1000  $\mu$ g of fluticasone propionate, to a 125-mL separatory funnel. Add to the separatory funnel 25 mL of *Diluent*. Stopper and shake vigorously until the Cream is completely dispersed. Add 25 mL of hexane, shake for an additional 3 min, and allow the phases to separate. Filter the lower layer through a 20-mL syringe containing a cotton plug into a 50-mL volumetric flask. Repeat the extraction with one 5-mL and one 2-mL aliquot of *Diluent*, filtering the lower layers into the volumetric flask. Wash the cotton plug with 1 mL of *Diluent*, and collect the washings into the volumetric flask. Dilute the sample with *Diluent* to volume.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** 240 nm**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1**Column temperature:** 50°**Flow rate:** 1.5 mL/min**Injection size:** 20  $\mu$ L**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements**

**Resolution:** NLT 1.4 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution* (calculated using the width of the peak at 10% of the height)

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fluticasone propionate ( $C_{25}H_{31}F_3O_5$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of fluticasone propionate from the *Sample solution*

$r_S$  = peak response of fluticasone propionate from the *Standard solution*

$C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of fluticasone propionate in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** <755>: Meets the requirements

**SPECIFIC TESTS**

- **pH** <791>: 4.5–6.5

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 10 cfu/g.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, protected from light. Store between 2° and 30°.

- **USP REFERENCE STANDARDS** <11>

USP Fluticasone Propionate RS

USP Fluticasone Propionate Nasal Spray Resolution Mixture RS

This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:  
*Fluticasone propionate:* 5-Fluoromethyl 6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.

*Fluticasone propionate related compound D:* 5-Methyl-6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.

**Fluticasone Propionate Nasal Spray****DEFINITION**

Fluticasone Propionate Nasal Spray is a white, opaque suspension of Fluticasone Propionate in water. It is supplied in a form suitable for nasal administration. It contains NLT 95.0% and NMT 115.0% of the labeled amount of fluticasone propionate ( $C_{25}H_{31}F_3O_5$ ).

**IDENTIFICATION****A. INFRARED ABSORPTION** (197M)

**Sample:** Transfer 30 g of Nasal Spray equally into two 50-mL centrifuge tubes. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. To one tube add 10 mL of methanol. Shake to disperse the residue, and transfer to the other tube. Shake the other tube for 1 min. Centrifuge at 3500 rpm for 10 min. Decant the supernatant into an agate mortar. Evaporate the methanol either by carefully blowing dry with compressed air or nitrogen, or by allowing the methanol to evaporate naturally. If using an air or nitrogen line, use a suitable in-line filter to avoid contamination. Allow the residue to dry overnight in a desiccator over silica gel.

**Acceptance criteria:** Meets the requirements

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Diluent:** Acetonitrile and 0.001 M hydrochloric acid (60:40)

**Buffer:** 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of  $3.5 \pm 0.05$ .

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (50:15:35)

**System suitability solution:** 50 µg/mL of USP Phenylethyl Alcohol RS and 10 µg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent*

**Standard solution:** 10 µg/mL of USP Fluticasone Propionate RS in *Diluent*

**Sample solution:** Nominally 10 µg/mL prepared as follows: Transfer an amount of the Nasal Spray containing 0.5 mg of fluticasone propionate to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate the flask for 10 min. Dilute with *Diluent* to volume, and shake. Allow to stand for 10 min until the supernatant is a clear solution. Inject the clear supernatant into the chromatograph.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 239 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

[NOTE—Record the peak areas at 210 nm for 5 min, then change the wavelength to 239 nm and record the peak areas.]

**Samples:** *System suitability solution* and *Standard solution*

Record the chromatogram at 210 nm for 5 min and then change the wavelength to 239 nm.

[NOTE—The relative retention times for phenylethyl alcohol, fluticasone propionate, and fluticasone propionate related compound D are about 0.42, 1.0, and 1.10, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the label claim of fluticasone propionate ( $C_{25}H_{31}F_3O_5S$ ) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of Fluticasone Propionate in the *Sample solution* (µg/mL)

**Acceptance criteria:** 95.0%–115.0%

**OTHER COMPONENTS****CONTENT OF PHENYLETHYL ALCOHOL**

**Diluent, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 0.05 mg/mL of USP Phenylethyl Alcohol RS in *Diluent*

**Sample solution:** Transfer 1.0 g of the Nasal Spray to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate for 10 min until supernatant is clear. Use the clear supernatant for analysis.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in mg/g, of phenylethyl alcohol in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/W)$$

$r_U$  = peak response of phenylethyl alcohol from the *Sample solution*

$r_S$  = peak response of phenylethyl alcohol from the *Standard solution*

$C_S$  = concentration of USP Phenylethyl Alcohol RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Sample solution*, 50 mL

$W$  = weight of the Nasal Spray in the *Sample solution* (g)

**Acceptance criteria**

For 50 sprays: 1.75–2.63 mg/g

For 120 sprays: 1.88–2.63 mg/g

**CONTENT OF BENZALKONIUM CHLORIDE**

**Buffer:** 250 mg/mL of citric acid. Adjust the solution with 2 N sodium hydroxide to a pH of  $3.5 \pm 0.05$ .

**Standard solution:** 200 µg/mL [0.02% (w/w)] of USP Benzalkonium Chloride RS in water

**Docusate sodium titrant:** Dissolve 0.22 g of USP Docusate Sodium RS in 100 mL of warm water. Dilute with water to make 1000 mL.

**Eosin Y indicator:** Dissolve 25 mg of eosin Y in 50 mL of acetone. Add 450 mL of chloroform and  $5.0 \pm 0.5$  g of citric acid. Shake thoroughly until no discoloration occurs. Filter the mixture to remove any undissolved citric acid. Store in an amber bottle.

**Titer value of docusate sodium:** Pipet 10 mL of the *Standard solution* into a 250-mL glass-stoppered flask containing 40 mL of water, 5 mL of *Eosin Y indicator*, and 2 mL of *Buffer*. Insert the stopper into the flask, and shake, releasing any build-up of pressure. Titrate with *Docusate sodium titrant* with vigorous shaking to a point when pink coloration is discharged from the chloroform layer. Perform a blank determination, substituting 10 mL of water for the *Standard solution*, and make any necessary correction (see *Titrimetry* (541)).

Calculate the titer value ( $T$ ) of the *Docusate sodium titrant*, in µg/mL, of benzalkonium chloride:

$$\text{Result} = C_S \times (V_S/V_0)$$

$C_S$  = concentration of USP Benzalkonium Chloride RS in the *Standard solution* (µg/mL)



$V_s$  = volume of the *Standard solution*, 10 mL  
 $V_D$  = volume of the *Docusate sodium titrant* used in the titration of the *Standard solution* (mL)

**Analysis**

**Sample:** 10 g of Nasal Spray

Transfer the *Sample* into a 250-mL glass-stoppered flask containing 40 mL of water, 5 mL of *Eosin Y indicator*, and 2 mL of *Buffer*. Repeat the procedure as given above for the *Standard solution*. To clarify the endpoint, place the flask in an ultrasonic bath for 1–2 min to separate the chloroform layer from the aqueous phase. Perform a blank determination.

Calculate the concentration of benzalkonium chloride, in  $\mu\text{g/g}$ , in the portion of Nasal Spray taken:

$$\text{Result} = TV/W$$

$T$  = titer value of the *Docusate sodium titrant*  
 $V$  = volume of the *Docusate sodium titrant* used in the titration of the Nasal Spray (mL)  
 $W$  = weight of the portion of Nasal Spray taken (g)

**Acceptance criteria:** 140–220  $\mu\text{g/g}$

**PERFORMANCE TESTS**

• **DELIVERED DOSE UNIFORMITY** (within container)

**Diluent:** Acetonitrile and 0.001 M hydrochloric acid (60:40)

**Buffer:** 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of  $3.5 \pm 0.05$ .

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (50:15:35)

**System suitability solution:** 5  $\mu\text{g/mL}$  of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent*

**Standard solution:** 4  $\mu\text{g/mL}$  of USP Fluticasone Propionate RS in *Diluent*

**Sample solution:** Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations (1 dose) into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations. Discharge actuations 3–48 (50-spray pack) or 3–118 (120-spray pack) to waste. Wipe the bottle clean, and collect the last two actuations (49 and 50 or 119 and 120) in a second 25-mL volumetric flask. Turn the flask to the upright position immediately after each actuation, and insert the stopper into the flask. Add 20 mL of *Diluent* to each flask, and shake well for 10 min to disperse the suspension. Dilute with *Diluent* to volume, and mix thoroughly. Allow the flask to stand until the excipients have settled. Inject the clear supernatant. Repeat this procedure with 4 additional bottles.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for fluticasone propionate and fluticasone propionate related compound D are about 1.0 and 1.10, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the label claim of fluticasone propionate ( $\text{C}_{25}\text{H}_{31}\text{F}_3\text{O}_5\text{S}$ ) in each dose:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** The mean dose delivered from 10 doses is within 85%–115% of the label claim. NMT 1 dose is outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim. If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 10 bottles. The mean dose delivered from 30 doses is within 85%–115% of the label claim. NMT 3 doses are outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim.

• **DELIVERED DOSE UNIFORMITY** (within batch)

**Diluent, Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability:** Prepare as directed in the test for *Delivered Dose Uniformity* (within container).

**Sample solution:** Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations (1 dose). Repeat this procedure with 9 additional bottles.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the label claim of fluticasone propionate ( $\text{C}_{25}\text{H}_{31}\text{F}_3\text{O}_5\text{S}$ ) in each dose:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** The mean dose delivered from 10 doses is within 85%–115% of the label claim. NMT 1 dose is outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim. If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 20 bottles. The mean dose delivered from 2 actuations in the beginning of the 30 bottles (30 doses) is within 85%–115% of the label claim. NMT 3 doses are outside 80%–120% of the label claim. No doses are outside 75%–125% of the label claim.

**IMPURITIES**

• **ORGANIC IMPURITIES**

**Diluent:** Acetonitrile and 0.001 M hydrochloric acid (60:40)

**Solution A:** Methanol and acetonitrile (77:23)

**Buffer:** 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of  $3.4 \pm 0.1$ .

**Mobile phase:** *Solution A* and *Buffer* (60:40)

**System suitability solution:** 0.1 mg/mL of USP Fluticasone Propionate Related Compounds Mixture RS and 0.5 mg/mL of USP Phenylethyl Alcohol RS in *Diluent*

**Control solution:** 0.5 mg/mL of USP Phenylethyl Alcohol RS and 0.08 mg/mL of USP Benzalkonium Chloride RS in a mixture of *Diluent* and water (4:1)

**Sample solution:** 0.2 g/mL of Nasal Spray in *Diluent*. Shake the flask vigorously to dissolve. Pass through a filter of 0.5- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.5 between fluticasone propionate related compound F and phenylethyl alcohol. NLT 2 between fluticasone propionate related compound D and fluticasone propionate

#### Analysis

**Samples:** *System suitability solution*, *Control solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*, excluding the peaks from the *Control solution*

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
S-Fluoromethyl 17 $\alpha$ -acetyloxy-6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxoandrosta-1,4-diene-17 $\beta$ -carbothioate; S-Fluoromethyl 9 $\alpha$ -fluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3,6-dioxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate	0.7	0.3
Fluticasone propionate related compound D	1.1	0.3
6 $\alpha$ ,9 $\alpha$ -Difluoro-11 $\beta$ ,17 $\alpha$ -dihydroxy-16 $\alpha$ -methyl-3-oxoandrosta-1,4-diene-17 $\beta$ -carboxylic acid-6 $\alpha$ ,9 $\alpha$ -difluoro-17 $\beta$ -(fluoromethylthio)carbonyl-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxoandrosta-1,4-dien-17 $\alpha$ -yl ester	2.1	0.3
Any unspecified related impurities	—	0.2
Total impurities	—	1.5

#### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the tests for absence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 25 cfu/mL, and the total combined molds and yeasts count does not exceed 25 cfu/mL.

- pH** <791>: 5.0–7.0

#### PARTICLE SIZE

**Analysis:** Remove the pump system after shaking the test bottle to ensure product uniformity. Transfer 1 drop of the Nasal Spray onto a clean microscope slide. Examine 10 random fields of view on the slide using 400 $\times$  magnification. Drug substance particles are irregular in

shape, whereas the excipient particles are elongated and angular. Record the number of individual drug substance particles that are less than 5  $\mu$ m in diameter, greater than 5  $\mu$ m but less than 15  $\mu$ m in diameter, and greater than 15  $\mu$ m in diameter. Calculate the percentage of each category by number.

**Acceptance criteria:** See *Table 2*.

**Table 2**

Particle Size	Acceptance Criteria
<5 $\mu$ m	NLT 98%
>5 $\mu$ m – <15 $\mu$ m	NMT 1.8%
>15 $\mu$ m	NMT 0.2%

#### FOREIGN PARTICULATES

**Analysis:** Shake the required number of bottles to ensure uniformity. Remove the pump system carefully to minimize contamination of the sample. Collect about 100 g of Nasal Spray, and pass it through a wetted 250- $\mu$ m screen. Rinse each bottle with a portion of water equal to twice the volume of each bottle. Pass the rinse through the 250- $\mu$ m screen. Visually observe the screen and filtrate for any foreign particulates. Also examine the screen under a microscope using transmitted light.

**Acceptance criteria:** No foreign particulates greater than 250  $\mu$ m are visible.

- DROPLET SIZE DISTRIBUTION:** Determine using a validated laser diffraction technique and method that measures the volume diameters of droplets. In preparation shake the bottle to ensure product uniformity. Prime the pump by discharging a predetermined (refer to the product label) number of actuations to waste, at which time a fine mist should appear. Measure and record the average of three sprays per bottle, and report the mean diameter defining the population of particles, by volume, below 10% ( $D_{10}$ ), 50% ( $D_{50}$ ), and 90% ( $D_{90}$ ) of five bottles.
- SPRAY PATTERN:** Determine the spray pattern using a validated method that measures the size of the pattern. Gently shake the bottle to ensure product uniformity. Prime the pump by discharging a predetermined number (refer to the product label) of actuations to waste, at which time a fine mist should appear. Measure and record the average of two sprays per bottle and report the longest axis (x axis), and the ratio of longest to shortest axes (x/y ratio) of two bottles.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 30°.

#### USP REFERENCE STANDARDS <11>

USP Benzalkonium Chloride RS

USP Docusate Sodium RS

USP Fluticasone Propionate RS

USP Fluticasone Propionate Nasal Spray Resolution Mixture RS

This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:

*Fluticasone propionate:* S-Fluoromethyl 6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.

*Fluticasone propionate related compound D:* S-Methyl-6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.

USP Fluticasone Propionate Related Compounds Mixture RS

This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compounds D and F, and the chemical names for all are given below:

*Fluticasone propionate:* S-Fluoromethyl 6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.

Fluticasone propionate related compound D: *S*-Methyl-6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxy-androsta-1,4-diene-17 $\beta$ -carbothioate.  
 Fluticasone propionate related compound F: 6 $\alpha$ ,9 $\alpha$ -Difluoro-11 $\beta$ ,17 $\alpha$ -dihydroxy-16 $\alpha$ -methyl-3-oxoandrosta-1,4-diene-17 $\beta$ -carboxylic acid.

USP Phenylethyl Alcohol RS

## Fluticasone Propionate Ointment

### DEFINITION

Fluticasone Propionate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate (C<sub>25</sub>H<sub>31</sub>F<sub>3</sub>O<sub>5</sub>S).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 0.17 mg/mL of USP Fluticasone Propionate RS in acetonitrile

**Test solution:** Transfer a quantity of Ointment, equivalent to 100  $\mu$ g of fluticasone propionate, to a 125-mL separatory funnel. Add 50 mL of hexane and 10 mL of acetonitrile to the separatory funnel. Stopper and shake the funnel until the Ointment is completely dispersed. Shake the separatory funnel for an additional min, and allow the phases to separate. Filter the lower layer through a 10-mL syringe containing a cotton plug into a 25-mL volumetric flask. Repeat the extraction with one 10-mL aliquot of acetonitrile, and filter the lower layer into the volumetric flask. Wash the cotton plug with 2 mL of acetonitrile, and collect the washings into the volumetric flask. Dilute the sample extract with acetonitrile to volume. Transfer one-half of the sample extract to a glass tube suitable for evaporation, and evaporate to dryness at about 40°. Transfer the remainder of the sample extract into the same tube, and continue evaporating to dryness. Dissolve the residue in 0.6 mL of acetonitrile. [NOTE—The *Test solution* may be cloudy because of the presence of undissolved excipients.]

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.2-mm layer of chromatographic silica gel mixture on a high-performance thin-layer chromatographic plate, 5- $\mu$ m particle size

**Application volume:** 40  $\mu$ L

**Developing solvent system:** Dichloromethane, ethyl acetate, and glacial acetic acid (30:8:1)

#### Analysis

**Samples:** *Standard solution* and *Test solution*  
 Separately apply the *Standard solution* and the *Test solution* to the plate. On the same plate, apply 20  $\mu$ L of the *Standard solution*, allow the application to dry, and apply 20  $\mu$ L of the *Test solution* on top of the dried 20- $\mu$ L *Standard solution* spot. Allow each of the applications to dry thoroughly. Place the plate in a tank equilibrated with the developing solvent, and allow the developing solvent to travel 8 cm from the point of application. Remove the plate and allow to air-dry. Examine the plate under ultraviolet light at 254 nm.

**Acceptance criteria:** The *R<sub>F</sub>* value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*. [NOTE—If the excipients in the Ointment interfere with the appearance of the principal spot obtained for the *Test solution*, use the *Standard solution* and the *Test solution* overspot to confirm identity.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

[NOTE—Protect the *Standard solution* and the *Sample solution* from direct light by using a light-protective volumetric flask and autosampler vials.]

**Buffer:** 1.20 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.50  $\pm$  0.03.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (46:14:40)

**Diluent:** Methanol and water (80:20)

**System suitability stock solution:** 0.5 mg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in methanol. [NOTE—USP Fluticasone Propionate Nasal Spray Resolution Mixture RS is a mixture of fluticasone propionate and fluticasone propionate related compound D.]

**System suitability solution:** 5  $\mu$ g/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent* from *System suitability stock solution*

**Standard stock solution:** 0.25 mg/mL USP Fluticasone Propionate RS. Dissolve the standard first in a volume of methanol, equivalent to 80% of the final volume, and dilute with water to volume.

**Standard solution:** 4  $\mu$ g/mL USP Fluticasone Propionate RS in *Diluent*, from *Standard stock solution*

**Sample solution:** Equivalent to 4  $\mu$ g/mL of fluticasone propionate. Transfer a quantity of Ointment, equivalent to about 100  $\mu$ g of fluticasone propionate, to a 125-mL separatory funnel. Add to the separatory funnel 45 mL of hexane previously heated to about 60° in a water bath. [NOTE—Hexane is highly flammable.] Stopper and shake vigorously until the Ointment is completely dispersed. Wash the stopper and neck of the separatory funnel with 5 mL of hexane heated to 60°, and allow the funnel to cool to room temperature. Transfer 10 mL of *Diluent* into the separatory funnel, and shake the contents for 1 min. Allow the phases to separate, and filter the lower aqueous layer via a cotton wool plug, previously washed with *Diluent*, into a 25-mL volumetric flask. Repeat the extraction with two 5-mL aliquots of *Diluent*, filtering the lower layers into the volumetric flask. Wash the cotton plug with 2 mL of *Diluent*, and collect the washings into the volumetric flask. Dilute the sample with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** 240 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 50°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.4 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution* (calculated using the width of the peak at 10% of the height)

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of fluticasone propionate (C<sub>25</sub>H<sub>31</sub>F<sub>3</sub>O<sub>5</sub>S) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of fluticasone propionate from the *Sample solution*

*r<sub>S</sub>* = peak response of fluticasone propionate from the *Standard solution*

- $C_S$  = concentration of USP Fluticasone Propionate RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of fluticasone propionate in the *Sample solution* ( $\mu\text{g/mL}$ )  
**Acceptance criteria:** 90.0%–110.0%

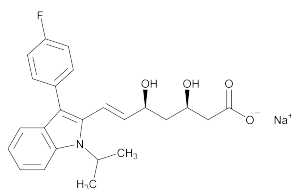
**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 10 cfu/g.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, protected from light. Store between 2° and 30°.
- USP REFERENCE STANDARDS** (11)  
 USP Fluticasone Propionate RS  
 USP Fluticasone Propionate Nasal Spray Resolution Mixture RS  
 This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:  
*Fluticasone propionate:* 5-Fluoromethyl 6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxyandrosta-1,4-diene-17 $\beta$ -carbothioate.  
*Fluticasone propionate related compound D:* 5-Methyl-6 $\alpha$ ,9 $\alpha$ -difluoro-11 $\beta$ -hydroxy-16 $\alpha$ -methyl-3-oxo-17 $\alpha$ -propionyloxy-androsta-1,4-diene-17 $\beta$ -carbothioate.

## Fluvastatin Sodium



$\text{C}_{24}\text{H}_{25}\text{FNNaO}_4$  433.45  
 6-Heptenoic acid, 7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-, monosodium salt,  $[R^*, S^*-(E)]-(\pm)-$ ;  
 Sodium  $(\pm)-(3R^*, 5S^*, 6E)$ -7-[3-(p-fluorophenyl)-1-isopropylindol-2-yl]-3,5-dihydroxy-6-heptenoate [93957-55-2].

**DEFINITION**

Fluvastatin Sodium contains NLT 98.0% and NMT 102.0% of  $\text{C}_{24}\text{H}_{25}\text{FNNaO}_4$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197K)  
 [NOTE—If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the sample specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a steam bath, protecting the solutions from light, and dry at 105° for 30 min. Repeat the test on the residues.]
- B. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements for the pyroantimonate precipitation test

**ASSAY****• PROCEDURE**

**Solution A:** Add 20 mL of 25% aqueous tetramethylammonium hydroxide solution to 880 mL of water. Adjust with about 2.3 mL of phosphoric acid to a pH of  $7.2 \pm 0.2$ . Add 100 mL of a mixture of methanol and acetonitrile (3:2).

**Solution B:** Add 20 mL of 25% aqueous tetramethylammonium hydroxide solution and 80 mL of water to 900 mL of a mixture of methanol and acetonitrile (3:2). Adjust with about 2.3 mL of phosphoric acid to a pH of  $7.2 \pm 0.2$ .

**Mobile phase:** See the gradient table.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
6	60	40
20	18	82
20.1	60	40
25.1	60	40

**System suitability solution:** 0.5 mg/mL of fluvastatin sodium from USP Fluvastatin for System Suitability RS, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

**Standard solution:** 0.5 mg/mL of USP Fluvastatin Sodium RS, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

**Sample solution:** 0.5 mg/mL of Fluvastatin Sodium, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 305 nm

**Column:** 4.6-mm  $\times$  5-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 35°

**Flow rate:** 3 mL/min

**Injection size:** 20  $\mu\text{L}$

[NOTE—Adjust the start time of the gradient step and the equilibration time for each instrument.]

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for fluvastatin and fluvastatin *anti*-isomer are about 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.6 between fluvastatin *anti*-isomer and fluvastatin, *System suitability solution*

**Column efficiency:** NLT 700 theoretical plates for the fluvastatin peak, *System suitability solution*

**Tailing factor:** NMT 3.0 for the fluvastatin peak, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $\text{C}_{24}\text{H}_{25}\text{FNNaO}_4$  in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **HEAVY METALS**, *Method II* (231): 20 ppm

**Organic Impurities****PROCEDURE**

[NOTE— Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

**Solution A, Solution B, Mobile phase, Standard solution, and Sample solution:** Proceed as directed in the *Assay*.

**System suitability solution A:** Prepare as directed for the *System suitability solution* in the *Assay*.

**System suitability solution B:** 0.1 mg/mL of USP Fluvastatin Related Compound B RS in a mixture of methanol and acetonitrile (3:2). Transfer about 0.5 mL of this solution to a 10-mL volumetric flask, and dilute with *System suitability solution A* to volume. [NOTE—*System suitability solution B* is stable for up to 6 months if stored in a refrigerator.]

**Chromatographic system:** Proceed as directed in the *Assay*, except to use a liquid chromatograph equipped with either a programmable variable-wavelength detector or two separate detectors capable of monitoring at 305 and 365 nm.

**System suitability**

**Samples:** *Standard solution* and *System suitability solution B*

[NOTE— Record the peak responses at 305 nm as directed for *Analysis*. Identify the peaks corresponding to fluvastatin, fluvastatin *anti*-isomer, and fluvastatin *t*-butyl ester.]

**Suitability requirements**

**Resolution:** NLT 1.6 between fluvastatin *anti*-isomer and fluvastatin, *System suitability solution B*

**Column efficiency:** NLT 700 theoretical plates for the fluvastatin peak, *System suitability solution B*

**Tailing factor:** NMT 3.0 for the fluvastatin peak, *System suitability solution B*

**Relative standard deviation:** NMT 1.0% at 305 nm, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms at 305 and 365 nm, identify the impurities listed in *Impurity Table 1*, and measure the peak responses. [NOTE— 3-Hydroxy-5-keto fluvastatin is monitored using a wavelength of 365 nm, and all other compounds are monitored at 305 nm.]

Calculate the percentage of each impurity, except for 3-hydroxy-5-keto fluvastatin, in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response at 305 nm of each impurity from the *Sample solution*

$r_S$  = peak response at 305 nm of fluvastatin from the *Standard solution*

$C_S$  = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

Calculate the percentage of 3-hydroxy-5-keto fluvastatin in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response at 365 nm of 3-hydroxy-5-keto fluvastatin from the *Sample solution*

$r_S$  = peak response at 365 nm of fluvastatin from the *Standard solution*

$C_S$  = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria**

**Individual Impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluvastatin <i>N</i> -ethyl analog <sup>a</sup>	0.7	1.2	0.1
Fluvastatin <i>anti</i> -isomer <sup>b</sup>	1.2	1.0	0.8
3-Hydroxy-5-keto fluvastatin <sup>c</sup>	1.5	27.0 <sup>d</sup>	0.1
Fluvastatin hydroxydiene <sup>e</sup>	2.0	0.92	0.1
Fluvastatin short-chain aldehyde <sup>f</sup>	3.0	1.4	0.1
Fluvastatin <i>t</i> -butyl ester (fluvastatin related compound B) <sup>g</sup>	3.4	0.94	0.2
Any other individual impurity	—	1.0	0.1

<sup>a</sup> [*R*\*, *S*\*-*E*]-(+)-7-[3-(4-Fluorophenyl)-1-ethyl-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid monosodium salt.

<sup>b</sup> [*R*\*, *R*\*-*E*]-(+)-7-[3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid monosodium salt.

<sup>c</sup> *E*-(+)-7-[3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indol-2-yl]-3-hydroxy-5-oxo-6-heptenoic acid monosodium salt.

<sup>d</sup> At 365 nm.

<sup>e</sup> [*E*,*E*]-(+)-7-[3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indol-2-yl]-3-hydroxy-4,6-heptadienoic acid monosodium salt.

<sup>f</sup> 3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indole-2-carboxaldehyde.

<sup>g</sup> [*R*\*, *S*\*-*E*]-(+)-7-[3-(4-Fluorophenyl)-1-methylethyl-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid 1,1-dimethylethyl ester.

**SPECIFIC TESTS**

- **pH** (791): 8.0–10.0, in a 10-mg/mL solution. Perform the test immediately after preparation.
- **WATER DETERMINATION**, *Method I* (921): NMT 4.0%; if labeled as a hydrated form, NMT 12.0%. [NOTE—For this monograph, the term “hydrated form” refers to several known forms of fluvastatin sodium that are not stoichiometric hydrates.]

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, protected from moisture. Store at a temperature not exceeding 40°.
- **LABELING:** Where it is a hydrated form, the label so indicates.
- **USP REFERENCE STANDARDS** (11)
  - USP Fluvastatin Sodium RS
  - USP Fluvastatin Related Compound B RS
  - Fluvastatin *t*-butyl ester.
  - USP Fluvastatin for System Suitability RS
  - Fluvastatin sodium, containing 1% to 2% of the fluvastatin sodium *anti*-isomer.

## Fluvastatin Capsules

» Fluvastatin Capsules contain an amount of Fluvastatin Sodium equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluvastatin ( $C_{24}H_{26}FNO_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, protected from moisture and from light. Store in a cool place or at controlled room temperature.

### USP Reference standards (11)—

USP Fluvastatin Sodium RS

USP Fluvastatin for System Suitability RS

Fluvastatin sodium and fluvastatin sodium anti-isomer.

[NOTE—USP Fluvastatin for System Suitability RS contains 1% to 2% of the fluvastatin sodium anti-isomer.]

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium*: water; 500 mL.

*Apparatus 2*: 50 rpm, sinkers not used.

*Time*: 30 minutes.

Determine the amount of  $C_{24}H_{26}FNO_4$  dissolved by employing the following method.

**Buffer solution**—Dissolve about 1.534 g of monobasic ammonium phosphate in about 800 mL of water, and adjust with phosphoric acid or ammonium hydroxide to a pH of 3.5.

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (7:3).

**Standard solution**—[NOTE—A volume of methanol, not exceeding 2% of the final volume of solution, may be used to aid in dissolving the USP Reference Standard.] Dissolve an accurately weighed quantity of USP Fluvastatin Sodium RS in *Medium* to obtain a solution having a known concentration of fluvastatin corresponding to that obtained when 1 Capsule is dissolved in 500 mL of solvent.

**Test solution**—Withdraw 20-mL portions of liquid under test from each vessel, and pass through a suitable filter, discarding the first 2 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector, a suitable 7- $\mu$ m guard column that contains packing L1, and a 4.6-mm  $\times$  10-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the fluvastatin peaks. From the measured peak areas, calculate the quantity of  $C_{24}H_{26}FNO_4$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{24}H_{26}FNO_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements, the *Chromatographic system* being prepared as directed for the *Dissolution* test.

**Chromatographic purity**—[NOTE—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

**pH 7.2 Buffer, Methanol-acetonitrile mixture, Solution A, Solution B, Mobile phase, and Diluent**—Proceed as directed in the *Assay*.

**System suitability solution**—Use the *System suitability preparation*, prepared as directed in the *Assay*.

**Standard solution**—Use the *Standard preparation*, prepared as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*, prepared as directed in the *Assay*.

**Chromatographic system**—Proceed as directed in the *Assay*, except use the liquid chromatograph equipped with either a programmable variable wavelength detector or two separate detectors capable of monitoring at 305 nm and at 365 nm. Chromatograph the *System suitability solution*, and record the peak responses at 305 nm as directed for *Procedure*. Identify the peaks corresponding to fluvastatin and fluvastatin anti-isomer. The resolution, *R*, between fluvastatin anti-isomer and fluvastatin is not less than 1.4; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms at 305 nm and 365 nm, identify the impurities listed in *Table 1*, and measure the peak responses. [NOTE—3-Hydroxy-5-keto fluvastatin is monitored using a wavelength of Fosinopril Sodium and 365 nm, and all other compounds are monitored at 305 nm.] Calculate the percentage of each impurity, except for 3-hydroxy-5-keto fluvastatin, in the portion of Capsules taken by the formula:

$$100(1/F)(411.48/433.45)(C_S / C_T)(r_{i(305)} / r_{S(305)})$$

in which *F* is the relative response factor as listed in *Table 1* [NOTE—Use *F* equal to 1.0 for unknown impurities]; 411.48 and 433.45 are the molecular weights of fluvastatin and fluvastatin sodium, respectively; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Fluvastatin Sodium RS in the *Standard solution*; *C<sub>T</sub>* is the concentration, in mg per mL, of fluvastatin in the *Test solution*, based on the label claim; *r<sub>i(305)</sub>* is the peak response at 305 nm for each impurity obtained from the *Test solution*; and *r<sub>S(305)</sub>* is the peak response at 305 nm for the fluvastatin peak, obtained from the *Standard solution*.

Calculate the percentage of 3-hydroxy-5-keto fluvastatin in the portion of Capsules taken by the formula:

$$100(1/F)(411.48/433.45)(C_S / C_T)(r_{i(365)} / r_{S(365)})$$

in which *F*, *C<sub>S</sub>*, and *C<sub>T</sub>* are as defined above; *r<sub>i(365)</sub>* is the peak response at 365 nm for 3-hydroxy-5-keto fluvastatin, obtained from the *Test solution*; and *r<sub>S(365)</sub>* is the peak response at 365 nm for the fluvastatin peak, obtained from the *Standard solution*. In addition to not exceeding the limits for each impurity in *Table 1*, not more than 0.5% of any unknown impurity is found; not more than 1.5% of total unknown impurities is found; and not more than 4.0% of total impurities is found.

Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Fluvastatin anti-isomer	1.2	1.0	1.5
3-Hydroxy-5-keto fluvastatin	1.6	27.0*	1.0
Fluvastatin hydroxydiene	2.2	0.92	1.0
Fluvastatin short-chain aldehyde	3.2	1.4	0.5

\*At 365 nm

### Assay—

**pH 7.2 Buffer**—Prepare a solution containing 40 mL of 25% aqueous tetramethylammonium hydroxide in 1 L of water, and adjust with approximately 4.5 mL of phosphoric acid to a pH of  $7.2 \pm 0.2$ .

**Methanol–acetonitrile mixture**—Prepare a mixture of methanol and acetonitrile (3:2).

**Solution A**—Prepare a filtered and degassed mixture of pH 7.2 Buffer and Methanol–acetonitrile mixture (87.5:12.5).

**Solution B**—Prepare a filtered and degassed mixture of Methanol–acetonitrile mixture and pH 7.2 Buffer (87.5:12.5).

**Mobile phase**—Use variable mixtures of Solution A and Solution B as directed for Chromatographic system.

**Diluent**—Prepare a mixture of pH 7.2 Buffer and Methanol–acetonitrile mixture (54:46).

**System suitability preparation**—Dissolve an accurately weighed quantity of USP Fluvastatin for System Suitability RS in Diluent to obtain a solution having a known concentration of about 0.42 mg of fluvastatin sodium per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluvastatin Sodium RS in Diluent to obtain a solution having a known concentration of about 0.42 mg of fluvastatin sodium per mL.

**Assay stock preparation**—Transfer the contents and the empty shells of 10 Capsules to a 200-mL glass-stoppered flask. Add 100.0 mL of methanol, and stir with a magnetic or mechanical stirrer for 45 minutes. Centrifuge a portion of this solution at 4000 rpm for 20 minutes.

**Assay preparation**—Quantitatively transfer an amount of the Assay stock preparation, containing 20.0 mg of fluvastatin based on the label claim, to a 50-mL volumetric flask, and dilute with Diluent to volume.

**Chromatographic system** (see Chromatography <621>)—The liquid chromatograph is equipped with a 305-nm detector and a 4.6-mm × 5-cm column that contains 5-μm packing L1. The flow rate is about 2.0 mL per minute. The chromatograph is programmed as follows. [NOTE—Adjust the start time of the gradient step and the equilibration time for each instrument.]

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6	54	46	isocratic
6–17	54→17	46→83	linear gradient
17–20	17	83	isocratic
20–20.1	17→54	83→46	linear gradient
20.1–26.1	54	46	equilibration

Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure: the retention time of the fluvastatin peak is about 5.4 minutes; the relative retention times are about 1.0 for fluvastatin and 1.2 for fluvastatin anti-isomer; the resolution,  $R_s$ , between fluvastatin anti-isomer and fluvastatin is not less than 1.4; and the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—If the retention time of the fluvastatin peak exceeds 5.7 minutes, adjust the isocratic step accordingly, so that both the fluvastatin peak and the anti-isomer peak elute within the isocratic region.]

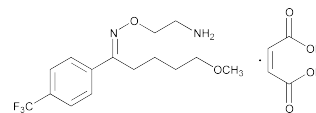
**Procedure**—Separately inject equal volumes (about 25 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the fluvastatin peaks. Calculate the quantity, in mg, of fluvastatin ( $C_{24}H_{26}FNO_4$ ) in the portion of Capsules taken by the formula:

$$5000(411.48/433.45)(C/V)(r_U/r_S)$$

in which 411.48 and 433.45 are the molecular weights of fluvastatin and fluvastatin sodium, respectively;  $V$  is the volume, in mL, of the Assay stock preparation taken to prepare the Assay preparation;  $C$  is the concentration, in mg per mL, of USP Fluvastatin Sodium RS in the Standard preparation; and  $r_U$  and  $r_S$  are the fluvastatin peak responses obtained

from the Assay preparation and the Standard preparation, respectively.

## Fluvoxamine Maleate



$C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$  434.41

1-Pentanone, 5-methoxy-1-[4-(trifluoromethyl)phenyl]-, O-(2-aminoethyl)oxime, (E)-, (Z)-2-butenedioate (1:1).

5-Methoxy-4'-(trifluoromethyl)valerophenone (E)-O-(2-aminoethyl)oxime, maleate (1:1) [61718-82-9].

» Fluvoxamine Maleate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

**USP Reference standards** <11>—

USP Fluvoxamine Maleate RS

**Identification**—

**A: Infrared Absorption** <197K>.

**B:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Melting temperature** <741>: between 121° and 123°.

**Loss on drying** <731>—Dry it in vacuum at 80° for 2 hours; it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method II** <231>: 0.001%.

**Related compounds**—

**Buffer solution, Mobile phase, Resolution solution, and Chromatographic system**—Proceed as directed in the Assay.

**Identification solution**—Dissolve a quantity of maleic acid in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.35 mg per mL.

**Standard solution**—Use the Standard preparation, prepared as directed in the Assay.

**Test solution**—Use the Assay stock preparation, prepared as directed in the Assay.

**Procedure**—Separately inject equal volumes (about 20 μL) of the Standard solution, the Test solution, and the Identification solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of impurities in the portion of Fluvoxamine Maleate taken by the formula:

$$5000(C/W)F(r_i/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Fluvoxamine Maleate RS in the Standard solution;  $W$  is the weight, in mg, of Fluvoxamine Maleate used to prepare the Test solution;  $F$  is the response factor of each impurity as given in Table 1;  $r_i$  is the individual peak area of each impurity in the Test solution; and  $r_S$  is the peak area of fluvoxamine maleate in the Standard solution. The limits of impurities are specified in Table 1. [NOTE—Disregard any peak due to maleic acid or the reagent blank.]

Table 1

Compound Name	Relative Retention Time	Response Factor	Limit (%)
Maleic acid	about 0.19	—	—
5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-(E)-O-[2-[(2-succinyl)amino]ethyl]oxime	about 0.50	1.0	0.3
5-Methoxy-4'-(trifluoromethyl)valerophenone(E)-O-(2-aminoethyl)aminoethyl oxime maleate	about 0.67	1.4	0.2
Z-isomer	about 0.79	1.0	0.5
Fluvoxamine	1.0	—	—
4'-(Trifluoromethyl)valerophenone(E)-O-2-(2-aminoethyl)aminoethyl oxime maleate	about 1.18	1.0	0.2
(E)-O-2-(2-Aminoethyl)-4-(trifluoromethyl)- $\alpha$ -phenyl-acetophenone oxime maleate	about 1.74	1.0	0.2
4'-(Trifluoromethyl)valerophenone(E)-O-(2-aminoethyl) oxime maleate	about 2.00	1.0	0.2
5-Methoxy-4'-(trifluoromethyl)valerophenone oxime	about 3.45	0.6	0.2
5-Methoxy-4'-(trifluoromethyl)valerophenone ketone	about 4.2	0.3	0.2
Unknown impurities	—	1.0	0.1
Total	—	—	1.5

**Assay—**

**Buffer solution**—Dissolve about 5 g of 1-pentanesulfonic acid sodium salt and 0.7 g of monobasic potassium phosphate in 620 mL of water. Adjust with phosphoric acid to a pH of  $3.00 \pm 0.05$ .

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (62:38). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Transfer about 6 mg of Fluvoxamine Maleate to a 50-mL volumetric flask. Heat the sample at 120° for 10 minutes. Cool down to room temperature, and add 3.0 mL of 0.1 N hydrochloric acid. Heat the solution in a water bath for 10 minutes. Cool down to room temperature, add 50 mg of Fluvoxamine Maleate, and dissolve in 25 mL of *Mobile phase*. Dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fluvoxamine Maleate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay stock preparation**—Transfer an accurately weighed quantity of about 50 mg of Fluvoxamine Maleate to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer 5.0 mL of the *Assay stock preparation* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 234-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1.7 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the Z-isomer and fluvoxamine maleate is not less than 3.0 and not less than 5.0 between 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-(E)-O-[2-[(2-succinyl)amino]ethyl]oxime and the Z-isomer. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Table 1*.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the fluvoxamine maleate peaks. Calculate the quantity, in mg, of  $C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$  in the

portion of Fluvoxamine Maleate taken by the formula:

$$1000C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Fluvoxamine Maleate RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fluvoxamine Maleate Tablets

**DEFINITION**

Fluvoxamine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fluvoxamine maleate ( $C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 5 g of 1-pentanesulfonic acid sodium salt and 0.7 g of monobasic potassium phosphate in 620 mL of water. Adjust with phosphoric acid to a pH of  $3.00 \pm 0.05$ .

**Mobile phase:** Acetonitrile and *Buffer* (19:31)

**System suitability solution:** 6 mg of fluvoxamine maleate to a 50-mL volumetric flask. Heat the sample at 120° for 10 min. Cool down to room temperature, and add 3.0 mL of 0.1 N hydrochloric acid. Heat the solution in a water bath for 10 min. Cool down to room temperature, add 50 mg of fluvoxamine maleate, and dissolve in 25 mL of *Mobile phase*. Dilute with *Mobile phase* to volume.

**Standard solution:** 0.05 mg/mL of USP Fluvoxamine Maleate RS in *Mobile phase*

**Sample stock solution:** Transfer a weighed quantity of finely powdered Tablets from NLT 20 Tablets to a suitable volumetric flask to obtain a nominal concentration of 1 mg/mL of fluvoxamine maleate. Add 50% of flask volume of *Mobile phase*. Sonicate for 15 min followed by mechanical shaking for 15 min. Dilute with *Mobile phase* to volume. Centrifuge a portion of this solution for 10 min.

**Sample solution:** 0.05 mg/mL from *Sample stock solution* diluted with *Mobile phase*. [NOTE—Pass a portion of this solution through a filter with a 0.45- $\mu$ m or finer pore size, and use the filtrate.]



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 234 nm**Column:** 4.6-mm × 25-cm; packing L7**Column temperature:** 40°**Flow rate:** 1.7 mL/min**Injection size:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*[NOTE—The relative retention times for maleic acid, 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-(*E*)-O-[2-[(2-succinyl)amino]ethyl]oxime, *Z*-isomer, and fluvoxamine maleate are 0.19, 0.5, 0.79, 1.0, respectively, *System suitability solution*.]**Suitability requirements****Resolution:** NLT 2.0 between the *Z*-isomer and fluvoxamine maleate; NLT 5.0 between 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-(*E*)-O-[2-[(2-succinyl)amino]ethyl]oxime and the *Z*-isomer, *System suitability solution***Column efficiency:** NLT 5000 theoretical plates, *Standard solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of  $C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of fluvoxamine maleate in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISSOLUTION** <711>**Medium:** Water; 900 mL, degassed**Apparatus 2:** 50 rpm**Time:** 30 min**Standard solution:** USP Fluvoxamine Maleate RS at a known concentration in *Medium***Sample solution:** Centrifuge portions of the solution under test at 2000 rpm for 10 min, and dilute with *Medium*, if necessary.**Spectrometric conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** UV**Analytical wavelength:** 246 nm**Analysis****Samples:** *Standard solution* and *Sample solution*

When there are known interferences due to excipients, excipient interference corrections may be applied, as necessary.

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$  is dissolved.• **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements**IMPURITIES****Organic Impurities**• **PROCEDURE**[NOTE— If (*E*)-5-methoxy-4'-difluoromethylvalerophenone-O-2-aminoethyl oxime is a known impurity, *Test 2* is recommended.]**Test 1****Buffer, Mobile phase, System suitability solution, Standard solution, and Chromatographic system:** Proceed as directed in the *Assay*.**Identification solution:** 0.35 mg/mL of maleic acid in *Mobile phase***Sample solution:** Use the *Sample stock solution*, prepared as directed in the *Assay*.**Analysis****Samples:** *Identification solution*, *Standard solution*, and *Sample solution*

[NOTE— Disregard any peak due to maleic acid or the reagent blank.]

Calculate the percentage of impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

 $r_U$  = individual peak area of each impurity from the *Sample solution* $r_S$  = peak area of fluvoxamine maleate from the *Standard solution* $C_S$  = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of fluvoxamine maleate from the *Sample solution* (mg/mL) $F$  = response factor of each impurity as given in *Impurity Table 1***Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 1.8%**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid	0.19	—	—
5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-( <i>E</i> )-O-[2-[(2-succinyl)amino]ethyl]oxime	0.50	1.0	0.8
5-Methoxy-4'-(trifluoromethyl)valerophenone-( <i>E</i> )-O-(2-aminoethyl)aminoethyl oxime maleate	0.67	1.4	0.2
<i>Z</i> -isomer	0.79	1.0	0.5
Fluvoxamine	1.0	—	—
4'-(Trifluoromethyl)valerophenone-( <i>E</i> )-O-2-(2-aminoethyl)aminoethyl oxime maleate	1.18	1.0	0.2
( <i>E</i> )-O-2-(2-Aminoethyl)-4-(trifluoromethyl)- $\alpha$ -phenyl-acetophenoneoxime maleate	1.74	1.0	0.2
4'-(Trifluoromethyl)valerophenone-( <i>E</i> )-O-(2-aminoethyl)oxime maleate	2.00	1.0	0.2

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methoxy-4'-(trifluoromethyl) valerophenone oxime	3.45	0.6	0.2
5-Methoxy-1-[4-(trifluoromethyl) phenyl]-1-pentanone-(E)-O-(2-aminoethyl)oxime maleic acid monoamide	4.3	1.0	0.2
5-Methoxy-4'-(trifluoromethyl) valerophenone ketone	4.2	0.3	0.2
Unknown impurities	—	1.0	0.1

**Test 2****Diluent:** Methanol and water (3:2)**Solution A:** 13.6 mg/mL of sodium acetate trihydrate in water**Mobile phase:** Acetonitrile, methanol, and *Solution A* (6:3:11). Add 2 mL of triethylamine. Adjust with glacial acetic acid to a pH of 4.5.**System suitability solution:** Proceed as directed in the *Assay*.**Standard solution:** 0.001 mg/mL of fluvoxamine maleate in *Diluent* prepared by dilution of the *Standard solution* in the *Assay***Sample stock solution:** Prepare as directed in the *Assay*.**Sample solution:** 0.1 mg/mL of fluvoxamine maleate from *Sample stock solution* and *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; packing L7**Column temperature:** 40°**Flow rate:** 2 mL/min**Injection size:** 100 µL; 20 µL for the *System suitability solution***System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.0 between the Z-isomer and fluvoxamine maleate, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Measure the responses for all the impurities and fluvoxamine maleate. Calculate the percentage of impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = individual peak area of each impurity from the *Sample solution*  
 $r_S$  = peak area of fluvoxamine maleate from the *Standard solution*  
 $C_S$  = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of fluvoxamine maleate in the *Sample solution* (mg/mL)  
 $F$  = relative response factor of each impurity as given in *Impurity Table 2*

**Acceptance criteria**Individual impurities: See *Impurity Table 2*.

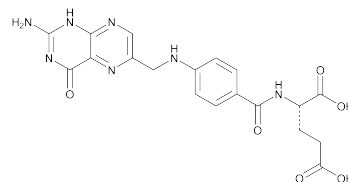
Total impurities: NMT 1.5%

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
(E)-5-methoxy-4'-difluoromethyl valerophenone-O-2-amino ethyloxime	0.58	1.0	0.2
(E)-N-[2[[[α-(4-methoxybutyl)-4-(trifluoromethyl) benzylidene]amino]oxy]ethyl]aspartic acid	0.70	1.0	1.2
(E)-5-methoxy-4'-trifluoromethyl valerophenone-O-[2-N-(aminoethyl) aminoethyl]oxime	0.75	1.0	0.2
Z-isomer	0.85	0.5	0.5
Fluvoxamine	1.0	—	—
(E)-4'-trifluoromethylvalerophenone-O-2-amino-ethyloxime	1.86	1.0	0.2
5-Methoxy-4'-trifluoromethyl valerophenone oxime	1.99	1.0	0.2
5-Methoxy-4-trifluoromethyl valerophenone	2.17	1.0	0.2
Unknown impurities	—	1.0	0.2

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- LABELING:** If a test in *Procedure* under *Organic Impurities* other than *Test 1* is used, then the labeling states with which test the article complies.
- USP REFERENCE STANDARDS (11)**  
USP Fluvoxamine Maleate RS

**Folic Acid**

$C_{19}H_{19}N_7O_6$  441.40  
 L-Glutamic acid, N-[4-[[[(2-amino-1,4-dihydro-4-oxo-6-pteridiny]methyl]amino]benzoyl]]-;

*N*-[*p*-[[*(2-Amino-4-hydroxy-6-pteridiny)*methyl]amino]benzoyl]-*L*-glutamic acid [59-30-3].

### DEFINITION

Folic Acid contains NLT 97.0% and NMT 102.0% of folic acid ( $C_{19}H_{19}N_7O_6$ ), calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

**Sample solution:** 10 µg/mL in 0.1 N sodium hydroxide solution

**Acceptance criteria:** Meets the requirements. The ratio  $A_{256}/A_{365}$  is 2.80–3.00.

### ASSAY

#### • PROCEDURE

[NOTE—Use low-actinic glassware throughout the following procedure.]

**3 N phosphoric acid:** 98 g/L of phosphoric acid in water  
**6 N ammonium hydroxide:** Dilute 40 mL of ammonium hydroxide with water to 100 mL.

**Mobile phase:** Transfer 2.0 g of monobasic potassium phosphate into a 1000-mL volumetric flask, and dissolve in 650 mL of water. Add 15.0 mL of a solution of 0.5 M tetrabutylammonium hydroxide in methanol, 7.0 mL of 3 N phosphoric acid, and 270 mL of methanol. Cool to room temperature, adjust with 3 N phosphoric acid or 6 N ammonium hydroxide to a pH of 5.0, and dilute with water to volume. Recheck the pH before use.

**Internal standard solution:** 2 mg/mL of methylparaben in *Mobile phase*. Dissolve the methylparaben first with methanol (about 4% of the final volume), and dilute with *Mobile phase* to volume.

**Standard stock solution:** 1 mg/mL of USP Folic Acid RS in *Mobile phase*. Dissolve the folic acid with the aid of 10% ammonium hydroxide (about 1% of the final volume), and dilute with *Mobile phase* to volume.

**Standard solution:** Transfer 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Transfer 100 mg of Folic Acid to a 100-mL volumetric flask, and dissolve in 40 mL of *Mobile phase* and 1 mL of 10% ammonium hydroxide. Dilute with *Mobile phase* to volume.

**Sample solution:** Transfer 4.0 mL of *Sample stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm × 25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.6 between methylparaben and folic acid

**Relative standard deviation:** NMT 2.0% for the ratios of the folic acid peak area to the internal standard peak area

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of folic acid ( $C_{19}H_{19}N_7O_6$ ) in the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Sample solution*

$R_S$  = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Standard solution*

$C_S$  = concentration of USP Folic Acid RS in the *Standard stock solution* (mg/mL)

$C_U$  = concentration of Folic Acid in the *Sample stock solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the anhydrous basis

### IMPURITIES

#### • RESIDUE ON IGNITION (281): NMT 0.3%

#### • RELATED COMPOUNDS

3 N phosphoric acid, 6 N ammonium hydroxide, *Internal standard solution*, *Standard stock solution*, *Standard solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

**Sample solution:** Use the *Sample stock solution*, prepared as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Allow the *Sample solution* to elute for NLT 2 times the retention time of folic acid. Record the chromatogram, and measure the areas of all the peaks.

Calculate the percentage of total secondary peaks in the portion of Folic Acid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = sum of the areas of all the peaks except that of the folic acid peak

$r_T$  = sum of the areas of all the peaks

**Acceptance criteria:** NMT 2.0%

### SPECIFIC TESTS

#### • WATER DETERMINATION, *Method I* (921)

**Analysis:** Proceed as directed in the chapter, except stir the methanol solvent before and during the addition of the test specimen and during the titration.

**Acceptance criteria:** NMT 8.5%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed, light-resistant containers.

#### • USP REFERENCE STANDARDS (11)

USP Folic Acid RS

## Folic Acid Injection

» Folic Acid Injection is a sterile solution of Folic Acid in Water for Injection prepared with the aid of Sodium Hydroxide or Sodium Carbonate. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

#### USP Reference standards (11)—

USP Folic Acid RS

USP Endotoxin RS

**Identification**—To a volume of the Injection equivalent to about 100 mg of folic acid add water to make about 25 mL. Adjust with hydrochloric acid to a pH of 3.0, cool to 5°, then filter, and wash the precipitate of folic acid with cold water until the last washing shows an absence of chloride. Then wash with acetone, and dry at 80° for 1 hour: the UV absorption spectrum of a 1 in 100,000 solution of the folic acid so obtained in sodium hydroxide solution (1 in 250)

exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid RS, concomitantly measured. The ratio  $A_{256}/A_{365}$  is between 2.80 and 3.00.

**Bacterial endotoxins** (85)—It contains not more than 357.1 USP Endotoxin Units per mg of folic acid.

**pH** (791): between 8.0 and 11.0.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Folic Acid Tablets*.

*Assay preparation*—Dilute an accurately measured volume of Injection, quantitatively and stepwise, with an aqueous solvent containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL, to obtain a solution having a concentration close to that of the *Standard preparation* and between 0.20 and 0.80 mg per mL.

*Procedure*—Proceed as directed in the Assay under *Folic Acid Tablets*, and calculate the quantity, in mg, of folic acid ( $C_{19}H_{19}N_7O_6$ ) in each mL of the Injection.

## Folic Acid Tablets

### DEFINITION

Folic Acid Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ).

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION

**Sample solution:** Digest the quantity of powdered Tablets, equivalent to 100 mg of folic acid, with 100 mL of 0.1 N sodium hydroxide, and filter. Adjust with hydrochloric acid to a pH of 3.0. Cool to 5°, filter, and wash the precipitate of folic acid with cold water until the last washing shows an absence of chloride. Then wash with acetone, and dry at 80° for 1 h. Dissolve the residue in 0.1 N sodium hydroxide to obtain a 10-μg/mL solution.

**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid RS, concomitantly measured. The absorbance ratio  $A_{256}/A_{365}$  is 2.80–3.00.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1-L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume, and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.

**Diluent:** Aqueous solution containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL.

**System suitability solution:** 0.2 mg/mL each of USP Folic Acid RS and USP Folic Acid Related Compound A RS in *Diluent*. [NOTE—Before use, pass through a filter of 1-μm or finer pore size.]

**Standard solution:** 0.20 mg/mL of USP Folic Acid RS, corrected for water content in *Diluent*.

**Sample solution:** Equivalent to 0.2 mg/mL of folic acid, from NLT 20 powdered Tablets in *Diluent*; shake gently to aid dissolution, and filter, discarding the first portion.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 25 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 3.6 between folic acid related compound A (calcium formyltetrahydrofolate) and folic acid, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of folic acid from the *Sample solution*

$r_S$  = peak area of folic acid from the *Standard solution*

$C_S$  = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of folic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of USP Folic Acid RS, corrected for water content, in *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the Assay, making any necessary modifications.

Calculate the percentage of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

$r_U$  = peak area of folic acid from the *Sample solution*

$r_S$  = peak area of folic acid from the *Standard solution*

$C_S$  = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Folic Acid RS

USP Folic Acid Related Compound A RS

Calcium formyltetrahydrofolate.

## Formaldehyde Solution

CH<sub>2</sub>O 30.03  
Formaldehyde.  
Formaldehyde [50-00-0].

» Formaldehyde Solution contains not less than 34.5 percent, by weight, of formaldehyde (CH<sub>2</sub>O), with methanol added (9.0% to 15.0%) to prevent polymerization.

**Packaging and storage**—Preserve in tight containers, and preferably store at a temperature not below 15°.

### Identification—

**A:** Dilute 2 mL with 10 mL of water in a test tube, and add 1 mL of silver-ammonia-nitrate TS: metallic silver is produced either in the form of a finely divided, gray precipitate, or as a bright, metallic mirror on the sides of the test tube.

**B:** Add 2 drops to 5 mL of sulfuric acid in which about 20 mg of salicylic acid has been dissolved, and warm the liquid very gently: a permanent, deep-red color appears.

**Acidity**—Measure 20.0 mL into a flask containing 20 mL of water, add 2 drops of bromothymol blue TS, and titrate with 0.1 N sodium hydroxide VS: not more than 10.0 mL of 0.1 N sodium hydroxide is consumed.

### Content of methanol—

**Internal standard solution**—Dilute 10 mL of dehydrated alcohol with water to 100 mL.

**Test solution**—To 10.0 mL of Solution add 10.0 mL of the Internal standard solution, and dilute with water to 100.0 mL.

**Standard solution**—To 1.0 mL of methanol add 10.0 mL of the Internal standard solution, and dilute with water to 100.0 mL.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 2- to 4-mm × 1.5- to 2.0-m column containing packing S3. The carrier gas is nitrogen or helium, flowing at a rate of 30 to 40 mL per minute. The column temperature is maintained at 120°. The injection port temperature and the detector temperature are maintained at 150°. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the resolution, *R*, between the peaks corresponding to methanol and alcohol is not less than 2.0.

**Procedure**—Separately inject equal volumes (1 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of methanol in the portion of Solution taken by the formula:

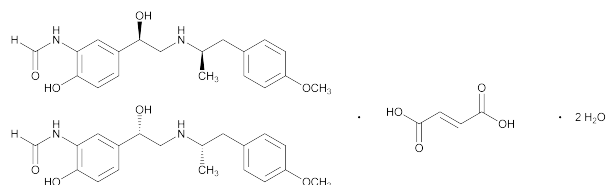
$$100 \times (V_M / V)(R_U / R_S)$$

in which *V<sub>M</sub>* is the volume, in mL, of methanol taken to prepare the Standard solution; *V* is the volume, in mL, of Solution taken to prepare the Test solution; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of methanol to that of the internal standard obtained from the Test solution and the Standard solution, respectively: between 9.0% and 15.0% (v/v) is found.

**Assay**—Into a 100-mL volumetric flask containing 2.5 mL of water and 1 mL of sodium hydroxide TS 2, introduce 1.0 g of the Solution to be examined, shake, and dilute with water to 100.0 mL. To 10.0 mL of the solution add 30.0 mL of 0.1 N iodine VS. Mix, and add 10 mL of sodium hydroxide TS 2. After 15 minutes, add 25 mL of diluted sulfuric acid and 4 mL of starch TS. Titrate with 0.1 N sodium thi-

osulphate VS. Each 1 mL of 0.05 M iodine is equivalent to 1.501 mg of CH<sub>2</sub>O.

## Formoterol Fumarate



(C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>)<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> · 2H<sub>2</sub>O 840.91  
(±)-2'-Hydroxy-5'-[(*R*\*)-1-hydroxy-2-[(*R*\*)-*p*-methoxy-α-methylphenethyl]amino]ethyl]formanilide fumarate (2:1) (salt), dihydrate [43229-80-7].

» Formoterol Fumarate contains not less than 98.5 percent and not more than 101.5 percent of (C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>)<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—The labeling states with which *Content of related compound I* the test article complies if a test other than *Content of related compound I*, *Test 1* is used.

### USP Reference standards <11>—

USP Formoterol Fumarate RS

USP Formoterol Fumarate System Suitability Mixture RS  
It is a mixture of USP Formoterol Fumarate RS and formoterol related compounds A, B, C, D, E, F, G, and H.

**Formoterol related compound A:** 1-(3-Amino-4-hydroxyphenyl)-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol.

**Formoterol related compound B:** *N*-[2-Hydroxy-5-[(1*RS*)-1-hydroxy-2-[[2-(4-methoxyphenyl)ethyl]amino]ethyl]phenyl]formamide.

**Formoterol related compound C:** *N*-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]acetamide.

**Formoterol related compound D:** *N*-[2-Hydroxy-5-[1-hydroxy-2-[methyl[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

**Formoterol related compound E:** *N*-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxy-3-methylphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

**Formoterol related compound F:** *N*-[2-Hydroxy-5-[1-[[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]amino]-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

**Formoterol related compound G:** (2*RS*)-1-(4-Methoxyphenyl)propan-2-amine.

**Formoterol related compound H:** *N*-[5-[(1*RS*)-2-[Benzyl[(1*RS*)-2-(4-methoxyphenyl)-1-methylethyl]amino]-1-hydroxyethyl]-2-hydroxyphenyl]formamide (monobenzyl analogue).

USP Formoterol Resolution Mixture RS

This standard is a mixture of formoterol and formoterol fumarate impurity I.

**Impurity I:** *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[(1*SR*)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide fumarate salt (2:1)(diastereoisomer).

**Identification, Infrared Absorption** (197K).

**Optical rotation, Angular Rotation** (781A): between  $-0.10^\circ$  and  $+0.10^\circ$ .

**Test solution:** 10 mg per mL, in methanol.

**pH** (791): between 5.5 and 6.5, in a solution in water containing 1 mg per mL.

**Water, Method I** (921): between 4.0% and 5.0%.

**Residue on ignition** (281): not more than 0.1%, determined on 1 g.

**Heavy metals, Method II** (31): not more than 0.002%.

**Related compounds—**

**Solution A**—Dissolve 3.73 g of sodium dihydrogen phosphate monohydrate and 0.35 g of phosphoric acid in water, dilute with water to 1000 mL, and mix. The pH of this solution is  $3.1 \pm 0.1$ .

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of **Solution A** and **Solution B** as directed for **Chromatographic system**. Make adjustments if necessary (see **System Suitability** under **Chromatography** (621)).

**Solution C**—Transfer 6.10 g of sodium dihydrogen phosphate monohydrate and 1.03 g of disodium hydrogen phosphate dihydrate to a 1000-mL volumetric flask, add 500 mL of water, and dissolve. Dilute with water to volume, and mix. The pH is  $6.0 \pm 0.1$ .

**Diluent**—Prepare a filtered and degassed mixture of **Solution C** and acetonitrile (84:16, v/v).

**System suitability solution**—Transfer about 5 mg of USP Formoterol Fumarate System Suitability Mixture RS (containing formoterol fumarate, and formoterol related compounds A, B, C, D, E, F, G, and H), accurately weighed, to a 25-mL volumetric flask, add 10 mL of **Diluent**, and sonicate to dissolve. Dilute with **Diluent** to volume, and mix.

**Test solution**—Transfer about 20.0 mg of Formoterol Fumarate, accurately weighed, to a 100-mL volumetric flask, add 50 mL of **Diluent**, and sonicate to dissolve. Dilute with **Diluent** to volume, and mix.

**Chromatographic system** (see **Chromatography** (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	84	16	equilibration
0–10	84	16	isocratic
10–37	84→30	16→70	linear gradient
37–40	30→84	70→16	linear gradient
40–55	84	16	isocratic

Chromatograph the **System suitability solution**, and record the peak responses as directed for **Procedure**: the resolution,  $R$ , between formoterol related compound G and formoterol related compound A is not less than 1.5; the peak-to-valley ratio ( $H_p / H_v$ ) of formoterol related compound C and formoterol is not less than 2.5, where  $H_p$  is the height above the baseline of the peak due to formoterol related compound C, and  $H_v$  is the height above the baseline of the lowest point of the curve separating this peak from the peak due to formoterol; and the relative retention times and limits are as provided in **Table 1**.

**Table 1**

Related Compound	Relative Retention Time	Relative Response Factor (F)	Limit (%)
G <sup>a</sup>	0.4	2.64	0.1
A <sup>b</sup>	0.5	1.75	0.3
B <sup>c</sup>	0.7	1.00	0.2
C <sup>d</sup>	1.2	1.10	0.2
D <sup>e</sup>	1.3	1.12	0.2
E <sup>f</sup>	1.8	0.67	0.1
F <sup>g</sup>	2.0	1.00	0.2
H <sup>h</sup>	2.2	1.24	0.1
Any other individual impurity			0.1
Total unspecified impurities			0.2
Total impurities			0.5

<sup>a</sup>(2RS)-1-(4-Methoxyphenyl)propan-2-amine.

<sup>b</sup>1-(3-Amino-4-hydroxyphenyl)-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol.

<sup>c</sup>N-[2-Hydroxy-5-[(1RS)-1-hydroxy-2-[[2-(4-methoxyphenyl)ethyl]amino]ethyl]phenyl]formamide.

<sup>d</sup>N-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]acetamide.

<sup>e</sup>N-[2-Hydroxy-5-[1-hydroxy-2-[methyl[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

<sup>f</sup>N-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxy-3-methylphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

<sup>g</sup>N-[2-Hydroxy-5-[1-[[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]amino]-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.

<sup>h</sup>N-[5-[(1RS)-2-[Benzyl[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]-1-hydroxyethyl]-2-hydroxyphenyl]formamide (monobenzyl analogue).

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the **System suitability solution** and the **Test solution** into the chromatograph, record the chromatograms, and measure all of the peak responses. Disregard any peak representing less than 0.05%. Calculate the percentage of each formoterol related compound in the portion of Formoterol Fumarate taken by the formula:

$$100F(r_i / r_s)$$

in which  $F$  is the relative response factor for each formoterol related compound according to **Table 1**;  $r_i$  is the peak response for each formoterol related compound; and  $r_s$  is the sum of the responses for all the peaks.

**Content of related compound I (diastereoisomer)—****TEST 1—**

**Standard solution**—Dissolve 10 mg of USP Formoterol Fumarate Resolution Mixture RS in 1 mL of dimethylformamide. Add 100  $\mu$ L of *N*-(trimethylsilyl)imidazole, and mix.

**Test solution**—Dissolve 10 mg of Formoterol Fumarate in 1 mL of dimethylformamide. Add 100  $\mu$ L of *N*-(trimethylsilyl)imidazole, and mix.

**Chromatographic system** (see **Chromatography** (621))—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm  $\times$  30-m fused-silica capillary column coated with a 0.25- $\mu$ m film of stationary phase G27, and a split injection system. The carrier gas is helium, flowing at a rate of about 2 mL per minute and a split ratio of about 75:1. The injection port and the detector temperatures are maintained at about 280° and 300°, respectively. The column temperature is programmed as follows. Initially the column temperature is equilibrated at 220° for 5 minutes, then the temperature is increased at a rate of 1° per minute to 250°, and maintained at 250° for 20 minutes. Chromatograph the **Standard solution**, and record the peak responses as directed for **Procedure**: the resolution,  $R$ , between

formoterol related compound I and formoterol is not less than 1.2.

**Procedure**—Separately inject equal volumes (about 2  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for formoterol related compound I and formoterol. Disregard all other peaks. Calculate the percentage of formoterol related compound I in the portion of Formoterol Fumarate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for formoterol related compound I, and  $r_s$  is the sum of the responses of both formoterol and formoterol related compound I peaks: not more than 0.3% of formoterol related compound I is found.

**TEST 2—**

**Potassium phosphate solution**—Dissolve 5.3 g of tribasic potassium phosphate, trihydrate, in 1000 mL of water, and mix. Adjust the pH with potassium hydroxide or phosphoric acid to  $12.0 \pm 0.1$ .

**Mobile phase**—Prepare a filtered degassed mixture of *Potassium phosphate solution* and acetonitrile (88:12).

**Standard solution**—Dissolve 5 mg of USP Formoterol Fumarate Resolution Mixture RS in water, dilute with water to 50 mL, and mix.

**Test solution**—Dissolve 5 mg of Formoterol Fumarate in water, dilute with water to 50 mL, and mix.

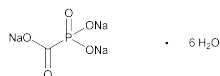
**Diluted test solution**—Dilute 1 mL of the *Test solution* with water to 20 mL. Dilute 1 mL of this solution with water to 25 mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L67 (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*). The flow rate is about 0.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for the *Procedure*: the peak-to-valley ratio ( $H_p / H_v$ ) of formoterol related compound I and formoterol is not less than 2.5, where  $H_p$  is the height above the baseline of the peak due to formoterol related compound I, and  $H_v$  is the height above the baseline of the lowest point of the curve separating this peak from the peak due to formoterol.

**Procedure**—Separately inject equal volumes (20  $\mu$ L) of the *Test solution* and the *Diluted test solution* into the chromatograph, record the chromatograms, and measure the peak responses for formoterol and formoterol related compound I. The area due to formoterol related compound I is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the *Diluted test solution*: not more than 0.3% of formoterol related compound I is found.

**Assay**—Transfer about 350 mg of Formoterol Fumarate, accurately weighed, to a titration vessel, dissolve in 50 mL of anhydrous acetic acid, and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M perchloric acid is equivalent to 40.24 mg of  $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4$ .

## Foscarnet Sodium



$CNa_3O_5P \cdot 6H_2O$  300.04

Phosphinocarboxylic acid, dihydroxy-, oxide, trisodium salt, hexahydrate.

Phosphonoformic acid, trisodium salt, hexahydrate [34156-56-4].

» Foscarnet Sodium contains not less than 98.5 percent and not more than 101.0 percent of  $CNa_3O_5P$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**USP Reference standards** <11>—

USP Foscarnet Sodium RS

USP Foscarnet Related Compound B RS

Disodium (ethoxyoxido-phosphanyl)formate.

$C_3H_5Na_2O_5P$  198.02

USP Foscarnet Related Compound D RS

O,O-Diethyl ethoxycarbonylphosphonate.

$C_7H_{15}O_5P$  210.16

**Identification**—

**A: Infrared Absorption** <197K>.

**B:** It meets the requirements of the test for *Sodium* <191>.

**pH** <791>: between 9.0 and 11.0, in a carbon dioxide-free aqueous solution containing 20 mg of Foscarnet Sodium per mL.

**Loss on drying** <731>—Dry about 0.1 g at  $150^\circ$  for at least 15 minutes and weigh: it loses between 35.0% and 37.0% of its weight.

**Heavy metals**—

**Lead standard stock solution (1000 ppm)**—Dissolve 0.4 g of lead nitrate in 250 mL of water. Prepare and store this solution in glass containers free from soluble lead salts.

**Lead standard solution**—Immediately before use, dilute *Lead standard stock solution (1000 ppm)*, quantitatively and stepwise if necessary, with water to obtain a solution having a known concentration of 1 ppm of lead.

**Sodium sulfide solution**—Dissolve 12 g of disodium sulfide nonahydrate with heating in 45 mL of a mixture of 10 volumes of water and 29 volumes of 85% glycerol, allow to cool, and dilute with the same mixture of solvents to 100 mL.

**Acetate buffer**—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

**Test solution**—Dissolve 1.25 g in 12.5 mL of 1 M hydrochloric acid. Heat on a boiling water bath for 3 minutes and cool to room temperature. Transfer to a beaker, adjust with 6 N ammonium hydroxide to a pH of about 3.5, and dilute with water to 25 mL.

**Standard solution**—Prepare a mixture of 5.0 mL of *Lead standard solution*, 5.0 mL of water, 2.0 mL of the *Test solution*, and 2.0 mL of *Acetate buffer*. Rapidly pour the solution into a test tube containing 1 drop of *Sodium sulfide solution*.

**Procedure**—To 12 mL of the *Test solution* add 2.0 mL of *Acetate buffer*. Rapidly pour the mixture into a test tube containing 1 drop of *Sodium sulfide solution*. The solution is not more intensely colored than the *Standard solution* prepared simultaneously (10 ppm).

**Limit of foscarnet related compound B, unknown impurities, and total impurities**—

**Solution A**—Dissolve 3.2 g of sodium sulfate decahydrate in water, add 3 mL of glacial acetic acid and 6 mL of 0.1 M sodium pyrophosphate, and dilute with water to 1000 mL.

**Solution B**—Dissolve 3.2 g of sodium sulfate decahydrate in water, add 6.8 g of sodium acetate and 6 mL of 0.1 M sodium pyrophosphate, and dilute with water to 1000 mL.

**Mobile phase**—Prepare a mixture of *Solution A* and *Solution B* (70:30). [NOTE—The pH of this solution is about 4.4.] To 1000 mL of this solution, add 0.25 g of tetrahexylam-

monium hydrogen sulfate and 100 mL of methanol, and mix.

**Test solution**—Dissolve an accurately weighed quantity of Fosfomycin Sodium in *Mobile phase* to obtain a solution having a known concentration of about 2.5 mg per mL.

**Standard solution**—Accurately dilute the *Test solution* in *Mobile phase*, stepwise if necessary, to obtain a solution having a known concentration of about 5.0 µg per mL.

**Resolution solution**—Transfer 2.0 mL of the *Test solution* into a 50-mL volumetric flask, add 5.0 mg of USP Fosfomycin Related Compound B RS, and dilute with *Mobile phase* to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between fosfomycin and fosfomycin related compound B is not less than 7.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, allow the chromatogram to run for 2.5 times of the retention time of fosfomycin, record the chromatograms, and measure the peak responses: the area of any peak, apart from the major peak, in the chromatogram obtained with the *Test solution* is not greater than the area of the major peak in the chromatogram obtained with the *Standard solution* (0.2%); the sum of the areas of all the peaks, apart from the major peak, is not greater than twice the area of the major peak in the chromatogram obtained with the *Standard solution* (0.4%). Disregard any peak with a relative retention time less than 0.6 and any peak with an area less than 0.2 times that of the peak in the chromatogram obtained with the *Standard solution*.

#### Limit of fosfomycin related compound D—

**Test solution**—Dissolve 0.25 g of Fosfomycin Sodium in 9 mL of 0.1 M acetic acid. Add 1 mL of alcohol, and mix.

**Standard solution**—Dissolve an accurately weighed quantity of USP Fosfomycin Related Compound D RS in alcohol, stepwise if necessary, to obtain a solution having a known concentration of about 25 µg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.31-mm × 25-m column coated with a 0.5-µm phase G27. The carrier gas is helium, flowing at a rate of 1 mL per minute. The split ratio is 1:20. The chromatograph is programmed as follows. The temperature of the column is increased from 100° to 180° at a rate of 10° per minute. The injection port temperature is maintained at 200°, and the detector temperature is maintained at 250°.

**Procedure**—Separately inject equal volumes (about 3 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses: the area of the peak due to fosfomycin related compound D in the chromatogram obtained with the *Test solution* is not greater than the area of the peak in the chromatogram obtained with the *Standard solution* (0.1%).

#### Limit of phosphate and phosphite—

**Mobile phase**—Dissolve about 0.1 g of potassium phthalate monobasic in water, add 2.5 mL of 1 M nitric acid, and dilute with water to 1000 mL.

**Test solution**—Dissolve an accurately weighed quantity of Fosfomycin Sodium in water to obtain a solution having a known concentration of about 2.4 mg per mL.

**Standard stock solution 1**—Dissolve an accurately weighed quantity of sodium dihydrogen phosphate monohydrate in water to obtain a solution having a known concentration of about 0.28 mg per mL.

**Standard stock solution 2**—Dissolve an accurately weighed quantity of sodium phosphite pentahydrate in water to ob-

tain a solution having a known concentration of about 0.43 mg per mL.

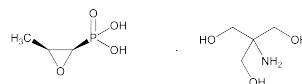
**Standard solution**—Transfer 1 mL each of *Standard stock solution 1* and *Standard stock solution 2* to a 25-mL volumetric flask, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 290-nm detector and a 4.6-mm × 5-cm column that contains packing L23 (see *Chromatographic Reagents*). The flow rate is about 1.4 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between phosphate and phosphite is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses: the area of any peak due to phosphate and phosphite in the chromatogram obtained with the *Test solution* is not greater than the area of the corresponding peak in the chromatogram obtained with the *Standard solution* (0.3% of phosphate and 0.3% of phosphite).

**Assay**—Dissolve about 0.2 g of Fosfomycin Sodium, accurately weighed, in 50 mL of water. Titrate with 0.05 M sulfuric acid, determining the endpoint potentiometrically at the first inflection point. Each mL of 0.05 M sulfuric acid is equivalent to 19.20 mg of CN<sub>3</sub>O<sub>3</sub>P.

## Fosfomycin Tromethamine



$C_3H_7O_4P \cdot C_4H_{11}NO_3$  259.19  
Phosphonic acid, (3-methyloxiranyl)-, (2*R*-cis)-, compd. with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1); (1*R*,2*S*)-(1,2-Epoxypropyl)phosphonic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) [78964-85-9].

#### DEFINITION

Fosfomycin Tromethamine contains NLT 98.0% and NMT 102.0% of  $C_3H_7O_4P \cdot C_4H_{11}NO_3$ , calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>
- **B.** The retention time of the fosfomycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the tromethamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

[NOTE—Prepare the solutions immediately before use.]

**Mobile phase:** 10.89 g/L of potassium dihydrogen phosphate in water

**Standard solution:** 120 mg/mL of USP Fosfomycin Tromethamine RS in *Mobile phase*

**Sample solution:** 120 mg/mL of Fosfomycin Tromethamine in *Mobile phase*

**Solution A:** Wet 300 mg of fosfomycin tromethamine with 60 µL of water, and heat in an oven at 60° for 24 h. Dissolve the residue, and dilute with *Mobile phase* to 20.0 mL.

**System suitability solution:** Dissolve 600 mg of fosfomycin tromethamine, and dilute with *Solution A* to 5.0 mL.



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** Differential refractometer at 35°**Column:** 4.6-mm × 25-cm; 5-μm packing L8**Flow rate:** 1 mL/min**Injection size:** 5 μL**Run time:** Twice the retention time of fosfomycin**System suitability****Sample:** *System suitability solution*[NOTE—The relative retention times are listed in *Impurity Table 1*.]**Suitability requirements****Resolution:** NLT 1.5 between fosfomycin open ring and fosfomycin**Peak-to-valley ratio:** NLT 1.5. [NOTE—Ratio is based on the height above the baseline due to the tromethamine phosphate peak and to the height above the baseline of the lowest point of the curve separating this peak from the peak due to the fosfomycin tromethamine adduct.]**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>3</sub>H<sub>7</sub>O<sub>4</sub>P · C<sub>4</sub>H<sub>11</sub>NO<sub>3</sub> in the portion of Fosfomycin Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of fosfomycin from the *Sample solution* $r_S$  = peak response of fosfomycin from the *Standard solution* $C_S$  = concentration of USP Fosfomycin Tromethamine RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Fosfomycin Tromethamine in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES****Inorganic Impurities**

- **HEAVY METALS, Method I** <231>: NMT 10 ppm

**Sample solution:** 100 mg/mL in water

- **LIMIT OF INORGANIC PHOSPHATES**

**Solution A:** Dissolve 4 g of finely powdered ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in 70 mL of water. Add 20 mL of nitric acid, and dilute with water to 100 mL.**Standard solution:** Dissolve 7.16 mg of potassium dihydrogen phosphate in 1000 mL of water (5 ppm PO<sub>4</sub>). [NOTE—Prepare immediately before use.]**Sample solution:** Dissolve 0.1 g of Fosfomycin Tromethamine in 3 mL of 2 N nitric acid, and dilute with water to 10 mL.**Analysis:** In separate containers, transfer 5 mL of *Standard solution* and 5 mL of *Sample solution*. Add 5 mL of water and 5 mL of *Solution A* to both solutions, and shake vigorously. After 5 min, any color in the *Sample solution* is not more intense than the *Standard solution*.**Acceptance criteria:** NMT 500 ppm**Organic Impurities**

- **PROCEDURE**

[NOTE— Prepare the solutions immediately before use.]

**Mobile phase, Sample solution, Solution A, System suitability solution, and System suitability:** Proceed as directed in the *Assay*.**Standard solution:** 0.36 mg/mL of USP Fosfomycin Tromethamine RS in *Mobile phase***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** Differential refractometer at 35°**Column:** 4.6-mm × 25-cm; 5-μm packing L8**Flow rate:** 1 mL/min**Injection size:** 10 μL**Run time:** Twice the retention time of fosfomycin**Analysis****Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of impurities in the portion of Fosfomycin Tromethamine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of any individual impurity in the *Sample solution* $r_T$  = peak responses of fosfomycin in the *Standard solution***Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tromethamine <sup>a</sup>	0.30	—
Fosfomycin tromethamine adduct <sup>b</sup>	0.48	0.3
Tromethamine phosphate <sup>c</sup>	0.54	0.1
Fosfomycin open ring <sup>d</sup>	0.88	0.3
Fosfomycin	1.00	—
Fosfomycin dimer tromethamine adduct <sup>e</sup>	1.27	0.1
Any other individual impurity	—	0.1

<sup>a</sup> 1,3-Propanediol, 2-amino-2-(hydroxymethyl)-; 2-Amino-2-(hydroxymethyl)-1,3-propanediol. [NOTE—Disregard two peaks due to tromethamine.]<sup>b</sup> 2-[2-Amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropylphosphonic acid.<sup>c</sup> 2-Amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogen phosphate.<sup>d</sup> (1,2-Dihydroxypropyl)phosphonic acid.<sup>e</sup> 2-[(2-[2-Amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl)hydroxyphosphoryloxy]-1-hydroxypropylphosphonic acid.**Acceptance criteria****Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any peak less than 0.05%.]**Total impurities:** NMT 0.5%**SPECIFIC TESTS**

- **PH** <791>: 3.5–5.5, in a carbon dioxide-free aqueous solution containing 50 mg/mL of fosfomycin tromethamine
- **OPTICAL ROTATION, Specific Rotation** <781S>: –13.5° to –12.5°

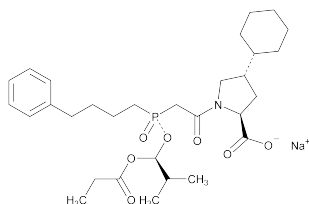
**Sample solution:** 50 mg/mL in a carbon dioxide-free water**Detection:** Mercury lamp at 365 nm

- **WATER DETERMINATION, Method 1c** <921>: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Fosfomycin Tromethamine RS

## Fosinopril Sodium



$C_{30}H_{45}NNaO_7P$  585.64  
 L-Proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]acetyl]-, sodium salt, [1  $[S^*(R^*)]$ , 2 $\alpha$ , 4 $\beta$ ]-;  
 (4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt [88889-14-9].

### DEFINITION

Fosinopril Sodium contains NLT 97.5% and NMT 102.0% of  $C_{30}H_{45}NNaO_7P$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** <197M>

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, phosphoric acid, and water (2000:1:10)

**System suitability solution:** 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL of USP Fosinopril Related Compound B RS in *Mobile phase*

**Standard solution:** 0.10 mg/mL of USP Fosinopril Sodium RS in *Mobile phase*

**Sample solution:** 0.10 mg/mL of Fosinopril Sodium in *Mobile phase*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm  $\times$  15-cm; packing L3

**Temperature:** 33°

**Flow rate:** 1.2 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between fosinopril related compound B and fosinopril sodium

**Relative standard deviation:** NMT 2.0% for Fosinopril peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{30}H_{45}NNaO_7P$  in the portion of Fosinopril Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Fosinopril Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Fosinopril Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.0% on the anhydrous basis

### IMPURITIES

#### Inorganic Impurities

- **HEAVY METALS**, *Method II* <231>: 20 ppm

#### Organic Impurities

##### • PROCEDURE 1

**Mobile phase, Sample solution and Chromatographic system:** Proceed as directed in the *Assay*. [NOTE—Record the chromatograms for four times the retention time of the fosinopril sodium peak.]

**System suitability solution:** 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL each of USP Fosinopril Related Compound A RS and USP Fosinopril Related Compound B RS in *Mobile phase*

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each individual related compound:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = individual peak response, other than the fosinopril sodium peak

$r_T$  = sum of all the peak responses

[NOTE— If present, two more diastereomers may not be resolved from fosinopril related compound B by this method. These peaks, appearing at a relative retention time of 0.7, should be integrated together to determine conformance with the limit in *Impurity Table 1*.]

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Procedure	Acceptance Criteria, NMT (%)
Fosinopril Related Compound A <sup>a</sup>	2.0	1	0.75
Fosinopril Related Compound B <sup>b</sup>	0.7	1	1.0
Fosinopril Related Compound C <sup>c</sup>	1.2	2	0.3
Fosinopril Related Compound D <sup>d</sup>	1.3	2	0.3
Fosinopril Related Compound E <sup>e</sup>	0.8	3	0.3
Fosinopril Related Compound F <sup>f</sup>	0.9	3	0.3
Impurity 1 <sup>g</sup>	0.53	1	0.3

<sup>a</sup> (4S)-4-Cyclohexyl-1-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

<sup>b</sup> (4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester).

<sup>c</sup> Mixture of (4S)-4-cyclohexyl-1-[(S)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt and (4S)-4-cyclohexyl-1-[(R)-[(R)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>d</sup> (4R)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>e</sup> (4S)-4-Phenyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>f</sup> (4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-propoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>g</sup> (2S,4S)-4-Cyclohexyl-1-pivaloylpyrrolidine-2-carboxylic acid.

<sup>h</sup> 2-((R)-((SR)-2-Methyl-1-(propionyloxy)propoxy)(4-phenylbutyl)phosphinyl)acetic acid.

<sup>i</sup> (S)-4-cyclohexyl-1-(3-oxopentanoyl)-L-proline.

**Impurity Table 1** (Continued)

Name	Relative Retention Time	Procedure	Acceptance Criteria, NMT (%)
Impurity 2 <sup>h</sup>	0.67	1	0.2
Impurity 3 (if present) <sup>i</sup>	0.37	1	0.15

<sup>a</sup> (4S)-4-Cyclohexyl-1-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

<sup>b</sup> (4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester).

<sup>c</sup> Mixture of (4S)-4-cyclohexyl-1-[(S)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt and (4S)-4-cyclohexyl-1-[(R)-[(R)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>d</sup> (4R)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>e</sup> (4S)-4-Phenyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>f</sup> (4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-propoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

<sup>g</sup> (2S,4S)-4-Cyclohexyl-1-pivaloylpyrrolidine-2-carboxylic acid.

<sup>h</sup> 2-[(R)-[(S)-2-Methyl-1-(propionyloxy)propoxy](4-phenylbutyl)phosphinyl]acetic acid.

<sup>i</sup> (S)-4-cyclohexyl-1-(3-oxopentanoyl)-L-proline.

## • PROCEDURE 2

**Sample solution:** Proceed as directed in the Assay.

**Mobile phase:** Acetonitrile, phosphoric acid, and water (4000:2:15)

**System suitability solution:** 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL each of USP Fosinopril Related Compound C RS and USP Fosinopril Related Compound D RS in the Mobile phase

### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm × 25-cm; packing L12

**Temperature:** 45°

**Flow rate:** 0.9 mL/min

**Injection size:** 20 µL

**Run time:** Twice the retention time of fosinopril sodium

### System suitability

**Sample:** System suitability solution

#### Suitability requirements

**Resolution:** NLT 1.5 between fosinopril sodium and fosinopril related compound C

### Analysis

**Sample:** Sample solution

Calculate the percentages of fosinopril related compound C and fosinopril related compound D only:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of fosinopril related compound C or fosinopril related compound D

$r_T$  = sum of all the peak responses

## • PROCEDURE 3

**Solution A:** 1-in-500 solution of phosphoric acid

**Mobile phase:** Acetonitrile and Solution A (14:11)

**System suitability solution:** 0.01 mg/mL each of USP Fosinopril Sodium RS, USP Fosinopril Related Compound E RS, and USP Fosinopril Related Compound F RS in Mobile phase

**Sample solution:** 0.2 mg/mL of Fosinopril Sodium in Mobile phase

### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 25-cm; packing L11

**Temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**Run time:** Four times the retention time of fosinopril sodium

### System suitability

**Sample:** System suitability solution

#### Suitability requirements

**Resolution:** NLT 1.5 between fosinopril related compound F and fosinopril sodium; NLT 1.5 between fosinopril related compound E and fosinopril related compound F

### Analysis

**Sample:** Sample solution

Measure the peak responses, carrying out the chromatography to four times the retention time of the fosinopril sodium peak.

Calculate the percentages of fosinopril related compound E and fosinopril related compound F only:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of fosinopril related compound E or fosinopril related compound F

$r_T$  = sum of all the peak responses

### Acceptance criteria

**Individual impurities:** See Impurity Table 1. In addition to not exceeding the limits for impurities in Impurity Table 1, NMT 0.1% of any other individual impurity is found (calculated as directed in Procedure 1).

**Total impurities:** NMT 1.5% for the total of Procedure 1, Procedure 2, and Procedure 3

## SPECIFIC TESTS

• **WATER DETERMINATION, Method I <921>:** NMT 0.2%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS <11>**

USP Fosinopril Sodium RS

USP Fosinopril Related Compound A RS

(4S)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

$C_{23}H_{34}NO_5P$  435.49

USP Fosinopril Related Compound B RS

(4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester), hemibarium salt, sesquihydrate.

$C_{30}H_{45}NO_7P \cdot 1/2Ba \cdot 1 1/2H_2O$  658.34

USP Fosinopril Related Compound C RS

(4S)-4-Cyclohexyl-1-[(RS)-[(RS)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

$C_{30}H_{45}NNaO_7P$  585.64

USP Fosinopril Related Compound D RS

(4R)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

$C_{30}H_{45}NNaO_7P$  585.64

USP Fosinopril Related Compound E RS

(4S)-4-Phenyl-1-[(R)-[(S)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

$C_{30}H_{39}NNaO_7P$  579.60

USP Fosinopril Related Compound F RS

(4S)-4-Cyclohexyl-1-[(R)-[(S)-1-hydroxy-propoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

$C_{29}H_{43}NNaO_7P$  571.62

## Fosinopril Sodium Tablets

» Fosinopril Sodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fosinopril sodium ( $C_{30}H_{45}NNaO_7P$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Fosinopril Sodium RS

USP Fosinopril Related Compound A RS

(4S)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

$C_{23}H_{34}NO_5P$  435.49

USP Fosinopril Related Compound G RS

(4-Phenylbutyl)phosphinylacetic acid, disodium salt.

$C_{12}H_{15}Na_2O_4P$  300.20

### Identification—

**A: Infrared Absorption** (197F)—

**Test specimen**—Transfer a portion of the finely powdered Tablets, equivalent to about 25 mg of fosinopril sodium, to a 100-mL beaker containing 40 mL of water. Heat at 25° for 5 minutes with stirring, and pass through a medium-porosity fritted-disc funnel. Centrifuge the filtrate at 2500 rpm for 30 minutes. Adjust the filtrate with phosphoric acid to a pH of 3 to precipitate the fosinopril, and pass through a fritted-disc funnel. Dissolve the precipitate by passing chloroform through the filter, and evaporate the chloroform solution to dryness under a current of air. Proceed as directed, using the oily residue so obtained and a similarly prepared residue from 25 mg of USP Fosinopril Sodium RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.2% phosphoric acid (64:36). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Prepare a solution in *Mobile phase* containing about 0.02 mg per mL each of USP Fosinopril Sodium RS and USP Fosinopril Related Compound G RS.

**Standard stock solution**—Accurately weigh about 20 mg of USP Fosinopril Sodium RS into a 200-mL volumetric flask, dissolve in 6-mL of methanol, sonicate briefly, and dilute with *Medium* to volume.

**Standard solution**—Dilute the *Standard stock solution* with *Medium* as directed in the following table.

Label claim	Standard stock solution (mL)	Final volume (Flask size)
5 mg	5.0	100
10 mg	10	100
20 mg	20	100
40 mg	40	100

**Test solution**—Use portions of the solution under test passed through a 1.2- $\mu$ m acrylic filter. [NOTE—Do not use glass filters.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm  $\times$  15-cm column, maintained at a temperature of 40°, that contains 5- $\mu$ m packing L1. The flow rate is

about 3 mL per minute. Chromatograph the *Resolution solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between fosinopril sodium and fosinopril related compound G is not less than 1.7; and the relative standard deviation for replicate injections of the *Standard solution* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Test solution* and the *Standard solution* having a known concentration of USP Fosinopril Sodium RS in the same *Medium*, and record the chromatograms. Measure the responses for the major peaks, and calculate the amount of  $C_{30}H_{45}NNaO_7P$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{30}H_{45}NNaO_7P$  is dissolved in 30 minutes.

**Uniformity of dosage units:** meet the requirements.

### Limit of related compound A—

**Mobile phase, Diluent, and Chromatographic system**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Fosinopril Related Compound A RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Dilute with *Diluent* to obtain a solution having a final known concentration of 0.0025 mg per mL.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the percentage of fosinopril related compound A in the portion of Tablets taken by the formula:

$$1000C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of the fosinopril related compound A in the *Standard solution*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 4% is found.

### Assay—

**Mobile phase**—Prepare a degassed mixture of methanol and 0.2% phosphoric acid (78:22). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of 0.2 M urea solution and acetonitrile (80:20).

**Resolution solution**—Prepare a solution in *Diluent* containing 30  $\mu$ g of USP Fosinopril Related Compound A RS and 70  $\mu$ g of USP Fosinopril Sodium RS per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fosinopril Sodium RS to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Tablets to a 500-mL volumetric flask, add 400 mL of *Diluent*, and stir for 40 minutes. Dilute with *Diluent* to volume, mix, and centrifuge. Quantitatively dilute an accurately measured volume ( $V_s$  mL) of the clear supernatant with *Diluent* to obtain a solution ( $V_A$  mL) containing about 0.1 mg of fosinopril sodium per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.0-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention time is 0.4 for fosinopril related compound A, and 1.0 for fosinopril sodium; the resolution,  $R$ , between the fosinopril sodium and fosinopril related compound A peaks is not less than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Continue the chromatography up to 1.5 times the retention time of the fosinopril sodium peak. Calculate the quantity, in mg, of fosinopril sodium ( $C_{30}H_{45}NNaO_7P$ ) in the portion of Tablets taken by the formula:

$$50C(V_A / V_S)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fosinopril Sodium RS in the *Standard preparation*;  $V_A$  is the volume, in mL, of the *Assay preparation*;  $V_S$  is the volume, in mL, of supernatant taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fosinopril Sodium and Hydrochlorothiazide Tablets

» Fosinopril Sodium and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fosinopril sodium ( $C_{30}H_{45}NNaO_7P$ ) and of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

- USP Benzothiadiazine Related Compound A RS  
4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $C_6H_8ClN_3O_4S_2$  285.73
- USP Chlorothiazide RS
- USP Fosinopril Sodium RS
- USP Fosinopril Related Compound A RS  
(4S)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.  
 $C_{23}H_{34}NO_5P$  435.49
- USP Fosinopril Related Compound H RS  
4-phenylbutyl phosphonic acid.  
 $C_{10}H_{15}O_3P$  214.20
- USP Hydrochlorothiazide RS

### Identification—

#### A: Infrared Absorption (197F)—

**FOSINOPRIL SODIUM**—Transfer a portion of the finely powdered Tablets, equivalent to about 25 mg of fosinopril sodium, to a 100-mL beaker containing 40 mL of water, heat at 30° for 5 minutes with stirring, and filter through a funnel having a medium-porosity fritted disk. Centrifuge the filtrate at 2500 rpm for 30 minutes. Adjust the filtrate with hydrochloric acid to a pH of 1 to precipitate the fosinopril, and filter through a fritted-disk funnel. Dissolve the precipitate by passing methyl isobutyl ketone through the filter, and evaporate the filtrate to dryness under a stream of nitrogen. Proceed as directed, using the oily residue so obtained and a similarly prepared residue from 25 mg of USP Fosinopril Sodium RS.

**HYDROCHLOROTHIAZIDE**—Transfer a portion of the finely powdered Tablets, equivalent to about 37.5 mg of hydrochlorothiazide, to a 250-mL beaker containing 120 mL of water, heat at 30° for 5 minutes with stirring, and filter through a funnel having a medium-porosity fritted disk. Wash the precipitate with 60 mL of methylene chloride and glacial acetic acid (90:10) mixture, and discard the filtrate. Dissolve the precipitate by passing 10 mL of methyl isobutyl ketone through the filter, and evaporate the filtrate to dryness under a stream of nitrogen. Proceed as directed, using

the waxy residue so obtained and a similarly prepared residue from 37 mg of USP Hydrochlorothiazide RS.

**B:** The retention times of the fosinopril sodium and hydrochlorothiazide peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Mobile phase**—Prepare a filtered and degassed mixture of 0.01 M monobasic potassium phosphate (pH 3.0), methanol, and acetonitrile (45:35:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solutions**—Separately dissolve about 20 mg of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS accurately weighed in 6 mL of methanol, and dilute with water to obtain solutions (*Standard stock solution A* and *B*) having known concentrations of about 0.1 mg per mL of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS, respectively.

**Standard solution**—Mix 25 mL of *Standard stock solution B* and x25 mL of *Standard stock solution A*, and dilute with water to 200 mL, x being the ratio of the respective labeled amounts, in mg, of fosinopril sodium to that of hydrochlorothiazide per Tablet.

**Resolution solution**—Transfer 5 mg of USP Fosinopril Related Compound H RS into a 100-mL volumetric flask, and dissolve in 5 mL of methanol. Add 2.0 mL of *Standard stock solution B*, dilute with water to volume, and mix.

**Test solution**—Use portions of the solution under test passed through a 1.2- $\mu$ m acrylic filter. [NOTE—Do not use glass filters.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a variable wavelength detector set at 210 nm and 272 nm and a 4.6-mm  $\times$  25-cm column, maintained at 40° that contains 5- $\mu$ m packing L10. The flow rate is about 1.3 mL per minute. With the detector set at 215 nm, chromatograph the *Resolution solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the fosinopril related compound H peak and the hydrochlorothiazide peak is not less than 1.5; and the relative standard deviation for replicate injections of the *Standard solution* is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Test solution* and the *Standard solution*, and record the chromatograms with the detector set at 272 nm from 0 to 5 minutes and at 210 nm from 5 to 9 minutes, for hydrochlorothiazide and fosinopril sodium, respectively. Measure the responses for the major peaks, and calculate the amount of  $C_{30}H_{45}NNaO_7P$  and of  $C_7H_8ClN_3O_4S_2$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{30}H_{45}NNaO_7P$  and not less than 75% (Q) of the labeled amount of  $C_7H_8ClN_3O_4S_2$  are dissolved in 30 minutes.

**Uniformity of dosage units:** meet the requirements.

### Related compounds—

**Solution A, Solution B, Diluent 2, and Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve accurately weighed quantities of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS in a suitable volumetric flask in *Diluent 2* to obtain a solution having a known concentration of about 0.004 mg per mL of each USP Reference Standard.

**System suitability solution**—Dissolve accurately weighed quantities of USP Chlorothiazide RS, USP Hydrochlorothiazide RS, USP Fosinopril Sodium RS, USP Fosinopril Related Compound A RS, and USP Benzothiadiazine Related Compound A RS in a suitable volumetric flask in *Diluent 2*. Dilute stepwise, if necessary, to obtain a solution having a known concentration of about 0.005 mg per mL of USP Chlorothia-

zide RS, USP Fosinopril Related Compound A RS, and USP Benzothiadiazine Related Compound A RS and about 0.5 mg per mL of USP Hydrochlorothiazide RS and of USP Fosinopril Sodium RS.

**Test solution**—Use the Assay preparation.

**Chromatographic system**—Proceed as directed in the Assay. Chromatograph the Standard solution and the System suitability solution, and record the peak responses as directed for Procedure: the resolution, *R*<sub>s</sub>, between chlorothiazide and hydrochlorothiazide is not less than 1.8; and the relative standard deviation for replicate injections is not more than 10.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses eluting between 2.7 minutes and 27 minutes. Calculate the percentage of any impurity or degradation product in the portion of Tablets taken by the formula:

$$100(r_U / r_S)(C_S / C_U)(1/F)$$

in which *r*<sub>U</sub> is the individual peak response for each impurity obtained from the Test solution; *r*<sub>S</sub> is the response of the fosinopril sodium or hydrochlorothiazide in the Standard solution; *F* is the relative response factor of each impurity relative to fosinopril sodium or hydrochlorothiazide as mentioned in Table 1; and *C*<sub>S</sub> and *C*<sub>U</sub> are the concentrations, in mg per mL, of fosinopril sodium or hydrochlorothiazide in the Standard solution and the Test solution, respectively. The limits of impurities are specified in Table 1.

**Assay—**

**Solution A**—Prepare a filtered and degassed solution of 0.01 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Diluent 1**—Use a mixture of water and acetonitrile (2:1).

**Diluent 2**—Use a mixture of 0.001 N hydrochloric acid and acetonitrile (2:1).

**Standard preparation**—Transfer into two separate suitable volumetric flasks accurately weighed quantities of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS. Dissolve USP Fosinopril Sodium RS in Diluent 1 and USP Hydrochlorothiazide RS in Diluent 2 respectively, to obtain stock solutions having known concentrations of about 2.0 mg per mL. Dilute quantitatively, and stepwise if necessary, portions of these two solutions with Diluent 2 to obtain a solution having a known concentration of 0.08 mg per mL of USP Fosinopril Sodium RS and of USP Hydrochlorothiazide RS.

**System suitability solution**—Dissolve accurately weighed quantities of USP Chlorothiazide RS, USP Hydrochlorothiazide RS, and USP Fosinopril Sodium RS in a suitable volumetric flask in Diluent 2 to obtain a solution having a known concentration of about 0.08 mg per mL of USP Hydrochlorothiazide RS and of USP Fosinopril Sodium RS and 0.025 mg per mL of the USP Chlorothiazide RS.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of fosinopril and 12.5 mg of hydrochlorothiazide, to a 100-mL volumetric flask, add about 75 mL of Diluent 2, and sonicate for about 10 minutes. Shake by mechanical means for 15 minutes. Dilute with Diluent 2 to volume, and mix. Pass a portion of this solution through a filter (PTFE or PVDF) having a 0.45-µm or finer porosity, and collect the rest of the filtrate. Dilute further, stepwise if necessary, to obtain a solution having a known concentration of about 0.075 mg of fosinopril sodium.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 206-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–2	88	12	isocratic
2–20	88→10	12→90	linear gradient
20–28	10	90	isocratic
28–37	10→88	90→12	linear gradient

Chromatograph the Standard preparation and the System suitability solution (about 20 µL), and record the peak responses as directed for Procedure: the resolution, *R*<sub>s</sub>, between the chlorothiazide and hydrochlorothiazide peaks is not less than 1.8; the tailing factor is less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of fosinopril sodium (*C*<sub>30</sub>H<sub>45</sub>NNaO<sub>7</sub>P) in the portion of the Tablets taken by the formula:

$$100(r_U / r_S)(C_S / C_U)$$

in which *r*<sub>U</sub> and *r*<sub>S</sub> are the peak responses of fosinopril sodium in the Assay preparation and the Standard preparation, respectively; and *C*<sub>S</sub> and *C*<sub>U</sub> are the concentrations, in mg per mL, of fosinopril sodium in the Standard preparation and the Assay preparation, respectively. Calculate the percentage

Table 1

Component	Relative Retention Time (RRT)	Relative Response Factor (RRF)	Limit (%)
Benzothiadiazine related compound A <sup>1</sup>	0.81 (relative to hydrochlorothiazide)	1.0	NMT 0.5
Fosinopril related compound A <sup>2</sup>	0.72 (relative to fosinopril)	1.2	NMT 4
Chlorothiazide <sup>3</sup>	0.90 (relative to hydrochlorothiazide)	1.7	NMT 0.3
Any other individual impurity	—	—	NMT 0.2 (relative to hydrochlorothiazide)
Total of all impurities	—	—	NMT 5.0

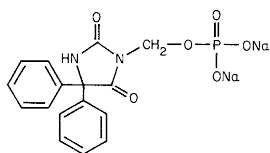
<sup>1</sup>4-Amino-6-chloro-1,3-benzenedisulfonamide.  
<sup>2</sup>(4S)-4-Cyclohexyl-1-[-(4-phenylbutyl)phosphinyl]acetyl-L-proline.  
<sup>3</sup>6-Chloro-2(H)-1,2,4-benzothiazine-7-sulfonamide.

of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of the Tablets taken by the formula:

$$100(r_U / r_S)(C_S / C_U)$$

in which  $r_U$  and  $r_S$  are the peak responses of hydrochlorothiazide in the *Assay preparation* and the *Standard preparation*, respectively; and  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of hydrochlorothiazide in the *Standard preparation* and the *Assay preparation*, respectively.

## Fosphenytoin Sodium



$C_{16}H_{13}N_2Na_2O_6P$  406.24

2,4-Imidazolidinedione, 5,5-diphenyl-3-[(phosphonoxy)methyl]-, disodium salt.

3-(Hydroxymethyl)-5,5-diphenylhydantoin, disodium phosphate (ester) [92134-98-0].

» Fosphenytoin Sodium contains not less than 98.0 percent and not more than 102.0 percent of  $C_{16}H_{13}N_2Na_2O_6P$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Fosphenytoin Sodium RS

USP Phenytoin RS

USP Phenytoin Related Compound A RS  
Diphenylglycine.

$C_{14}H_{15}NO_2$  227.26

USP Phenytoin Related Compound B RS

Diphenylhydantoic acid.

$C_{15}H_{14}N_2O_3$  270.29

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** It meets the requirements of the tests for *Sodium* (191).

**pH** (791): between 8.5 and 9.5, in a solution containing 75 mg per mL.

**Water**, *Method I* (921): between 21.7% and 25.7%.

**Heavy metals**, *Method I* (231): 0.002%.

**Related compounds**—

*Buffer solution* and *Mobile phase*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve accurately weighed quantities of USP Phenytoin Related Compound A RS, USP Phenytoin Related Compound B RS, and USP Phenytoin RS in *Mobile phase*; and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 3.0 µg per mL, 3.0 µg per mL, and 1.5 µg per mL, respectively.

*Test solution*—Transfer about 150 mg of Fosphenytoin Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system*—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak response as directed for *Procedure*: identify the peaks due to phenytoin related compound A, phenytoin, and phenytoin related compound B; the order of elution is phenytoin related compound A, phenytoin, followed by phenytoin related compound B; the resolution,  $R$ , between phenytoin and phenytoin related compound B is not less than 2.0; and the relative standard deviation is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for not less than six times the retention time of the major peak, and measure all the peak responses. The order of elution is phenytoin related compound A, phenytoin, followed by phenytoin related compound B, followed by the major peak due to fosphenytoin. Calculate the percentage of phenytoin, phenytoin related compound B, and phenytoin related compound A, if present, in the portion of Fosphenytoin Sodium taken by the formula:

$$100(C_S / C_U)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the USP Reference Standard of the respective impurity in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of Fosphenytoin Sodium in the *Test solution*; and  $r_i$  and  $r_S$  are the peak responses for each impurity obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% of phenytoin is found; not more than 0.1% of any other impurity is found; and not more than 0.5% of total impurities is found. [NOTE—Use the peak area and concentration of USP Phenytoin RS in the *Standard solution* as  $r_S$  and  $C_S$ , respectively, to calculate the percentage of the unknown impurities.]

**Assay**—

*Buffer solution*—Dissolve 6.80 g of monobasic potassium phosphate and 30 mL of 0.5 M dodecyltriethylammonium phosphate in 900 mL of water, adjust with 1.5 M phosphoric acid to a pH of about 5.0, dilute with water to 1000 mL, mix, and filter.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Fosphenytoin Sodium RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL.

*Assay preparation*—Transfer about 150 mg of Fosphenytoin Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a nominal concentration of about 0.15 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from fosphenytoin sodium is not less than 5000 theoretical plates; the tailing factor for the fosphenytoin sodium peak is not more than 1.6; and the relative standard deviation for replicate injections is not more than 0.5%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in percentage, of  $C_{16}H_{13}N_2Na_2O_6P$  in the portion of Fosphenytoin Sodium taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which  $C_s$  is the concentration, in mg per mL, of USP Fosphenytoin Sodium RS in the *Standard preparation*;  $C_u$  is the nominal concentration of fosphenytoin, in mg per mL, in the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fosphenytoin Sodium Injection

» Fosphenytoin Sodium Injection is a sterile solution of Fosphenytoin Sodium in Water for Injection. Fosphenytoin Sodium is a prodrug. Injection containing 1 mg per mL of Fosphenytoin Sodium is equivalent to 0.667 mg per mL of Phenytoin Sodium after injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fosphenytoin sodium ( $C_{16}H_{13}N_2Na_2O_6P$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store between 2° and 8°. Do not store at room temperature for more than 48 hours.

**Labeling**—Both the actual content of Fosphenytoin Sodium and the content of Phenytoin Sodium, expressed in terms of phenytoin sodium equivalents, are stated prominently on the label.

### USP Reference standards (11)—

USP Fosphenytoin Sodium RS

USP Phenytoin RS

USP Phenytoin Related Compound A RS

Diphenylglycine.

$C_{14}H_{13}NO_2$  227.26

USP Phenytoin Related Compound B RS

Diphenylhydantoic acid.

$C_{15}H_{14}N_2O_3$  270.29

### Identification—

**A: Infrared Absorption** (197K)—

*Test specimen*—Transfer a 5-mL aliquot of Injection to a 100-mL beaker, add 30 mL of acetone to form a white precipitate, and stir for 20 minutes using a magnetic stirrer. Filter in vacuum, and collect the precipitate using suitable filter paper. Allow to dry in vacuum for 15 minutes.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85): not more than 14 USP Endotoxin Units per mL.

**pH** (791): between 8.3 and 9.3.

### Related compounds—

*Buffer solution*, *Mobile phase*, *Standard stock solution 1*, *Standard stock solution 2*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Procedure*—Inject a volume (about 40  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentages of phenytoin, phenytoin related

compound A, phenytoin related compound B, and unknown impurities in each mL of Injection taken by the formula:

$$200,000(C/VL)(r_i / r_s)$$

in which  $C$  is the concentration, in mg per mL, of the respective impurity in the *Standard solution*;  $V$  is the volume, in mL, of the Injection taken to prepare the *Test solution*;  $L$  is the labeled amount, in mg per mL, of fosphenytoin sodium in the Injection; and  $r_i$  and  $r_s$  are the individual peak responses of the impurities in the chromatograms obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.5% of phenytoin related compound B is found; not more than 0.2% of phenytoin is found; not more than 0.2% of phenytoin related compound A is found; not more than 0.1% of any individual unknown impurity is found; and not more than 2.0% total impurities is found. [NOTE—Use the peak area and concentration of the USP Phenytoin RS in the *Standard solution* as  $r_s$  and  $C$ , respectively, to calculate the percentage of the unknown impurities.]

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

*Buffer solution*—Dissolve about 8.2 g of monobasic potassium phosphate in 1 L of water. Adjust with 6 N potassium hydroxide solution to a pH of  $6.5 \pm 0.05$ .

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution*, methanol, and acetonitrile (73:25:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard stock solution 1*—Dissolve an accurately weighed quantity of USP Fosphenytoin Sodium RS in methanol, and dilute quantitatively, and stepwise if necessary, with *Buffer solution* to obtain a solution having a known concentration of about 0.75 mg per mL.

*Standard stock solution 2*—Dissolve an accurately weighed quantity of USP Phenytoin RS, USP Phenytoin Related Compound A RS, and USP Phenytoin Related Compound B RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.0075 mg per mL, 0.0075 mg per mL, and 0.015 mg per mL, respectively.

*Standard preparation*—Transfer 10.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 50-mL volumetric flask. Dilute with *Buffer solution* to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of the Injection, equivalent to about 300 mg of fosphenytoin, to a 200-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask. Dilute with *Buffer solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 1.25 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for phenytoin related compound B, about 0.5 for phenytoin related compound A, 1.0 for fosphenytoin, and about 3.8 for phenytoin; the resolution,  $R$ , between phenytoin related compound B and phenytoin related compound A is not less than 4.0; the column efficiency is not less than 2250 theoretical plates for the fosphenytoin peak; the tailing factor is not more than 1.8 for the fosphenytoin peak; and the relative standard deviation for replicate injections is not more than 1.0% for the fosphenytoin peak and not more than 5.0% for the phenytoin related compound B, phenytoin related compound A, and phenytoin peaks.

*Procedure*—Separately inject equal volumes (about 40  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

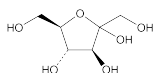


ure the peak areas for fosphenytoin. Calculate the quantity, in mg, of fosphenytoin sodium ( $C_{16}H_{13}N_2Na_2O_6P$ ) in each mL of the Injection taken by the formula:

$$2000(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Fosphenytoin Sodium RS in the *Standard preparation*; V is the volume, in mL, of the Injection taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the fosphenytoin peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Fructose



$C_6H_{12}O_6$  180.16  
D-Fructose [57-48-7].

### DEFINITION

Fructose, dried in vacuum at 70° for 4 h, contains NLT 98.0% and NMT 102.0% of fructose ( $C_6H_{12}O_6$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197K>  
Sample: Previously dried  
Acceptance criteria: Meets the requirements

### ASSAY

- **PROCEDURE**  
Sample: 10 g, previously dried  
Optical rotation  
(See *Optical Rotation*, *Angular Rotation* <781A>.)  
Tube length: 100 mm  
Temperature: 25°  
Analysis: Transfer the *Sample* to a 100-mL volumetric flask, and dissolve in 50 mL of water. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume. After 30 min, determine the angular rotation. The observed rotation, in degrees, represents the weight, in g.  
Calculate the percentage of fructose ( $C_6H_{12}O_6$ ) in the Fructose taken.

$$\text{Result} = [(a \times F)/W] \times 100$$

$a$  = observed rotation of the sample (°)

$F$  = correction factor, -1.124

$W$  = weight of the *Sample* (g)

Acceptance criteria: 98.0%–102.0% on a previously dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** <281>: NMT 0.5%
- **CHLORIDE AND SULFATE**, *Chloride* <221>  
Sample: 2.0 g  
Control: 0.50 mL of 0.020 N hydrochloric acid  
Acceptance criteria: NMT 0.018%; the *Sample* shows no more chloride than corresponds to the *Control*.

- **CHLORIDE AND SULFATE**, *Sulfate* <221>  
Sample: 2.0 g  
Control: 0.50 mL of 0.020 N sulfuric acid  
Acceptance criteria: NMT 0.025%; the *Sample* shows no more sulfate than corresponds to the *Control*.
  - **ARSENIC**, *Method II* <211>  
Test preparation: Prepare by heating the acidified mixture nearly to dryness. Cool, and add 30% hydrogen peroxide.  
Acceptance criteria: NMT 1 µg/g
  - **CALCIUM AND MAGNESIUM (AS CALCIUM)**  
Sample: 20 g  
Titrimetric system  
Mode: Direct titration  
Titrant: 0.005 M edetate disodium VS  
Endpoint detection: Visual  
Analysis: Dissolve the *Sample* in 200 mL of water. Add 2 drops of hydrochloric acid, 5 mL of ammonia–ammonium chloride buffer TS, and 8 drops of eriochrome black TS, and mix. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 200.4 µg of Ca.  
Acceptance criteria: 0.005% of Ca; NMT 5.0 mL of *Titrant* is consumed.
  - **HEAVY METALS**, *Method I* <231>  
Test preparation: 4 g of Fructose in 23 mL of water. Add 2 mL of 1 N acetic acid.  
Acceptance criteria: NMT 5 µg/g
  - **LIMIT OF HYDROXYMETHYLFURFURAL**  
Resorcinol solution: 10 mg/mL of resorcinol in hydrochloric acid  
Sample solution: 100 mg/mL  
Analysis: To 10 mL of *Sample solution* add 5 mL of ether, and shake vigorously. Transfer 2 mL of the ether layer to a test tube, and add 1 mL of *Resorcinol solution*.  
Acceptance criteria: A slight pink color may develop, but no cherry-red color appears immediately.
- ### SPECIFIC TESTS
- **COLOR OF SOLUTION**  
Sample solution: 25 g of Fructose in water to make 50 mL  
Comparison solution: Mix 1.0 mL of cobaltous chloride CS, 3.0 mL of ferric chloride CS, and 2.0 mL of cupric sulfate CS with water to make 10 mL, and dilute 3 mL of this solution with water to make 50 mL.  
Analysis: View the *Sample solution* and *Comparison solution* downward in matched color-comparison tubes against a white surface.  
Acceptance criteria: The *Sample solution* has no more color than the *Comparison solution*.
  - **ACIDITY**  
Sample: 5.0 g  
Titrimetric system  
Mode: Direct titration  
Titrant: 0.02 N sodium hydroxide VS  
Endpoint detection: Visual  
Analysis: Dissolve the *Sample* in 50 mL of carbon dioxide-free water. Add phenolphthalein TS, and titrate with *Titrant* to a distinct pink color.  
Acceptance criteria: NMT 0.50 mL of *Titrant* is needed for neutralization.

• **Loss on Drying** (731)

Analysis: Dry under vacuum at 70° for 4 h.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** (11)  
USP Fructose RS

## Fructose Injection

» Fructose Injection is a sterile solution of Fructose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of fructose ( $C_6H_{12}O_6$ ). Fructose Injection contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Fructose RS

**Identification**—

*Silver nitrate solution*—Dissolve 0.6 g of silver nitrate in 2.0 mL of water in a 100-mL volumetric flask, dilute with acetone to volume, and mix.

*Sodium hydroxide solution*—Dissolve 2.0 g of sodium hydroxide in 5.0 mL of water in a 100-mL volumetric flask, dilute with alcohol to volume, and mix.

*Alcoholic monobasic sodium phosphate*—Dissolve 4.0 g of monobasic sodium phosphate in 150 mL of water, and add, with mixing, 500 mL of alcohol. Use the entire mixture, even if there are two phases. Prepare the solution fresh daily.

*Standard preparation*—Prepare an aqueous solution containing 2.5 mg of USP Fructose RS per mL.

*Test preparation*—Dilute an accurately measured volume of Injection with water to obtain a solution having a known concentration of about 2.5 mg of fructose per mL.

*Procedure*—Immerse a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel, coated side down, in *Alcoholic monobasic sodium biphosphate* for 2.0 minutes. Remove the plate from the solution, place it, coated side up, on a clean, absorbent towel, dry it in a current of warm air, and activate it at 105° for 30 minutes. Cool to room temperature, and apply 5  $\mu$ L each of the *Standard preparation* and the *Test preparation*. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone, *n*-butyl alcohol, and water (50:40:10). Remove the plate, allow the solvent to evaporate, and spray the plate with *Silver nitrate solution*. Allow the plate to dry for 30 seconds, then spray with *Sodium hydroxide solution*: the  $R_f$  value of the spot appearing within 3 minutes from the *Test preparation* corresponds to that obtained from the *Standard preparation*.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 3.0 and 6.0, determined on a portion to which 0.30 mL of a saturated potassium chloride solution

has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of fructose.

**Heavy metals, Method I** (231)—Place a volume of Injection, equivalent to 4 g of fructose, in a porcelain dish, and evaporate to a volume of about 10 mL. Cool, and dilute with water to 25 mL: the limit is 5 ppm.

**Limit of hydroxymethylfurfural**—It meets the requirements of the test for *Hydroxymethylfurfural* under *Fructose*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to 5 g of fructose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. After 30 minutes determine the angular rotation (see *Optical Rotation* (781)), and record the observed rotation,  $a$ , as an absolute number. Calculate the quantity, in mg, of fructose ( $C_6H_{12}O_6$ ) in each mL of the Injection taken by the formula:

$$1124a / lV$$

in which  $l$  is the length, in dm, of the polarimeter tube; and  $V$  is the volume, in mL, of Injection taken.

## Fructose and Sodium Chloride Injection

» Fructose and Sodium Chloride Injection is a sterile solution of Fructose and Sodium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of  $C_6H_{12}O_6$  (fructose) and of NaCl (sodium chloride). Fructose and Sodium Chloride Injection contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Fructose RS

**Identification**—It responds to the *Identification* tests under *Fructose Injection* and to the tests for *Sodium* (191) and for *Chloride* (191).

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

**pH** (791): between 3.0 and 6.0.

**Heavy metals, Method I** (231)—Proceed as directed in the test for *Heavy metals* under *Fructose Injection*: the limit is 5 ppm.

**Limit of hydroxymethylfurfural**—It meets the requirements of the test for *Hydroxymethylfurfural* under *Fructose*.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for fructose**—Transfer an accurately measured volume of Injection, containing about 5 g of fructose, to a 100-mL volumetric flask, add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. After 30 minutes determine the angular rotation (see *Optical Rotation* (781)), and record the observed rotation,  $a$ , as an abso-

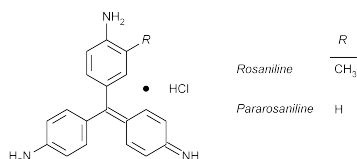
lute number. Calculate the quantity, in mg, of fructose ( $C_6H_{12}O_6$ ) in each mL of the Injection taken by the formula:

$$1124a / IV$$

in which  $l$  is the length, in dm, of the polarimeter tube; and  $V$  is the volume, in mL, of Injection taken.

**Assay for sodium chloride**—Transfer an accurately measured volume of Injection, equivalent to about 90 mg of sodium chloride, to a conical flask, evaporate to a volume of about 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

## Basic Fuchsin



Benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl-2-methyl]-, monohydrochloride.

C.I. Basic Violet 14 monohydrochloride [632-99-5].

» Basic Fuchsin is a mixture of rosaniline and pararosaniline hydrochlorides. It contains the equivalent of not less than 88.0 percent of rosaniline hydrochloride ( $C_{20}H_{19}N_3 \cdot HCl$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### Identification—

**A:** To 5 mL of a solution (1 in 1000) add a few drops of hydrochloric acid: a yellow color is produced (*distinction from acid fuchsin*).

**B:** To 5 mL of a solution (1 in 500) add a few drops of tannic acid TS: a red precipitate is formed.

**C:** To 10 mL of a solution (1 in 500) add 10 mL of ammonia TS and 500 mg of zinc dust, and agitate the mixture: the solution becomes decolorized. Place a few drops of the decolorized solution on filter paper, and nearby on the same paper place a few drops of 3 N hydrochloric acid: a red color develops at the zone of contact.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 5.0% of its weight.

**Residue on ignition** (281)—Ignite 1 g with 0.5 mL of sulfuric acid: the weight of the residue is not more than 0.3%.

**Alcohol-insoluble substances**—Boil 1 g, accurately weighed, with 50 mL of alcohol under a reflux condenser for 15 minutes, filter through a tared filtering crucible, wash the residue on the filter with hot alcohol until the washings cease to be colored violet, and dry the crucible at 105° for 1 hour: the amount of insoluble residue is not more than 1.0%.

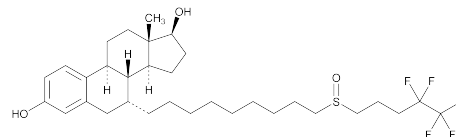
**Arsenic, Method II** (211): 8 ppm.

**Lead** (251): Place 1 g in a small Kjeldahl flask, add 5 mL of sulfuric acid, and insert a small funnel into the flask. Gently rotate the flask until the sulfuric acid has completely wetted the Basic Fuchsin, then heat with a small flame until carbonization is complete. Allow to cool, and add, in small quantities, 5 mL of nitric acid. Again heat gently until fumes

of sulfur trioxide are evolved. Allow to cool, add another 5 mL of nitric acid, and heat to the evolution of sulfur trioxide. Allow to cool, add about 25 mL of water, and boil for a few minutes. Cool, neutralize with stronger ammonia water, using litmus paper as the indicator, and add 5 mL of nitric acid. Transfer the solution to a 100-mL volumetric flask, dilute to volume, and mix. A 20-mL portion of this solution contains not more than 30 ppm of lead.

**Assay**—Dissolve about 100 mg of Basic Fuchsin, accurately weighed, in 175 mL of water in a 500-mL closed system titration vessel fitted with a gas inlet tube, a gas outlet tube, an upright reflux condenser, and a buret. Add about 25 mL of sodium tartrate solution (30 in 100) and a polytetrafluoroethylene-coated magnetic stirring bar, and heat to boiling. Flush this titration vessel for 15 minutes with nitrogen that has been passed through two successive gas washing bottles each containing 500 mL of a mixture of water, titanium trichloride solution (20 in 100), and hydrochloric acid (400:40:40) to which about 10 mg of safranin O has been added. Continue the heating and nitrogen flow, and while stirring titrate with 0.05 N titanium trichloride VS to a yellow endpoint. Each mL of 0.05 N titanium trichloride is equivalent to 3.379 mg of  $C_{20}H_{19}N_3 \cdot HCl$ .

## Fulvestrant



$C_{32}H_{47}F_5O_3S$  606.77

Estra-1,3,5(10)-triene-3,17-diol, 7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-, (7 $\alpha$ ,17 $\beta$ ); 7 $\alpha$ -[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17 $\beta$ -diol [129453-61-8].

» Fulvestrant is a mixture of the diastereoisomers A and B. It contains not less than 97.0 percent and not more than 102.0 percent of  $C_{32}H_{47}F_5O_3S$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store refrigerated at 2° to 8°.

### USP Reference standards (11)—

USP Fulvestrant RS

USP Fulvestrant System Suitability Mixture RS

Contains fulvestrant isomer A, fulvestrant isomer B, and fulvestrant  $\beta$ -isomer.

### Identification—

**A:** Infrared Absorption (197K)—

*Spectral range:* 4000 to 400  $cm^{-1}$ .

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between +108° and +115° measured at 365 nm.

*Test solution:* 20 mg per mL, in methanol.

**Water, Method 1c** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

### Related compounds—

*Mobile phase and System suitability solution*—Prepare as directed in the *Assay*.

*Standard solution*—Prepare as directed for the *Standard preparation* in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fulvestrant taken by the formula:

$$100(CV/W)(1/F)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Fulvestrant RS in the *Standard solution*; *V* is the volume, in mL, of the *Test solution*; *W* is the weight, in mg, of Fulvestrant taken to prepare the *Test solution*; *F* is the relative response factor as listed in the accompanying table; *r<sub>i</sub>* is the individual peak response for each impurity obtained from the *Test solution*; and *r<sub>s</sub>* is the fulvestrant peak response obtained from the *Standard solution*. Disregard impurity peaks less than 0.05%. The limits are as shown in the accompanying table.

Compound	Relative Retention Time	Relative Response Factor	Limit (%)
6-Keto-fulvestrant <sup>1</sup>	0.5	2.9	0.1
$\Delta$ 6,7-Fulvestrant <sup>2</sup>	0.9	3.3	0.1
Fulvestrant	1.0	1.0	—
Fulvestrant sulfone <sup>3</sup>	1.2	1.0	0.2
Fulvestrant extended <sup>4</sup>	1.7	1.0	0.3
Fulvestrant sterol dimer <sup>5</sup>	1.9	1.0	0.8
Fulvestrant $\beta$ -isomer <sup>6</sup>	1.1	—	*
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

<sup>1</sup>Estra-1,3,5(10)-triene-6-one-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7 $\alpha$ ,17 $\beta$ )

<sup>2</sup>Estra-1,3,5(10),6-tetraene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7 $\alpha$ ,17 $\beta$ )

<sup>3</sup>Estra-1,3,5(10)-triene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfonyl]nonyl]-(7 $\alpha$ ,17 $\beta$ )

<sup>4</sup>Estra-1,3,5(10)-triene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7 $\alpha$ ,17 $\beta$ )

<sup>5</sup>7,7-Nonamethylene-bis(estra-1,3,5(10)-triene-3,17-diol-(7 $\alpha$ ,17 $\beta$ ))

<sup>6</sup>Estra-1,3,5(10)-triene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7 $\beta$ ,17 $\beta$ )

\*Fulvestrant  $\beta$ -isomer, a component of USP Fulvestrant System Suitability Mixture RS, is not a specified impurity.

### Diastereoisomer ratio—

**Mobile phase**—Prepare a filtered and degassed mixture of 2-methylpentane and dehydrated alcohol (880:120). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve a suitable quantity of USP Fulvestrant System Suitability Mixture RS in *Mobile phase* to obtain a solution containing 1 mg of USP Fulvestrant System Suitability Mixture RS per mL.

**Test solution**—Transfer about 20 mg of Fulvestrant, accurately weighed, to a 20-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu$ m packing L51. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between fulvestrant isomer A and fulvestrant isomer B is not less than 1.3; and the tailing factor for fulvestrant isomer B is not more than 1.5.

[NOTE—For the purpose of peak identification, the retention times are about 20 minutes for fulvestrant isomer B and 23 minutes for fulvestrant isomer A.]

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the two fulvestrant isomer peaks. Calculate the content of fulvestrant isomer A or fulvestrant isomer B, as a percentage, by the formula:

$$100(r_U / r_S)$$

in which *r<sub>U</sub>* is the peak response of either fulvestrant isomer A or fulvestrant isomer B; and *r<sub>S</sub>* is the total peak response of both fulvestrant isomer A and fulvestrant isomer B: between 42% and 48% of fulvestrant isomer A and between 52% and 58% of fulvestrant isomer B is obtained.

### Assay—

**Solution A**—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (410:320:270).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (490:410:100).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve suitable quantities of USP Fulvestrant System Suitability Mixture RS in methanol to obtain a solution containing about 10 mg of USP Fulvestrant System Suitability Mixture RS per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Fulvestrant RS in methanol to obtain a solution having a known concentration of about 10 mg per mL.

**Assay preparation**—Transfer about 100 mg of Fulvestrant, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm  $\times$  15-cm column that contains 3.5- $\mu$ m packing L7. The flow rate is about 2 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–25	100	0	isocratic
25–55	100→0	0→100	linear gradient
55–65	0	100	isocratic
65–66	0→100	100→0	linear gradient
66–70	100	0	equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.1 for fulvestrant  $\beta$ -isomer and 1.0 for fulvestrant; the resolution, *R*, between fulvestrant and fulvestrant  $\beta$ -isomer is not less than 1.5; and the tailing factor for the fulvestrant peak is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

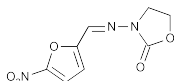
**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the fulvestrant peaks. Calculate the quantity, in mg, of C<sub>32</sub>H<sub>47</sub>F<sub>5</sub>O<sub>3</sub>S in the portion of Fulvestrant taken by the formula:

$$CV(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Fulvestrant RS in the *Standard preparation*; *V* is the volume, in mL, of the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak

responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Furazolidone



$C_8H_7N_3O_5$  225.16

2-Oxazolidinone, 3-[[[5-nitro-2-furanyl)methylene]amino]-3-[(5-Nitrofurfurylidene)amino]-2-oxazolidinone [67-45-8].

» Furazolidone contains not less than 97.0 percent and not more than 103.0 percent of  $C_8H_7N_3O_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight.

### USP Reference standards <11>—

USP Furazolidone RS

#### Identification—

**A:** *Infrared Absorption* <197K>: previously dried.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

**C:** Add about 50 mg to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution becomes purple, immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.

**Loss on drying** <731>—Dry it at 100° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.25%.

**Assay**—Transfer about 100 mg of Furazolidone, accurately weighed, to a 250-mL volumetric flask, dilute with dimethylformamide to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furazolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 400 µg per mL. Transfer 5.0 mL of this stock solution to a 250-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 50) as the blank. Calculate the quantity, in mg, of  $C_8H_7N_3O_5$  in the Furazolidone taken by the formula:

$$12.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Furazolidone RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

## Furazolidone Oral Suspension

» Furazolidone Oral Suspension is a suspension of Furazolidone in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more

than 110.0 percent of the labeled amount of furazolidone ( $C_8H_7N_3O_5$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

### USP Reference standards <11>—

USP Furazolidone RS

**Identification**—Add a quantity of Oral Suspension, equivalent to about 50 mg of furazolidone, to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution turns purple, immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.

**pH** <791>: between 6.0 and 8.5.

**Assay**—Transfer an accurately measured volume of Oral Suspension, equivalent to about 160 mg of furazolidone, to a suitable flask. Add 5 mL of water, and mix. Transfer the mixture with the aid of dimethylformamide to a 1000-mL volumetric flask. Add about 500 mL of dimethylformamide, shake by mechanical means for 10 minutes, dilute with dimethylformamide to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furazolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 160 µg per mL. Transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 20) as the blank. Calculate the quantity, in mg, of furazolidone ( $C_8H_7N_3O_5$ ) in each mL of the Oral Suspension taken by the formula:

$$20(C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Furazolidone RS in the Standard solution; V is the volume, in mL, of Oral Suspension taken; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

## Furazolidone Tablets

» Furazolidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_8H_7N_3O_5$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

### USP Reference standards <11>—

USP Furazolidone RS

**Identification**—Add a quantity of powdered Tablets, equivalent to about 50 mg of furazolidone, to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution turns purple, immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.

**Uniformity of dosage units** <905>: meet the requirements.

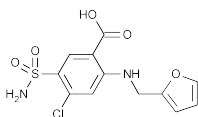
**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of furazolidone, to a 250-mL volumetric flask. Add about 150 mL of dimethylformamide, warm to about 50°, and sonicate to aid in dissolving the furazolidone. Cool, dilute with dimethylformamide to volume, mix, and centrifuge a portion of the mixture. Transfer

5.0 mL of the clear solution so obtained to a 250-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furozolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 400 µg per mL. Transfer 5.0 mL of this stock solution to a 250-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 50) as the blank. Calculate the quantity, in mg, of  $C_8H_7N_3O_5$  in the portion of Tablets taken by the formula:

$$12.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Furozolidone RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

## Furosemide



$C_{12}H_{11}ClN_2O_5S$  330.74

Benzoic acid, 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]-.

4-Chloro-N-furfuryl-5-sulfamoylanthranilic acid [54-31-9].

» Furosemide contains not less than 98.0 percent and not more than 101.0 percent of  $C_{12}H_{11}ClN_2O_5S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Furosemide RS

USP Furosemide Related Compound A RS

2-Chloro-4-N-furfurylamino-5-sulfamoylbenzoic acid.

$C_{12}H_{11}ClN_2O_5S$  330.74

USP Furosemide Related Compound B RS

4-Chloro-5-sulfamoylanthranilic acid.

$C_7H_7ClN_2O_4S$  250.66

### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 8 µg per mL.

*Medium:* 0.02 N sodium hydroxide.

Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Dissolve about 5 mg in 10 mL of methanol. Transfer 1 mL of this solution to a flask, add 10 mL of 2.5 N hydrochloric acid, and reflux on a steam bath for 15 minutes. Cool, and add 15 mL of 1 N sodium hydroxide and 5 mL of sodium nitrite solution (1 in 1000). Allow the mixture to stand for 3 minutes, add 5 mL of ammonium sulfamate solution (1 in 200), mix, and add 5 mL of freshly prepared N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000): a red to red-violet color is produced.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

**Related compounds**—[NOTE—Protect Furosemide solutions from exposure to light.]

**Mobile phase**—Prepare a filtered and degassed mixture of water, tetrahydrofuran, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluting solution**—Dilute 22 mL of glacial acetic acid with a mixture of acetonitrile and water (50:50) to 1000 mL, and mix.

**System suitability solution**—Dissolve suitable quantities of USP Furosemide RS and USP Furosemide Related Compound A RS in *Diluting solution* to obtain a solution containing about 20 µg per mL and 12 µg per mL, respectively.

**Standard solution**—Prepare a solution in *Diluting solution* containing 5.0 µg each of USP Furosemide Related Compound A RS and USP Furosemide Related Compound B RS per mL.

**Test solution**—Transfer an accurately weighed quantity of Furosemide to a suitable volumetric flask, dissolve in and dilute with *Diluting solution* to volume to obtain a solution having a concentration of about 1.0 mg per mL, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector capable of recording at both 254 nm and 272 nm and a 4.6-mm × 25-cm column that contains packing L1. [NOTE—The 2,4-dichloro-5-sulfamoylbenzoic acid impurity does not respond at 272 nm and the 2,4-bis(furfurylamino)-5-sulfamoylbenzoic acid impurity has a very intense absorbance at 254 nm.] The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between furosemide and furosemide related compound A is not less than 2.5; and the relative standard deviation determined from furosemide is not more than 2.0%. [NOTE—The response for furosemide is at 254 nm.]

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. [NOTE—The chromatographic run time is not less than 2.5 times the retention time of the furosemide peak.] The sum of the responses at 254 nm of those peaks eluting before furosemide in the chromatogram obtained from the *Test solution* is not more than the response at 254 nm of the furosemide related compound B peak in the chromatogram obtained from the *Standard solution* (0.5%). The sum of the responses at 272 nm of those peaks eluting after furosemide in the chromatogram obtained from the *Test solution* is not more than the response at 272 nm of the furosemide related compound A peak in the chromatogram obtained from the *Standard solution* (0.5%).

**Assay**—Dissolve about 600 mg of Furosemide, accurately weighed, in 50 mL of dimethylformamide to which has been added 3 drops of bromothymol blue TS, and which previously has been neutralized with 0.1 N sodium hydroxide. Titrate with 0.1 N sodium hydroxide VS to a blue endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 33.07 mg of  $C_{12}H_{11}ClN_2O_5S$ .

## Furosemide Injection

» Furosemide Injection is a sterile solution of Furosemide in Water for Injection prepared with the aid of Sodium Hydroxide or, where intended solely for veterinary use, Diethanolamine or Monoethanolamine. It contains not less than

90.0 percent and not more than 110.0 percent of the labeled amount of furosemide ( $C_{12}H_{11}ClN_2O_5S$ ).

**Packaging and storage**—Store in single-dose or multiple-dose, light-resistant containers, of Type I glass.

**Labeling**—Injection intended solely for veterinary use is so labeled.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Furosemide RS

USP Furosemide Related Compound A RS

2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.

$C_{12}H_{11}ClN_2O_5S$  330.74

USP Furosemide Related Compound B RS

4-Chloro-5-sulfamoylanthranilic acid.

$C_7H_7ClN_2O_4S$  250.66

**Identification**—Transfer to a 100-mL volumetric flask a volume of Injection, equivalent to about 40 mg of furosemide, dilute with water to volume, and mix. Dilute 2.0 mL of this solution with 0.02 N sodium hydroxide in a second 100-mL volumetric flask to volume, and mix. Dissolve about 10 mg of USP Furosemide RS in 6.0 mL of 0.1 N sodium hydroxide in a 25-mL volumetric flask, and dilute with water to volume. Dilute 2.0 mL of the resulting solution quantitatively with 0.02 N sodium hydroxide to obtain a Standard solution having a concentration of about 8 µg per mL. Concomitantly determine the UV absorption spectra of both solutions: the UV absorption spectra so obtained exhibit maxima and minima at the same wavelengths.

**Bacterial endotoxins** (85)—It contains not more than 3.6 USP Endotoxin Units per mg of furosemide.

**pH** (791): between 8.0 and 9.3 or, where labeled as intended solely for veterinary use, between 7.0 and 7.8 if it contains diethanolamine, or between 8.0 and 9.3 if it contains monoethanolamine.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Limit of furosemide related compound B**—[NOTE—Protect furosemide solutions from exposure to light.]

*Mobile phase, Diluting solution, System suitability solution and Chromatographic system*—Prepare as directed in the test for Related compounds under Furosemide.

*Standard solution*—Prepare a solution in Diluting solution containing 10.0 µg of USP Furosemide Related Compound B RS per mL.

*Test solution*—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add Diluting solution to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. The response at 254 nm obtained for any peak observed in the chromatogram of the Test solution at a retention time corresponding to that of the Reference Standard in the Standard solution is not greater than the response at 254 nm obtained for the peak in the chromatogram of the Standard solution, corresponding to not more than 1.0% of furosemide related compound B. Where the Injection is labeled as intended solely for veterinary use, the response at 254 nm obtained in the chromatogram of the Test solution at a retention time corresponding to that of the Reference Standard in the Standard solution is not greater than 2.5 times the response at 254 nm obtained for the peak in the chromatogram of the Standard solution, corresponding to not more than 2.5% of furosemide related compound B.

**Other requirements**—It meets the requirements under Injections (1).

**Assay**—[NOTE—Protect furosemide solutions from exposure to light.]

*Mobile phase, Diluting solution, System suitability solution, and Chromatographic system*—Prepare as directed in the test for Related compounds under Furosemide.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Furosemide RS in Diluting solution to obtain a solution having a known concentration of about 1.0 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add Diluting solution to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Using the response at 254 nm, calculate the quantity, in mg, of furosemide ( $C_{12}H_{11}ClN_2O_5S$ ) in each mL of the Injection taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Furosemide RS in the Standard preparation; V is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Furosemide Oral Solution

» Furosemide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furosemide ( $C_{12}H_{11}ClN_2O_5S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Furosemide RS

USP Furosemide Related Compound A RS

2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.

$C_{12}H_{11}ClN_2O_5S$  330.74

USP Furosemide Related Compound B RS

4-Chloro-5-sulfamoylanthranilic acid.

$C_7H_7ClN_2O_4S$  250.66

**Identification**—

**A: Ultraviolet Absorption** (197U)—

*Solution*: 6 µg per mL.

*Medium*: 0.01 N sodium hydroxide.

Absorptivities are not significantly different.

**B:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**Minimum fill** (755): meets the requirements.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 7.0 and 10.0.

**NOTE**—Throughout the following procedures, protect test or assay specimens, the USP Reference Standards, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**Limit of furosemide related compound B—**

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Furosemide Related Compound B RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 15.0 µg per mL.

*Test solution*—Transfer an accurately measured portion of Oral Solution, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks: the peak response of furosemide related compound B obtained from the *Test solution* is not greater than the corresponding peak response obtained from the *Standard solution*.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (165:35:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Diluting solution*—Prepare a mixture consisting of water, acetonitrile, and glacial acetic acid (22:22:1).

*System suitability solution*—Dissolve suitable quantities of USP Furosemide RS, USP Furosemide Related Compound A RS, and USP Furosemide Related Compound B RS in *Diluting solution* to obtain a solution containing about 0.1 mg of each per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Furosemide RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 1 mg per mL.

*Assay preparation*—Transfer an accurately measured portion of Oral Solution, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.2 for furosemide related compound B, 1.0 for furosemide, and 1.1 for furosemide related compound A; the resolution, *R*, between furosemide and furosemide related compound A is not less than 1.5; and the tailing factor for the furosemide peak is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of furosemide (C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S) in the portion of Oral Solution taken by the formula:

$$10C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Furosemide RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Furosemide Tablets**

» Furosemide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furosemide (C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—The labeling indicates with which *Dissolution* test the product complies. Tablets intended solely for veterinary use are so labeled.

**USP Reference standards** <11>—

USP Furosemide RS

USP Furosemide Related Compound A RS

2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.

C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S 330.74

USP Furosemide Related Compound B RS

4-Chloro-5-sulfamoylanthranilic acid.

C<sub>7</sub>H<sub>7</sub>ClN<sub>2</sub>O<sub>4</sub>S 250.66

**Identification**—Transfer a portion of finely powdered Tablets, equivalent to about 40 mg of furosemide, to a 100-mL volumetric flask. Add 25 mL of 0.1 N sodium hydroxide, and allow to stand for 30 minutes with occasional shaking. Dilute with water to volume, and mix. Filter the solution, discarding the first 10 mL of the filtrate, and transfer 2.0 mL to a second 100-mL volumetric flask. Add 0.02 N sodium hydroxide to volume, and mix. Proceed as directed in the *Identification* test under *Furosemide Injection*, beginning with "Dissolve about 10 mg of USP Furosemide RS."

**Dissolution** <711>—

*Test 1:* If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

*Medium:* pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 60 minutes.

*Procedure*—Determine the amount of C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S dissolved from UV absorbances at the isosbestic point at 274 nm on filtered portions of the solution under test, suitably diluted with pH 5.8 phosphate buffer, in comparison with a *Standard solution* having a known concentration of USP Furosemide RS in the same *Medium*.

*Tolerances*—Not less than 80% (*Q*) of the labeled amount of C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S is dissolved in 60 minutes.

*Test 2:* If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Tablets labeled as intended for veterinary use comply with this test.

*Apparatus 2:* 65 rpm.

*Medium, Time, and Procedure*—Proceed as directed under *Test 1*.

*Tolerances*—Not less than 80% (*Q*) of the labeled amount of C<sub>12</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>5</sub>S is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Limit of furosemide related compound B**—[NOTE—Protect furosemide solutions from exposure to light.]

*Mobile phase, Diluting solution, System suitability solution, and Chromatographic system*—Prepare as directed in the test for *Related compounds* under *Furosemide*.

*Standard solution*—Prepare a solution in *Diluting solution* containing 8.0 µg of USP Furosemide Related Compound B RS per mL.

*Test solution*—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add *Diluting solution* to volume, and mix.



**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The response at 254 nm obtained for any peak observed in the chromatogram of the *Test solution* at a retention time corresponding to that of the Reference Standard in the *Standard solution* is not greater than the response at 254 nm obtained for the peak in the chromatogram of the *Standard solution*, corresponding to not more than 0.8% of furosemide related compound B.

**Assay**—[NOTE—Protect furosemide solutions from exposure to light.]

*Mobile phase, Diluting solution, System suitability solution, and Chromatographic system*—Prepare as directed in the test for *Related compounds* under *Furosemide*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Furosemide RS in *Diluting solution* to obtain a solution having a known concentration of about 1.0 mg per mL.

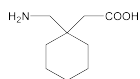
*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of furosemide, to a 50-mL volumetric flask, add 30 mL of *Diluting solution*, and sonicate for 10 minutes. Add *Diluting solution* to volume, mix, and filter, discarding the first 10 mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Using the response at 254 nm, calculate the quantity, in mg, of furosemide ( $C_{12}H_{11}ClN_2O_5S$ ) in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Furosemide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gabapentin



$C_9H_{17}NO_2$  171.24  
Cyclohexanecarboxylic acid, 1-(aminomethyl)-;  
1-(Aminomethyl)cyclohexanecarboxylic acid [60142-96-3].

### DEFINITION

Gabapentin contains NLT 98.0% and NMT 102.0% of gabapentin ( $C_9H_{17}NO_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Diluent:** 2.32 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 2.0.

**Buffer:** 0.58 g/L of monobasic ammonium phosphate and 1.83 g/L of sodium perchlorate in water. Adjust with perchloric acid to a pH of 1.8.

**Mobile phase:** Acetonitrile and *Buffer* (24:76)

**Standard solution:** 14.0 mg/mL of USP Gabapentin RS in *Diluent*

**System suitability solution:** 2.3 mg/mL of USP Gabapentin RS from the *Standard solution* in *Diluent*

**Sample solution:** 14 mg/mL of Gabapentin in *Diluent*

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Column efficiency:** NLT 1900 theoretical plates for the gabapentin peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the gabapentin peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of gabapentin ( $C_9H_{17}NO_2$ ) in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Gabapentin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS**, *Method II* (231): NMT 20 ppm
- EARLY-ELUTING IMPURITIES**

**Diluent, Buffer, and Mobile phase:** Prepare as directed in the *Assay*.

**Impurities solution:** 1.4 mg/mL of USP Gabapentin Related Compound A RS and 0.84 mg/mL of USP Gabapentin Related Compound B RS in methanol

**System suitability solution:** Dissolve a suitable quantity of USP Gabapentin RS in *Diluent* in a suitable volumetric flask, and add an appropriate volume of *Impurities solution* to obtain a solution containing 14.0 mg/mL of USP Gabapentin RS, 14 µg/mL of USP Gabapentin Related Compound A RS, and 8.4 µg/mL of USP Gabapentin Related Compound B RS in *Diluent*.

**Standard solution:** 8.4 µg/mL of USP Gabapentin Related Compound E RS in *Diluent*

**Sample solution:** 14 mg/mL of Gabapentin in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.3 between gabapentin related compound A and gabapentin related compound B

**Relative standard deviation:** NMT 2.0% for gabapentin

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of any impurity from the *Sample solution*

$r_S$  = peak area of gabapentin related compound E from the *Standard solution*

$C_S$  = concentration of gabapentin related compound E in the *Standard solution* (mg/mL)

$C_U$  = concentration of Gabapentin in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time <sup>a</sup>	Relative Response Factor <sup>b</sup>	Acceptance Criteria, NMT (%)
Gabapentin related compound E <sup>c</sup>	2.9	1.0	0.10
Gabapentin related compound A <sup>d</sup>	3.5	5.3	0.1
Gabapentin related compound B <sup>e</sup>	3.8	0.35	0.06
Gabapentin	1.0	—	—
Any other individual, unidentified impurity	—	0.41	0.10

<sup>a</sup> The relative retention times are calculated based on the retention time of gabapentin.

<sup>b</sup> The relative response factors are calculated based on the response of gabapentin related compound E due to the low absorptivity of gabapentin at the monitoring wavelength (215 nm).

<sup>c</sup> Carboxymethyl-cyclohexanecarboxylic acid.

<sup>d</sup> 2-Aza-spiro[4.5]decan-3-one.

<sup>e</sup> (1-Cyano-cyclohexyl)-acetic acid.

### LATE-ELUTING IMPURITIES

**Diluent and Buffer:** Prepare as directed in the *Assay*.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (35:30:35)

**Standard solution:** 2.8 µg/mL of USP Gabapentin Related Compound D RS in *Diluent*. Initially dissolve the USP Gabapentin Related Compound D RS in a small amount of methanol, then dilute with *Diluent*.

**Sample solution:** 14 mg/mL of Gabapentin in *Diluent*

**Chromatographic system**  
(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 13,600 theoretical plates

**Relative standard deviation:** NMT 7.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Disregard peaks with a relative retention time of NMT 0.35, relative to gabapentin related compound D.]

Calculate the percentage of any impurity in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of any impurity from the *Sample solution*

$r_S$  = peak area of gabapentin related compound D from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of Gabapentin in the *Sample solution* (mg/mL)

$F$  = relative response factor: gabapentin related compound D, 1; all other impurities, 0.025

**Acceptance criteria**

**Individual impurities:** NMT 0.10%

**Total impurities:** NMT 0.5% (including impurities found in *Early-Eluting Impurities*)

## SPECIFIC TESTS

### • PH <791>

**Sample solution:** 20 mg/mL in water

**Acceptance criteria:** 6.5–8.0

### • WATER DETERMINATION, *Method I* <921>: NMT 0.5%

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers. Store at room temperature.

### • USP REFERENCE STANDARDS <11>

USP Gabapentin RS

USP Gabapentin Related Compound A RS

2-Aza-spiro[4.5]decan-3-one.

$C_9H_{15}NO$  153.22

USP Gabapentin Related Compound B RS

(1-Cyano-cyclohexyl)-acetic acid.

$C_9H_{13}NO_2$  167.21

USP Gabapentin Related Compound D RS

(1-(3-Oxo-2-aza-spiro[4.5]dec-2-ylmethyl)-cyclohexyl)-acetic acid.

$C_{18}H_{29}NO_3$  307.43

USP Gabapentin Related Compound E RS

Carboxymethyl-cyclohexanecarboxylic acid.

$C_9H_{14}O_4$  186.21

## Gabapentin Capsules

### DEFINITION

Gabapentin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of gabapentin ( $C_9H_{17}NO_2$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION <197K>

**Sample:** Empty the contents of NLT 10 Capsules, and grind to a fine powder. Use a quantity of the powder, equivalent to 2 mg of gabapentin, and 200 mg of potassium bromide.

**Acceptance criteria:** Meet the requirements

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** 1.2 g/L of monobasic potassium phosphate in water. Adjust with 5 N potassium hydroxide to a pH of 6.9.

**Mobile phase:** Dissolve 1.2 g of monobasic potassium phosphate in 940 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile and stir.

**Standard solution:** 4.0 mg/mL of USP Gabapentin RS in *Diluent*

**Sample solution:** Nominally 4.0 mg/mL of gabapentin, from the contents of NLT 20 Capsules, equivalent to 100 mg of gabapentin, in *Diluent*. Sonication for about 60 s may be necessary to dissolve the contents.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.2 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 7000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gabapentin ( $C_9H_{17}NO_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Medium:** 0.06 N hydrochloric acid (prepared by adding 51 mL of hydrochloric acid to 10 L of water); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 20 min

**Sample solution:** Filter a portion of the solution under test using a suitable filter of 0.45-µm pore size.

**Mobile phase:** Prepare as directed in the *Assay*.

**Standard solution:** 0.0011L mg/mL of USP Gabapentin RS in the *Medium*, where L is the label claim in mg/Capsule

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Flow rate:** 1.2 mL/min**Injection volume:** 100 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 7000 theoretical plates**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of gabapentin (C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $V$  = volume of the *Medium* in the dissolution vessel, 900 mL $L$  = label claim (mg/Capsule)**Tolerances:** NLT 80% (Q) of the labeled amount of gabapentin (C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES****Diluent:** Prepare as directed in the *Assay*.**Solution A:** Dissolve 1.2 g of monobasic potassium phosphate in 940 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile and stir.**Solution B:** Dissolve 1.2 g of monobasic potassium phosphate in 700 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 300 mL of acetonitrile and stir.**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.0	100	0
45.0	0	100
45.1	100	0
50.0	100	0

**Standard solution:** 0.04 mg/mL each of USP Gabapentin RS and USP Gabapentin Related Compound A RS in *Diluent***Sample solution:** Nominally 20 mg/mL of gabapentin, from the contents of NLT 20 Capsules, equivalent to 500 mg of gabapentin, in *Diluent*. Sonication for about 30 s may be necessary.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Flow rate:** 1.5 mL/min**Injection volume:** 50 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for the gabapentin peak**Relative standard deviation:** NMT 5.0% for gabapentin and gabapentin related compound A**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of gabapentin related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response for gabapentin related compound A from the *Sample solution* $r_S$  = peak response for gabapentin related compound A from the *Standard solution* $C_S$  = concentration of USP Gabapentin Related Compound A RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product, relative to gabapentin content, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response for each unspecified impurity from the *Sample solution* $r_S$  = peak response for gabapentin from the *Standard solution* $C_S$  = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)**Acceptance criteria****Gabapentin related compound A:** NMT 0.4%**Any individual unspecified impurity:** NMT 0.1%**Total impurities:** NMT 1.0%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Gabapentin RS

USP Gabapentin Related Compound A RS

2-Aza-spiro[4.5]decan-3-one.

C<sub>9</sub>H<sub>15</sub>NO 153.22**Gabapentin Tablets****DEFINITION**Gabapentin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of gabapentin (C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub>).**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)**Sample:** Grind at least 20 Tablets to a fine powder. Use an amount of powder equivalent to 2 mg of gabapentin and 200 mg of dry potassium bromide.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE****Diluent:** 1.2 g/L of monobasic potassium phosphate in water. Adjust with 5 N potassium hydroxide to a pH of 6.9.**Mobile phase:** Dissolve 1.2 g of monobasic potassium phosphate in 940 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile, and stir. Filter and degas.**Standard solution:** 4.0 mg/mL of USP Gabapentin RS in *Diluent***Sample solution:** 4.0 mg/mL of gabapentin from NLT 20 finely powdered Tablets in *Diluent*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Flow rate:** 1.2 mL/min**Injection size:** 50 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 7000 theoretical plates**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0% of gabapentin**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION <711>****Test 1****Medium:** 0.06 N hydrochloric acid (51 mL of hydrochloric acid in 10 L of water); 900 mL**Apparatus 2:** 50 rpm**Time:** 45 min**Sample solution:** Pass a portion of the solution under test through a suitable 0.45-μm filter.Determine the amount of C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> dissolved by using the following method.**Mobile phase:** Prepare as directed in the *Assay*.**Standard solution:** 0.0011 × L mg/mL of USP Gabapentin RS in the *Medium*, where L is the label claim in mg/Tablet**Chromatographic system:** Proceed as directed for the *Assay*.**Injection size:** 100 μL for the Tablets labeled to contain 100, 300, or 400 mg; 50 μL for Tablets labeled to contain 600 or 800 mg**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 5000 theoretical plates**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 3%**Analysis****Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $V$  = volume of the *Medium*, 900 mL $L$  = Tablet label claim in mg**Tolerances:** NLT 80% (Q) of the labeled amount of C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.**Medium, Apparatus 2, Mobile phase, Standard solution, Sample solution, Chromatographic system, and Analysis:** Proceed as directed for *Test 1*.**Time:** 30 min**Tolerances:** NLT 80% (Q) of the labeled amount of C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**IMPURITIES****Organic Impurities****• PROCEDURE****Diluent:** Prepare as directed in the *Assay*.**Solution A:** Dissolve 1.2 g of monobasic potassium phosphate in 940 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile, and stir. Filter and degas.**Solution B:** Dissolve 1.2 g of monobasic potassium phosphate in 700 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 300 mL of acetonitrile and stir. Filter and degas.**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.0	100	0
45.0	0	100
45.1	100	0
50.0	100	0

**Standard solution:** 0.04 mg/mL each of USP Gabapentin RS and USP Gabapentin Related Compound A RS in *Diluent***Sample solution:** Equivalent to 20 mg/mL of gabapentin, from NLT 20 powdered Tablets, in *Diluent*. [NOTE—Sonication for about 30 s may be necessary.]**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Flow rate:** 1.5 mL/min**Injection size:** 50 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for the gabapentin peak**Relative standard deviation:** NMT 5.0% for gabapentin and gabapentin related compound A peaks**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of gabapentin related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response for gabapentin related compound A from the *Sample solution* $r_S$  = peak response for gabapentin related compound A from the *Standard solution* $C_S$  = concentration of USP Gabapentin Related Compound A RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product relative to the gabapentin content in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response for each unspecified impurity from the *Sample solution* $r_S$  = peak response for gabapentin from the *Standard solution*

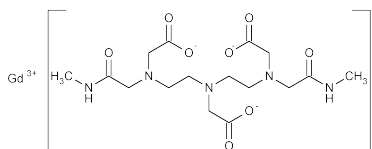
- $C_S$  = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Gabapentin related compound A:** NMT 0.4%  
**Any individual unspecified impurity:** NMT 0.1%  
**Total impurities:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
 USP Gabapentin RS  
 USP Gabapentin Related Compound A RS  
 2-Aza-spiro[4.5]decan-3-one.  
 $C_9H_{15}NO$  153.22

**Gadodiamide**

$C_{16}H_{26}GdN_5O_8$  573.66  
 [5,8-Bis(carboxymethyl)-11-[2-(methylamino)-2-oxoethyl]-3-oxo-2,5,8,11-tetraazatridecan-13-oato(3-)]gadolinium.  
 [N,N-Bis[2-[(carboxymethyl)](methylcarbamoyl)methyl]-amino]ethyl]glycinato(3-)]gadolinium [131410-48-5].

» Gadodiamide contains not less than 97.0 percent and not more than 103.0 percent of  $C_{16}H_{26}GdN_5O_8$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

- USP Gadodiamide RS  
 USP Gadodiamide Related Compound A RS  
 Gadolinium sodium diethylenetriamine pentaacetic acid monomethylamide.  
 $C_{15}H_{22}GdN_4NaO_9$  582.60  
 USP Gadodiamide Related Compound B RS  
 Gadolinium disodium diethylenetriamine pentaacetic acid.  
 $C_{14}H_{18}GdN_3Na_2O_{10}$  591.54  
 USP Endotoxin RS

**Clarity of solution**—*Reference solution*—

**REFERENCE SOLUTION A**—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours. [Caution—Hydrazine sulfate is highly toxic. Avoid skin contact.]

**REFERENCE SOLUTION B**—Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25 mL of water, and mix to dissolve.

**PRIMARY OPALESCENT MIXTURE**—To the flask containing *Reference solution B*, add 25.0 mL of *Reference solution A*, mix, and allow to stand for 24 hours. [NOTE—The suspension is stable for 2 months. Mix before use, and do not use if it adheres to the container.]

**OPALESCENCE STANDARD**—Dilute 15.0 mL of the *Primary opalescent mixture* with water to 1000.0 mL, and mix. This standard must be freshly prepared.

**PROCEDURE**—Transfer 10.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 40 mL of this solution (*Reference solution*) to a 50-mL color comparison tube.

**Test solution**—Transfer an accurately weighed quantity of Gadodiamide, equivalent to about 15 g of anhydrous gadodiamide, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 40 mL of this solution to a 50-mL color comparison tube.

**Blank**—Transfer 40 mL of water to a 50-mL color comparison tube.

**Procedure**—Five minutes after preparation of the *Reference solution*, view the *Reference solution*, the *Test solution*, and the *Blank* against a black background: the *Test solution* is not more opalescent than the *Reference solution*. [NOTE—If the *Test solution* is more opalescent than the *Reference solution*, heat the *Test solution* to 60° to 70° for 2 to 3 minutes, cool to room temperature, and view again.]

**Identification**—

**A: Infrared Absorption** (197K).

**B:** It exhibits the maximum absorption at the relevant wavelength specified when tested as directed under the test for *Content of gadolinium*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count is not more than 500 cfu per g. The total combined molds and yeasts count is not more than 50 cfu per g.

**Bacterial endotoxins** (85)—It contains not more than 3.5 USP Endotoxin Units per g.

**Water, Method I** (921): between 3.0% and 14.0%.

**Limit of free gadolinium (III)**—

**Arsenazo III indicator**—Transfer 150 mg of arsenazo III disodium to a 100-mL volumetric flask, dilute with water to volume, and mix.

**MES buffer**—Transfer 48.8 g of 2-(N-morpholino)ethanesulfonic acid (MES) to a 250-mL volumetric flask. Add 180 mL of water and 25 mL of 2 N sodium hydroxide, and mix. Adjust with 2 N sodium hydroxide to a pH of 6.0, dilute with water to volume, and mix.

**Edetate disodium titrant**—Pipet 100 mL of 0.02 M edetate disodium VS into a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Transfer about 1 g of Gadodiamide, accurately weighed, to a 125-mL conical flask, add 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator*, and mix. A turquoise color indicates the presence of excess gadolinium.

**Procedure**—Titrate the *Test solution* with *Edetate disodium titrant* to a violet-pink endpoint. Each mL of *Edetate disodium titrant* is equivalent to 0.3145 mg of excess gadolinium: not more than 0.3% of free gadolinium is found, calculated on the anhydrous basis.

**Limit of free diethylenetriamine pentaacetic acid bis-methylamide**—

**Arsenazo III indicator and MES buffer**—Proceed as directed for *Limit of free gadolinium (III)*.

**0.002 M gadolinium (III) titrant**—Transfer 18.6 g of gadolinium chloride to a 1000-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Pipet 10 mL of this solution into a conical flask, and add 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator*. Titrate with 0.02 M edetate disodium VS to a violet-pink endpoint, and determine the molarity. Pipet 40 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Blank solution**—Transfer 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator* into a suitable flask, and mix.

**Test solution**—Transfer about 1 g of Gadodiamide, accurately weighed, to a 125-mL conical flask. Add 25 mL of MES buffer and 0.1 mL of Arsenazo III indicator, and mix. A violet-pink color indicates the presence of excess diethylenetriamine pentaacetic acid bismethylamide.

**Procedure**—Concomitantly titrate the *Blank solution* and the *Test solution* with 0.002 M gadolinium (III) titrant to a turquoise endpoint. Calculate the percentage of free diethylenetriamine pentaacetic acid bismethylamide in the portion of Gadodiamide taken by the formula:

$$100(V_U - V_B) M_T (419.43) / W$$

in which  $V_U$  is the volume of gadolinium (III) in the *Test solution*;  $V_B$  is the volume of gadolinium (III) in the *Blank solution*;  $M_T$  is the molarity of the 0.002 M gadolinium (III) titrant; 419.43 is the molecular weight of diethylenetriamine pentaacetic acid bismethylamide; and  $W$  is the weight, in mg, of Gadodiamide taken to prepare the *Test solution*: not more than 0.7% of diethylenetriamine pentaacetic acid bismethylamide is found, calculated on the anhydrous basis.

#### Limit of methylamine—

**Borate buffer**—Transfer 12.4 g of boric acid to a 500-mL volumetric flask, and suspend it in 300 mL of water. Add 100 mL of 1 N potassium hydroxide, and mix. Adjust with 1 N potassium hydroxide to a pH of 10.0, dilute with water to volume, and mix. Store in a closed plastic container.

**OPA reagent**—Transfer 100 mg of o-phthalaldehyde to an amber bottle, add 3 mL of methanol, and mix. Add 220 mL of Borate buffer and 0.1 mL of mercaptoethanol, and mix. [NOTE—This solution must be freshly prepared.]

**Standard solutions**—Transfer about 110 mg of methylamine hydrochloride, accurately weighed, to a 500-mL volumetric flask, and dilute with water to volume to obtain a solution having a concentration of about 100 µg of methylamine per mL. Pipet 1, 5, 10, and 20 mL of this solution into separate 100-mL volumetric flasks, dilute the contents of each flask with water to volume, and mix.

**Test solution**—Transfer about 1.5 g of Gadodiamide, accurately weighed, to a 10-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as follows for each of the *Standard solutions*. Add 3.0 mL of OPA reagent, mix, and within 1 minute measure the absorbance at 335 nm. Compare to a blank consisting of 3.0 mL of water and 3.0 mL of OPA reagent. Plot a calibration curve of absorbance versus standard concentration, in µg of methylamine per mL. Mix 3.0 mL of the *Test solution* with 3.0 mL of OPA reagent, and proceed as directed above. [NOTE—If the absorbance obtained with the *Test solution* exceeds the absorbance of the highest *Standard solution*, perform an additional quantitative dilution of the *Test solution*, and repeat the analysis.] Determine the concentration, in µg per mL, of methylamine in the *Test solution* by interpolation from the calibration curve. Calculate the amount of methylamine in the portion of Gadodiamide taken: not more than 0.05% of methylamine is found.

#### Limit of acetone, ethyl alcohol, and isopropyl alcohol—

**Internal standard solution**—Transfer about 500 mg of methyl ethyl ketone to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

**Stock solution**—Transfer about 1000 mg each of acetone, ethyl alcohol, and isopropyl alcohol, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Diluted stock solution A**—Pipet 5 mL of the *Stock solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

**Diluted stock solution B**—Pipet 10 mL of the *Stock solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

**System suitability solution**—Pipet 10 mL of the *Internal standard solution* and 15 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test solution 1**—Pipet 10 mL of the *Internal standard solution* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

**Test solution 2**—Pipet 10 mL of the *Internal standard solution* and 2.5 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

**Test solution 3**—Pipet 10 mL of the *Internal standard solution* and 15 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

**Test solution 4**—Pipet 10 mL of the *Internal standard solution* and 25 mL of *Diluted stock solution B* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m capillary column coated with a 1.8-µm phase G43. The column temperature is maintained at 40°. Helium is used as the carrier gas at a flow rate of about 1.5 mL per minute, and the split ratio is 1:15. The injection port and detector block are maintained at about 250°. Chromatograph the *System suitability solution* as directed for *Procedure*: the order of elution is ethyl alcohol, acetone, isopropyl alcohol, and methyl ethyl ketone; the resolution,  $R$ , between the ethyl alcohol and acetone peaks is not less than 1.0 and between the acetone and isopropyl alcohol peaks is not less than 1.0; and the relative standard deviation is not more than 3.0% for each of the three analytes.

**Procedure**—Separately inject equal volumes (about 1 µL) of each of the four *Test solutions* into the chromatograph, record the chromatograms, and measure the areas for the major peaks relative to the area for the internal standard peak. Plot the responses of the *Test solutions* versus the content, in µg per mL, of the relevant analyte in each vial, draw the straight line best fitting the four points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. Calculate the percentage of each analyte in the portion of Gadodiamide taken by the formula:

$$a/(10,000b)$$

in which  $a$  is the intercept and  $b$  is the slope of the straight line, evaluated by linear regression analysis. [NOTE—If  $a$  is negative, report the result as none detected.] Not more than 0.2% of acetone, ethyl alcohol, and isopropyl alcohol is found, calculated on the anhydrous basis; and the sum of all three analytes is not more than 0.2%, calculated on the anhydrous basis.

**Related compounds—**

*Mobile phase*—Prepare as directed in the Assay.

*Postcolumn reagent*—Dissolve 120 mg of arsenazo III acid in 400 mL of water previously acidified with 6.3 mL of nitric acid. Add 650 mg of urea, and mix to dissolve. Pass the solution through a 0.45- $\mu$ m porosity filter, washing the filter with 600 mL of water. Dilute with water to 1000 mL, mix, and degas.

*System suitability solution*—Prepare an aqueous solution containing about 0.01 mg of USP Gadodiamide Related Compound A RS, 0.01 mg of USP Gadodiamide Related Compound B RS, and 2 mg of USP Gadodiamide RS in each mL.

*Test solution*—Transfer about 200 mg of Gadodiamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—Proceed as directed in the Assay, except to use the *Postcolumn reagent* prepared as directed above. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the gadodiamide and gadodiamide related compound A peaks is not less than 1.0, and between gadodiamide related compound A and gadodiamide related compound B peaks is not less than 1.5; and the relative standard deviation for replicate injections is not more than 10%.

*Procedure*—Inject about 10  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. [NOTE—The tail of the gadodiamide peak may contain a small shoulder due to an isomer; the area of the shoulder should be included in the gadodiamide peak area.] Calculate the percentage of each impurity in the portion of Gadodiamide taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each impurity; and  $r_s$  is the sum of all peaks having a percentage greater than 0.10%: not more than 2.0% of gadodiamide related compounds A and B is found; no individual impurity is more than 0.2%; and the sum of all impurities, other than gadodiamide related compounds A and B, is not more than 0.5%.

**Content of gadolinium—**

*Standard solutions*—Prepare three separate solutions in 0.2 M nitric acid to obtain concentrations of 100, 150, and 200  $\mu$ g of gadolinium per mL.

*Test solution*—Transfer about 600 mg of Gadodiamide, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with 0.2 M nitric acid to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the gadolinium resonance line of 342.3 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>), using 0.2 M nitric acid as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in  $\mu$ g per mL, of gadolinium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in  $\mu$ g per mL, of gadolinium in the *Test solution*: the content of gadolinium is between 26.0% and 29.0%, calculated on the anhydrous basis.

**Assay—**

*Mobile phase*—Transfer 14.0 mL of triethylamine, 5.7 mL of glacial acetic acid, and 5.7 mL of water to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 900 mL of water, and mix. Adjust with 1 N acetic acid or 1 N sodium hydroxide to a pH between 6.5 and 7.0. Dilute with water to volume, mix, filter, and degas (see *System Suitability* under *Chromatography* <621>).

*Postcolumn reagent*—Dissolve 325 mg of urea in a solution of 60 mg of arsenazo III acid in 550 mL of water previously acidified with 3.2 mL of nitric acid. Pass the solution through a 0.45- $\mu$ m porosity filter, wash the filter with 400 mL of water, dilute with water to 1000 mL, mix, and degas.

*Standard preparation*—Prepare an aqueous solution of USP Gadodiamide RS having a known concentration of about 0.6 mg per mL.

*Assay preparation*—Transfer about 60 mg of Gadodiamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 658-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m base-deactivated packing L1. A second pump mixes the *Mobile phase* with the *Postcolumn reagent* prior to detection via a T-junction. The system is maintained at a constant temperature between 20° and 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.5%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{16}H_{26}GdN_5O_8$  in the portion of Gadodiamide taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Gadodiamide RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the gadodiamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gadodiamide Injection

**DEFINITION**

Gadodiamide Injection is a sterile solution of Gadodiamide in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of gadodiamide ( $C_{16}H_{26}GdN_5O_8$ ). It may contain stabilizers and buffers. Gadodiamide Injection intended for intravascular use contains no antimicrobial agents.

**IDENTIFICATION**

- A. ULTRAVIOLET ABSORPTION (197U)**

Wavelength range: 240–300 nm

Solution: 57 mg/mL of gadodiamide in water

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- PROCEDURE**

**Solution A:** Dissolve 14 mL of triethylamine and 5.7 mL of glacial acetic acid in 1 L of water.

**Mobile phase:** Transfer 50 mL of *Solution A* to a 1-L volumetric flask. Add 900 mL of water. Adjust with 1 N acetic acid or 1 N sodium hydroxide to a pH between 6.5 and 7.0. Dilute with water to volume.

**Postcolumn reagent:** Dissolve 325 mg of urea in a solution of 60 mg of arsenazo III acid in 550 mL of water previously acidified with 3.2 mL of nitric acid. Pass the solution through a filter of 0.45- $\mu$ m pore size, wash the filter with 400 mL of water, and dilute with water to 1000 mL.



**Standard solution:** 0.18 mg/mL of USP Gadodiamide RS

**Sample solution:** Equivalent to a nominal concentration of 0.18 mg/mL of gadodiamide from Injection

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Vis 658 nm

**Column:** 4.6-mm × 25-cm; 5-μm base-deactivated packing L1. [NOTE—A second pump mixes the *Mobile phase* with the *Postcolumn reagent* prior to detection via a T-junction.]

**Column temperature:** 20°–35° (system maintained at constant temperature)

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of gadodiamide

(C<sub>16</sub>H<sub>26</sub>GdN<sub>5</sub>O<sub>8</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of gadodiamide from the *Sample solution*

$r_S$  = peak response of gadodiamide from the *Standard solution*

$C_S$  = concentration of USP Gadodiamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of gadodiamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A, Mobile phase, and Chromatographic system:** Prepare as directed in the *Assay*.

**Postcolumn reagent:** Dissolve 120 mg of arsenazo III acid in 400 mL of water previously acidified with 6.3 mL of nitric acid. Add 650 mg of urea, and mix to dissolve. Pass the solution through a filter of 0.45-μm pore size, washing the filter with 600 mL of water. Dilute with water to 1000 mL.

**System suitability solution:** 10 μg/mL of USP Gadodiamide Related Compound A RS, 10 μg/mL of USP Gadodiamide Related Compound B RS, and 2 mg/mL of USP Gadodiamide RS in an aqueous solution

**Sample solution:** Equivalent to a nominal concentration of 2 mg/mL of gadodiamide from Injection

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.0 between the gadodiamide and gadodiamide related compound A peaks; NLT 1.5 between gadodiamide related compound A and gadodiamide related compound B peaks

**Relative standard deviation:** NMT 10%

[NOTE—The tail of the gadodiamide peak may contain a small shoulder due to an isomer. The area of the shoulder should be included in the gadodiamide peak area.]

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the volume of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity

$r_T$  = sum of all peaks having a percentage greater than 0.10%

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Acceptance Criteria, NMT (%)
Gadodiamide related compound A	2.0
Gadodiamide related compound B	2.0
Any other individual impurity	0.2
Total impurities (sum of all impurities other than gadodiamide related compounds A and B)	0.5

#### SPECIFIC TESTS

• **OSMOLALITY AND OSMOLARITY, Osmolality <785>:** 650–1000 mOsm/kg

• **PH <791>:** 5.5–7.0

• **BACTERIAL ENDOTOXINS TEST <85>:** NMT 0.029 USP Endotoxin Unit/mg of gadodiamide

• **INJECTIONS <1>:** Meets the requirements

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.

• **LABELING:** Label containers of Injection to direct the user to discard any unused portion. Label it to state its routes of administration. Label it to indicate “serious injury can occur if given by intrathecal route.”

• **USP REFERENCE STANDARDS <11>**

USP Endotoxin RS

USP Gadodiamide RS

USP Gadodiamide Related Compound A RS

Gadolinium sodium diethylenetriamine pentaacetic acid monomethylamide.

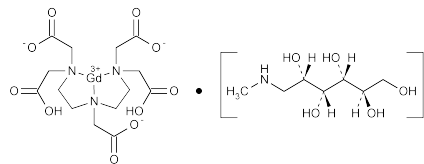
C<sub>15</sub>H<sub>22</sub>GdN<sub>4</sub>NaO<sub>9</sub> 582.60

USP Gadodiamide Related Compound B RS

Gadolinium disodium diethylenetriamine pentaacetic acid.

C<sub>14</sub>H<sub>18</sub>GdN<sub>3</sub>Na<sub>2</sub>O<sub>10</sub> 591.54

## Gadopentetate Dimeglumine Injection



» Gadopentetate Dimeglumine Injection is a sterile solution of gadopentetate dimeglumine in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gadopentetate dimeglumine (C<sub>14</sub>H<sub>20</sub>GdN<sub>3</sub>O<sub>10</sub> · 2C<sub>7</sub>H<sub>17</sub>NO<sub>5</sub>). It may contain small amounts of Meglumine and Pentetic Acid as stabilizers, and it may contain suitable buffers. Gadopentetate Dimeglumine Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage—**Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Gadopentetate Monomeglumine RS

**Identification**—

**A:** *Ultraviolet Absorption* (197U)—

**Solutions:** 74 mg of USP Gadopentetate Monomeglumine RS per mL, and 94 mg of Gadopentetate Dimeglumine per mL.

**Medium:** water.

**B:** Dilute a volume of Injection with water to obtain a test solution having a concentration of 35 mg per mL. Separately apply 10  $\mu$ L of this solution and 10  $\mu$ L of a Standard solution containing 28 mg of USP Gadopentetate Monomeglumine RS per mL in a meglumine solution (0.075 in 1000) to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of 1,4-dioxane, water, and ammonium hydroxide (70:30:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Dip the plate in a detection reagent prepared by mixing 100 mg of ninhydrin and 250 mg of cadmium acetate with 1.0 mL of glacial acetic acid and diluting with alcohol to 50 mL. Heat the plate at 120° for 10 minutes, and locate the spots by examining the plate in daylight: the principal spot obtained from the test solution corresponds in appearance and  $R_f$  value to the principal spot obtained from the Standard solution (presence of meglumine).

**C:** Prepare the test solution and Standard solution as directed for *Identification* test A. Transfer 500 mL of 1 N sulfuric acid at 0° to a 1000-mL conical flask, immerse in an ice bath, and add 50 g of ceric sulfate tetrahydrate. Mix until dissolved, filter, and refrigerate (*Stock solution A*). Transfer 325 mL of 2 N sulfuric acid at 0° to a 500-mL volumetric flask. To 25 g of sodium arsenite, add 150 mL of 1 N sodium hydroxide, mix, and add in small portions to the sulfuric acid. Dilute with water to volume, and refrigerate (*Stock solution B*). Just prior to use, prepare a spray reagent by mixing equal volumes of *Stock solution A* and *Stock solution B* at a temperature not lower than 10°. [NOTE—Use the spray reagent within five minutes.] Proceed as directed under *Thin-layer Chromatographic Identification Test* (201), except to spray the plate first with the spray reagent and then with a 1% solution of 1,2-phenylenediamine in acetone. Locate the spots by examining the plate in daylight: the principal spot obtained from the test solution corresponds in appearance and  $R_f$  value to the principal spot obtained from the Standard solution (presence of gadopentetate).

**Bacterial endotoxins** (85)—It contains not more than 25 USP Endotoxin Units per mL of Injection.

**pH** (791): between 6.5 and 8.0.

**Heavy metals** (231)—

**Standard preparation**—Proceed as directed in the test for Heavy metals under *Diatrizoate Meglumine*.

**Test preparation**—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of gadopentetate dimeglumine, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix.

**Procedure**—Proceed as directed for *Procedure* in the test for Heavy metals under *Diatrizoate Meglumine*: the limit is 0.002%.

**Meglumine content**—Proceed as directed in the test for *Meglumine content* under *Diatrizoate Meglumine Injection*. The meglumine content is between 37.4% and 45.8% of the labeled amount of gadopentetate dimeglumine.

**Content of gadolinium**—

**Cesium chloride solution**—Dissolve 10.0 g of cesium chloride in 100.0 mL of water, and mix.

**Blank solution**—Transfer 10.0 mL of *Cesium chloride solution* and 1.0 mL of hydrochloric acid (spectrophotometric grade) to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard solutions**—Transfer about 1.153 g of gadolinium (III) oxide, accurately weighed, to a 100-mL volumetric flask, add 2.0 mL of hydrochloric acid to dissolve, dilute with water to volume, and mix. Transfer 3.0, 4.0, and 5.0 mL of this stock solution to separate 50-mL volumetric flasks, and to each flask add 5.0 mL of *Cesium chloride solution* and 0.5 mL of hydrochloric acid (spectrophotometric grade). Dilute the contents of each flask with water to volume, and mix. These *Standard solutions* contain, respectively, 600, 800, and 1000  $\mu$ g of gadolinium per mL.

**Test solution**—Treat an accurately measured volume of Injection, equivalent to about 469 mg of gadopentetate dimeglumine, with 0.2 mL of nitric acid in a porcelain crucible, concentrate on a hot plate, char with a burner, and ignite in a muffle furnace at 800° until all black particles disappear (approximately 1 hour). Allow the residue to cool on a refractory surface for about 5 minutes, then equilibrate to room temperature in a desiccator. Dissolve the white residue so obtained in a mixture of 1.0 mL of water and 1.0 mL of hydrochloric acid (spectrophotometric grade) with heating. Transfer this solution to a 100-mL volumetric flask, add 10.0 mL of *Cesium chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the gadolinium emission line at 368.4 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a gadolinium hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus their concentrations, in  $\mu$ g per mL, of gadolinium, and draw the straight line best fitting the three plotted points. From the graph so obtained and the absorbance of the *Test solution*, determine the concentration, in  $\mu$ g per mL, of gadolinium in the *Test solution*. Calculate the quantity, in  $\mu$ g, of gadolinium in each mL of the Injection taken by the formula:

$$100C/V$$

in which C is the concentration, in  $\mu$ g per mL, of gadolinium in the *Test solution*; and V is the volume, in mL, of Injection taken. The gadolinium content is between 15.1% and 18.4% of the labeled amount of gadopentetate dimeglumine.

**Content of pentetic acid**—

**Stock solution A**—Transfer about 50 g of sodium acetate and 10 mL of glacial acetic acid to a 1000-mL volumetric flask, and dilute with degassed water to volume. Adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5.

**Stock solution B**—Transfer about 50.8 mg of xylenol orange to a 100-mL volumetric flask, and add degassed water to volume.

**Diluting solution**—Transfer 30 mL of *Stock Solution A* and 3 mL of *Stock Solution B* to a 200-mL volumetric flask, and dilute with degassed water to volume.

**Procedure**—Transfer an accurately measured volume of Injection, equivalent to about 938 mg of gadopentetate dimeglumine, to a suitable container, add 20 mL of water and 10 mL of *Diluting solution*, and mix. Adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5. Titrate with 0.001 M gadolinium sulfate solution until the color changes from yellow to reddish violet. Each mL of 0.001 M gadolinium sulfate consumed is equivalent to 0.7867 mg of pentetic acid ( $C_{14}H_{23}N_3O_{10}$ ). The pentetic acid content is between 0.027% and 0.04%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture containing about 1.37 g of tetrabutylammonium perchlorate in a mixture of acetonitrile and water (120:880). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 46.43 mg of USP Gadopentetate monomeglumine RS, accurately weighed, to a 25-mL volumetric flask containing 12.5 mL of 0.1% meglumine solution. Dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 469 mg of gadopentetate dimeglumine, to a 200-mL volumetric flask. Dilute with water to volume, and mix.

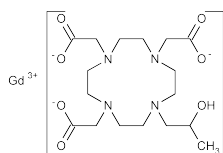
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 195-nm detector and a 4.6-mm × 12.5-cm column that contains 5-μm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 800 theoretical plates, the tailing factor is not more than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{20}GdN_3O_{10} \cdot 2C_7H_{17}NO_5$  in the portion of Injection taken by the formula:

$$(938.02 / 742.80)(200C)(R_U / R_S)$$

in which 938.02 and 742.80 are the molecular weights of gadopentetate dimeglumine and gadopentetate monomeglumine, respectively; C is the concentration, in mg per mL, of USP Gadopentetate Monomeglumine RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gadoteridol



$C_{17}H_{29}GdN_4O_7$  558.68

Gadolinium, [10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)- $N^1, N^4, N^7, N^{10}, O^1, O^4, O^7, O^{10}$ ].

(±)-[10-(2-Hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)]gadolinium [120066-54-8].

» Gadoteridol contains not less than 97.0 percent and not more than 101.0 percent of  $C_{17}H_{29}GdN_4O_7$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Gadoteridol RS

USP Gadoteridol Related Compound A RS

10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.

$C_{17}H_{32}N_4O_7$  404.46

USP Gadoteridol Related Compound B RS

1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid gadolinium salt.

USP Gadoteridol Related Compound C RS

1,4,7,10-tetraaza-11-oxo-bicyclo[8.2.2]tetradecane-4,7-diacetic acid.

**Identification—**

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 10 mg per mL.

*Medium:* water.

**Water, Method I** (921): not more than 15%.

**Heavy metals, Method II** (231): not more than 0.001%.

**Limit of gadoteridol related compound A—**

**Buffer solution**—Dissolve 0.60 g of tromethamine and 3.72 g of edetate disodium in 3 L of water, adjust with 5 N sodium hydroxide to a pH of 7.0, dilute with water to 4 L, and mix. Filter, and chill between 5° and 8°.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution*, acetonitrile, and tetrahydrofuran (98.8:1.0:0.2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Cupric acetate solution**—Transfer 3.99 g of cupric acetate and 12.11 g of tromethamine to a 2-L volumetric flask, dissolve in 1500 mL of water, adjust with glacial acetic acid to a pH of 7.0, dilute with water to volume, and mix. Filter, and chill between 5° and 8°.

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Gadoteridol Related Compound A RS in *Buffer solution*, and dilute quantitatively and stepwise with *Buffer solution* to obtain a solution having a known concentration of about 6 μg per mL.

**Standard solution**—Just prior to injection onto the column, dilute 1.0 mL of the *Standard stock solution* with 1.0 mL of *Cupric acetate solution* to obtain a solution having a known concentration of about 3 μg of USP Gadoteridol Related Compound A RS per mL. [NOTE—The copper–gadoteridol related compound A complex is stable for at least 2 hours, if maintained at about 5°.]

**Test solution**—Transfer about 30 mg of Gadoteridol, accurately weighed, to a test tube, add 1.0 mL of *Buffer solution*, and mix on a vortex mixer to dissolve. Add 1.0 mL of *Cupric acetate solution*, and mix. Immediately inject the solution into the chromatograph. [NOTE—The copper–gadoteridol related compound A complex is stable for at least 2 hours, if maintained at about 5°.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 15-cm column that contains packing L21. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for gadoteridol related compound A and 2.0 for copper–edetate; the resolution,  $R$ , between gadoteridol related compound A and copper–edetate is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of gadoteridol related compound A in the portion of Gadoteridol taken by the formula:

$$100(C / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Gadoteridol Related Compound A RS in the *Standard stock*

solution;  $W$  is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the gadoteridol related compound A peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.01% is found, calculated on the anhydrous basis.

#### Limit of free gadolinium (III)—

*Buffer solution, Mobile phase, Diluent, Standard stock solution 1, Standard stock solution 2, Standard solution, and Chromatographic system*—Proceed as directed for *Chromatographic purity, Test 1*.

*Test solution*—Transfer about 60 mg of Gadoteridol, accurately weighed, to a vial, add 2.0 mL of *Diluent*, and mix. Immediately place the vial in a bath maintained at about 5°.

*Procedure*—Proceed as directed for *Procedure under Chromatographic purity, Test 1*. Calculate the percentage of free gadolinium (III) in the portion of Gadoteridol taken by the formula:

$$200(157.25 / 334.38)(C / W)(r_U / r_S)$$

in which 157.25 is the molecular weight of gadolinium and 334.38 is the molecular weight of gadolinium acetate;  $C$  is the concentration, in mg per mL, of gadolinium acetate, calculated on the anhydrous basis, in the *Standard solution*;  $W$  is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the free gadolinium (III) peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.01% is found.

**Limit of regioisomer**—Using the chromatogram of the *Assay preparation* as obtained in the *Assay*, calculate the percentage of regioisomer peak at a relative retention time of about 1.2 in relation to that of the gadoteridol peak by the formula:

$$100r_R / (r_R + r_G)$$

in which  $r_R$  and  $r_G$  are the regioisomer and gadoteridol peak responses, respectively, in the *Assay preparation*: not more than 2.5% is found.

#### Chromatographic purity—

##### TEST 1: GADOLINIUM-CONTAINING IMPURITIES—

*Buffer solution*—Dissolve 10.2 g of monobasic potassium phosphate, 0.165 g of dibasic potassium phosphate, and 1.76 g of edetate disodium in 3000 mL of water, and filter.

*Mobile phase*—Prepare a degassed mixture of *Buffer solution* and acetonitrile (98:2). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Diluent*—Transfer 3.40 g of monobasic potassium phosphate, 4.21 g of dibasic potassium phosphate, and 0.584 g of edetate disodium to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Standard stock solution 1*—Quantitatively dissolve an accurately weighed quantity of gadolinium (Gd III) acetate in water to obtain a solution having a concentration of about 0.4 mg per mL.

*Standard stock solution 2*—Prepare a solution of USP Gadoteridol Related Compound B RS in water to obtain a solution having a known concentration of about 0.6 mg per mL.

*Standard solution*—Transfer 1.0 mL of *Standard stock solution 1* and 2.0 mL of *Standard stock solution 2* to a 10-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution to a vial, add 3.0 mL of *Diluent*, and mix.

*Test solution*—Transfer about 60 mg of Gadoteridol, accurately weighed, to a vial, add 2.0 mL of *Diluent*, and mix. Immediately place the vial in a bath maintained at about 5°.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm, and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is

about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for free gadolinium (III), 1.6 for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid gadolinium sodium salt (gadoteridol related compound D), and 2.2 for gadoteridol related compound B; and the relative standard deviation for replicate injections is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Allow the *Test solution* to elute for not less than 1.3 times the retention time of the gadoteridol peak. Calculate the percentage of gadoteridol related compound D in the portion of Gadoteridol taken by the formula:

$$200(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Gadoteridol Related Compound B RS, calculated on the anhydrous basis, in the *Standard solution*;  $W$  is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the gadoteridol related compound D peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Calculate the percentage of gadoteridol related compound B in the portion of Gadoteridol taken by the formula:

$$200(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Gadoteridol Related Compound B RS, calculated on the anhydrous basis, in the *Standard solution*;  $W$  is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the gadoteridol related compound B peak responses obtained from the *Test solution* and *Standard solution*, respectively. Calculate the percentage of any other impurity in the portion of Gadoteridol taken by the formula:

$$200(C / W)(r_i / r_S)$$

in which  $r_i$  is the peak response of any other impurity obtained from the *Test solution*; and  $r_S$  is the peak response for gadoteridol related compound B obtained from the *Standard solution*: not more than 0.1% of any individual impurity is found, and not more than 0.3% of gadolinium-containing impurities is found.

##### TEST 2 (NONGADOLINIUM-CONTAINING IMPURITIES)—

*50 mM Ammonium phosphate buffer*—Dissolve 17.25 g of monobasic ammonium phosphate in 3000 mL of water, and filter.

*pH 5.0 Buffer*—Transfer 2000 mL of *50 mM Ammonium* to a 2-L beaker, and adjust with ammonium hydroxide to a pH of 5.0.

*pH 7.0 Buffer*—Transfer 1000 mL of *50 mM Ammonium* to a 1-L beaker, and adjust with ammonium hydroxide to a pH of 7.0.

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 5.0 buffer* and acetonitrile (87:13). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP Gadoteridol Related Compound C RS in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 5 μg per mL.

*System suitability solution*—Prepare a solution in *pH 7.0 buffer* containing about 2 mg of USP Gadoteridol RS and 0.002 mg of USP Gadoteridol Related Compound C RS per mL.

*Test solution*—Transfer about 50 mg of Gadoteridol to a 25-mL volumetric flask, dissolve in and dilute with *pH 7.0 Buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L18. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*: the relative retention times are about 1.0 for gadoteridol, 1.2 for 1,4,7,10-tetraaza-1,4,7-tris-(carboxymethyl)-11-oxo-bicyclo [8.2.2] tetradecanium chloride (gadoteridol related compound F), 1.5 for 1,4,7,10-tetraaza-13-oxo-bicyclo [8.2.1] tridecane-4,7-diacetic acid (gadoteridol related compound E), and 1.7 for gadoteridol related compound C; and the resolution, *R*, between gadoteridol and gadoteridol related compound C is not less than 5. Chromatograph the *Standard solution* as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 4.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Allow the *Test solution* to elute for not less than twice the retention time of the gadoteridol peak. Calculate the percentage of gadoteridol related compound C in the portion of Gadoteridol taken by the formula:

$$2.5(C / W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Gadoteridol Related Compound C RS in the *Standard solution*; *W* is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for gadoteridol related compound C obtained from the *Test solution* and *Standard solution*, respectively. Calculate the percentages of gadoteridol related compound F and gadoteridol related compound E in the portion of Gadoteridol taken by the formula:

$$2.5(1 / F)(C / W)(r_i / r_S)$$

in which *F* is the relative response factor for gadoteridol related compound F (0.68) or for gadoteridol related compound E (1.7); *r<sub>i</sub>* is the peak response for gadoteridol related compound E or gadoteridol related compound F in the *Test solution*; and *C*, *W*, and *r<sub>S</sub>* are as defined above. Calculate the percentage of any other impurity in the portion of Gadoteridol taken by the formula:

$$2.5(C / W)(r_i / r_S)$$

in which *r<sub>i</sub>* is the response of any other impurity in the chromatogram of the *Test solution*; and *C*, *W*, and *r<sub>S</sub>* are as defined above: not more than 0.1% of any other impurity is found, and the total of nongadolinium-containing impurities is not more than 0.3%, calculated on the anhydrous basis.

**Other requirements**—Where the label states that Gadoteridol is sterile, it meets the requirements for *Sterility Tests* <71> and *Bacterial endotoxins* under *Gadoteridol Injection*. Where the label states that Gadoteridol must be subjected to further processing during the preparation of injectable dosage form, it meets the requirements for *Bacterial endotoxins* under *Gadoteridol Injection*.

#### Assay—

**Buffer solution**—Dissolve 10.2 g of monobasic potassium phosphate and 0.165 g of dibasic potassium phosphate in 3 L of water, and filter.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (98:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Gadoteridol RS in *Buffer solution* to obtain a solution having a known concentration of about 0.6 mg per mL.

**Assay preparation**—Transfer about 60 mg of Gadoteridol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Buffer solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm, and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the gadoteridol peak and the peak with a relative retention time of about 1.2 is not less than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>17</sub>H<sub>29</sub>GdN<sub>4</sub>O<sub>7</sub> in the portion of Gadoteridol taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration of USP Gadoteridol RS, in mg per mL, in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the gadoteridol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gadoteridol Injection

» Gadoteridol Injection is a sterile solution of Gadoteridol in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gadoteridol (C<sub>17</sub>H<sub>29</sub>GdN<sub>4</sub>O<sub>7</sub>). It may contain buffers and stabilizers. Gadoteridol Injection intended for intravenous use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose *Containers for Injections* as described under *Injections* <1>, preferably of Type I glass. Store at controlled room temperature, and protect from light.

**Labeling**—Label containers of Injection intended for intravenous injection to direct the user to examine the product to ensure that all solids are dissolved, to discard the product if solids persist, and to discard any unused portion remaining in the container.

#### USP Reference standards <11>—

USP Endotoxin RS  
USP Gadoteridol RS  
USP Gadoteridol Related Compound A RS  
10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.  
C<sub>17</sub>H<sub>32</sub>N<sub>4</sub>O<sub>7</sub> 404.46

#### Identification—

**A: Ultraviolet Absorption** (197U)—

*Solution*: 10 mg per mL.

*Medium*: water.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 8.3 USP Endotoxin Units per mL of gadoteridol.

**pH** <791>: between 6.5 and 8.0.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Limit of free gadolinium (III)—**

*Buffer solution, Mobile phase, and Diluent*—Proceed as directed for *Test 1* in the *Chromatographic purity* test under *Gadoteridol*.

*Standard solution*—Prepare a solution of gadolinium (Gd III) acetate in water to obtain a solution having a known concentration of about 0.4 mg of gadolinium acetate per mL. Transfer 1.0 mL of the solution to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 1.0 mL of this solution to a small vial, add 3.0 mL of *Diluent*, and mix.

*Test solution*—Transfer an accurately measured volume of Injection, equivalent to about 150 mg of gadoteridol, to a small vial, dilute with *Diluent* to 5.0 mL, and mix. Immediately place in a bath maintained at about 5°.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for about 1.5 times the retention time of the gadoteridol peak, and measure the peak responses. Calculate the percentage, by weight, of free gadolinium (III) in the volume of Injection taken by the formula:

$$500(157.25/334.38)(C/VP)(r_U / r_S)$$

in which 157.25 and 334.38 are the molecular weights of gadolinium and gadolinium acetate, respectively; C is the concentration, in mg per mL, of gadolinium (Gd III) acetate, calculated on the anhydrous basis, in the *Standard solution*; V is the volume, in mL, of Injection taken for the *Test solution*; P is the labeled potency of gadoteridol, in mg per mL, in the Injection; and  $r_U$  and  $r_S$  are the peak responses for free gadolinium (III) obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.02% is found.

**Limit of gadoteridol related compound A—**

*Buffer solution, Mobile phase, Cupric acetate solution, Standard stock solution, Standard solution, and Chromatographic system*—Proceed as directed for the *Limit of gadoteridol related compound A* test under *Gadoteridol*.

*Test solution*—Transfer an accurately measured volume of Injection, equivalent to about 30 mg of gadoteridol, to a test tube, dilute with chilled *Buffer solution* to 1.0 mL, and mix. Add 1.0 mL of chilled *Cupric acetate solution*, mix on a vortex mixer for about 10 seconds, and immediately inject as directed for *Procedure*.

*Procedure*—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of gadoteridol related compound A in the Injection by the formula:

$$0.2(C/VP)(r_U / r_S)$$

in which C is the concentration, in μg per mL, of USP Gadoteridol Related Compound A RS in the *Standard solution*; V is the volume, in mL, of Injection taken for the *Test solution*; P is the labeled potency, in mg per mL, of gadoteridol in the Injection; and  $r_U$  and  $r_S$  are the peak responses of gadoteridol related compound A in the *Test solution* and the *Standard solution*, respectively: not more than 0.02% is found.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay—**

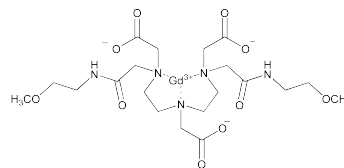
*Buffer solution, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Gadoteridol*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 150 mg of gadoteridol, to a 250-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg per mL, of gadoteridol ( $C_{17}H_{29}GdN_4O_7$ ) in the volume of Injection taken by the formula:

$$250(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Gadoteridol RS in the *Standard preparation*; V is the volume of Injection taken, in mL; and  $r_U$  and  $r_S$  are the gadoteridol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Gadoversetamide**

$C_{20}H_{34}GdN_5O_{10}$  661.76  
(Gadoversetamide) [8,11-bis(carboxymethyl)-14-[2-[(2-methoxyethyl)amino]-2-oxoethyl]-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(3-)], gadolinium.  
[N,N-Bis[2-[(carboxymethyl)[[(2-methoxyethyl)carbamoyl]methyl]amino]ethyl]glycinato(3-)]gadolinium [131069-91-5].

» Gadoversetamide contains not less than 97.0 percent and not more than 102.0 percent of  $C_{20}H_{34}GdN_5O_{10}$ , calculated on an anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards <11>—**

USP Endotoxin RS

USP Gadodiamide Related Compound B RS

Gadolinium disodium diethylenetriamine pentaacetic acid.

$C_{14}H_{18}GdN_3Na_2O_{10}$  591.54

USP Gadoversetamide RS

USP Gadoversetamide Related Compound A RS

Hydrogen [8, 11, 14-tris(carboxymethyl)-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(4-)]gadolinium.

**Identification—**

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** The lanthanide selectivity test detects gadolinium (III) in 0.1 N nitric acid with arsenazo (III). Prepare  $1.5 \times 10^{-4}$  M arsenazo (III) solution by dissolving 30 mg of arsenazo (III) and 160 mg of urea in 100 mL water, adding 1.6 mL of nitric acid, and diluting with water to 250 mL. Add 10 mg of Gadoversetamide to 1.0 mL of the  $1.5 \times 10^{-4}$  M arsenazo

(III) solution, and mix. The color changes from a wine red to green-blue, indicating the presence of gadolinium.

**Bacterial endotoxins** (85): not more than 15 USP Endotoxin Units per g of gadoversetamide.

**Water, Method 1a** (921): not more than 10.0% (w/w), a solvent mixture of methanol and formamide (9:1) being used.

**Limits of free gadolinium (III) and total chelatable material—**

**MES buffer**—Dissolve 97.6 g of 2-morpholinoethanesulfonic acid (MES) in about 950 mL of water, and mix. Adjust with 20% sodium hydroxide to a pH of 6, dilute with water to 1000 mL, and mix.

**Edetate titrant:** 0.002 M edetate disodium VS.

**0.003 M Gadolinium (III) titrant**—Transfer 0.790 g of gadolinium chloride to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Transfer about 5 g of Gadoversetamide, accurately weighed, into a 250-mL flask, and add 20 mL of water and 2 mL of hydrochloric acid. Stir, and heat to boiling. Rinse the sides of the flask with water. Add 50 mL of MES buffer and 100 to 150  $\mu$ L of xylene orange TS to impart a light yellow color. Heat to boiling, adjust with ammonium hydroxide to a pH of 6, and continue boiling for 2 minutes. If the solution is yellow, proceed as directed under *Uncomplexed chelatable material*. If the color is red-violet, proceed as directed under *Free gadolinium (III)*.

**Uncomplexed chelatable material**—Continue boiling, and titrate with 0.003 M Gadolinium (III) titrant to a red-violet endpoint that undergoes no further color change upon addition of more titrant. Record the volume of titrant used to reach the endpoint.

**Free gadolinium (III)**—Continue boiling and titrate with Edetate titrant to a yellow or yellow-orange endpoint that undergoes no further color change upon addition of more titrant. Record the volume of titrant used to reach the endpoint.

**Calculations**—If the Test solution was titrated with Edetate titrant, calculate the percentage of Free gadolinium (III) in the portion of Gadoversetamide taken by the formula:

$$(66.18/W)(V_{EU})(M_E)$$

in which *W* is the weight, in g, of Gadoversetamide taken; *V<sub>EU</sub>* is the volume, in mL, of Edetate titrant used to titrate the Test solution; and *M<sub>E</sub>* is the molarity of the Edetate titrant. If the sample was titrated with 0.003 M Gadolinium (III) titrant, calculate the percentage of Uncomplexed chelatable material in the portion of Gadoversetamide taken by the formula:

$$(66.18/W)(V_{GU})(M_G)$$

in which *W* is as defined herein; *V<sub>GU</sub>* is the volume, in mL, of 0.003 M Gadolinium (III) titrant used to titrate the Test solution; and *M<sub>G</sub>* is the molarity of the 0.003 M Gadolinium (III) titrant. Not more than 0.05% of free gadolinium III and not more than 0.1% of uncomplexed chelatable material, both calculated on the anhydrous basis, are found.

**Limit of 2-methoxyethylamine—**

**Mobile phase**—Add 2 mL of 5 M phosphoric acid to 550 mL of water, mix, and adjust with 10% (w/w) ammonium hydroxide to a pH of 5.0. Add 450 mL of acetonitrile, mix, filter, and degas.

**0.4 M Borate buffer**—Add 12.4 g of boric acid to 300 mL of water, and swirl to suspend. Add 100 mL of 1 N potassium hydroxide, and mix. Adjust with about 60 mL of 1 N potassium hydroxide to a pH of 10.0, dilute with water to 500 mL, and mix. Test the pH, and adjust if necessary.

**o-Phthalaldehyde reagent**—Dissolve 25 mg of o-phthalaldehyde in 0.75 mL of methanol, add 25 mL of 0.4 M Borate buffer having a pH of 10.0 and 25  $\mu$ L of 2-mer-

captoethanol, and mix. [NOTE—Protect from light. Discard after 3 days.]

**Standard solutions**—Prepare aqueous solutions of 2-methoxyethylamine having known concentrations of about 1, 20, and 50  $\mu$ g per mL, respectively. Derivatize by adding an equal volume of o-Phthalaldehyde reagent to each solution immediately before injection.

**Test solution**—Transfer about 250 mg of Gadoversetamide, accurately weighed, to a 5-mL volumetric flask, and dissolve in and dilute with water to volume. Derivatize the solution by combining equal volumes of o-Phthalaldehyde reagent and Test solution immediately before injection.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 335-nm detector and a 250-mm  $\times$  4.6-mm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard solutions, and record the chromatograms as directed for Procedure: the relative retention times of o-phthalaldehyde and 2-methoxyethylamine are about 0.6 and 1.0, respectively. Plot the concentration of 2-methoxyethylamine in each Standard solution versus its peak area, and perform a regression analysis to obtain a slope and an intercept. The correlation coefficient, *r*, is not less than 0.995, and the relative standard deviation for replicate injections of the 50  $\mu$ g per mL Standard solution is not more than 5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the Test solution and the Standard solutions into the chromatograph, record the chromatograms, and measure the peak responses. Determine the concentration, in  $\mu$ g per mL, of 2-methoxyethylamine in the Test solution from the standard response line. Calculate the percentage of 2-methoxyethylamine by the formula:

$$0.5C/W$$

in which *C* is the concentration, in  $\mu$ g per mL, of 2-methoxyethylamine obtained from the Standard response line; and *W* is the weight, in mg, of Gadoversetamide taken. Not more than 0.10% (w/w) of 2-methoxyethylamine is present, calculated on the anhydrous basis.

**Limit of residual solvents—**

**Internal standard solution**—Dilute butyl alcohol with water (3:5000).

**Standard solutions**—To four separate 5-mL volumetric flasks, transfer the following designated compositions:

Flask	Isopropyl alcohol	Acetonitrile	Internal standard
1	25 $\mu$ g	25 $\mu$ g	1.0 mL
2	100 $\mu$ g	100 $\mu$ g	1.0 mL
3	250 $\mu$ g	250 $\mu$ g	1.0 mL
4	500 $\mu$ g	500 $\mu$ g	1.0 mL

Dilute each flask with water to volume, and mix. The resulting Standard solutions contain about 5, 20, 50, and 100  $\mu$ g of isopropyl alcohol and acetonitrile per mL.

**Test solution**—Transfer about 500 mg of Gadoversetamide, accurately weighed, to a 5-mL volumetric flask. Add 1.0 mL of Internal standard solution, dissolve in and dilute with water to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm  $\times$  30-m capillary column with a 1.0- $\mu$ m thickness of phase G35. Helium is used as the carrier gas, at a flow rate of about 5 mL per minute. The column temperature is maintained at 35° for 5 minutes, then increased at a rate of 15° per minute to 110°. The injection port temperature is maintained at 150°, and the detector temperature is maintained at 300°. Chromatograph the Standard solutions, and record the peak area ratios as di-

rected for *Procedure*: the relative retention times are about 0.5 for isopropyl alcohol, 0.7 for acetonitrile, and 1.0 for butyl alcohol. Plot the concentration for each standard versus its peak area ratio, and perform a regression analysis. The correlation coefficient,  $r$ , is not less than 0.995 for each analyte; and the relative standard deviation for replicate injections of the 100  $\mu\text{g}$  per mL *Standard solution* is not more than 5%.

*Procedure*—Separately inject equal volumes (about 2  $\mu\text{L}$ ) of the *Test solution* and the *Standard solutions* into the chromatograph, record the chromatograms, and measure the peak area ratios of the standard peak to the internal standard peak. Determine the concentration of isopropyl alcohol and acetonitrile from the respective standard response lines. Calculate the percentage (w/w) of each solvent in the portion of Gadoversetamide taken by the formula:

$$0.5C/W$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL (obtained from the respective standard response line) of isopropyl alcohol and acetonitrile in the *Test solution*; and  $W$  is the weight (anhydrous), in mg, of the portion of Gadoversetamide taken: not more than 0.1% (w/w) of isopropyl alcohol is found; and not more than 0.025% (w/w) of acetonitrile is found. The total residual solvent content (sum of the % w/w isopropyl alcohol and the % w/w acetonitrile) does not exceed 0.1% w/w.

#### Related compounds—

*Solution A*—Dissolve 2.06 g of monobasic potassium phosphate and 18.6 mL of 20% w/w tetraethylammonium hydroxide in 950 mL of water. Adjust with phosphoric acid to a pH of 7, dilute with water to make 1000 mL, mix, filter, and degas.

*Solution B*—Prepare a filtered and degassed mixture of *Solution A* and acetonitrile (475:25).

*Mobile phase*—Use a mixture of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard stock solution*—Transfer about 50 mg each of USP Gadoversetamide Related Compound A RS and USP Gadodiamide Related Compound B RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. [NOTE—The solution may be stored for a week.]

*Standard solutions*—Prepare aqueous solutions of diluted *Standard stock solution* containing about 25, 150, and 250  $\mu\text{g}$  of each Reference Standard per mL.

*Test solution*—Transfer about 250 mg of Gadoversetamide, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 205-nm detector and a metal-free 4.6-  $\times$  150-mm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is 1 mL per minute. The chromatograph is programmed to pump a mixture of *Solution A* to *Solution B* (97:3). The column temperature is maintained at about 25°. The relative retention times are about 0.6 for gadodiamide related compound B and 0.7 for gadoversetamide related compound A; the resolution,  $R_s$ , between gadodiamide related compound B and gadoversetamide related compound A is not less than 1.0; and the relative standard deviation for replicate injections is not more than 5% for the 250  $\mu\text{g}$  per mL *Standard solution*.

*Procedure*—Separately inject equal volumes (about 50  $\mu\text{L}$ ) of the *Test solution* and the *Standard solutions* into the chromatograph. Allow 1 hour between injections to remove slow-eluting impurities. Determine the quantities, in  $\mu\text{g}$  per mL, of gadoversetamide related compound A and gadodiamide related compound B from the respective Standard response lines. Calculate the percentage of gadoversetamide

related compound A in the portion of Gadoversetamide taken by the formula:

$$100C/V$$

in which  $C$  is the concentration of gadoversetamide related compound A, in  $\mu\text{g}$  per mL, obtained from the Standard response line; and  $V$  is the concentration of gadoversetamide, in  $\mu\text{g}$  per mL, in the *Test solution*. Not more than 1.0% (w/w) of gadoversetamide related compound A is found, calculated on the anhydrous basis. Calculate the percentage of gadodiamide related compound B in the portion of Gadoversetamide taken by the following formula:

$$92.2C/V$$

in which  $C$  is the concentration of gadodiamide related compound B, in  $\mu\text{g}$  per mL, obtained from the Standard response line; and  $V$  is as described herein. Not more than 0.5% (w/w) of gadodiamide related compound B is found, calculated on the anhydrous basis.

#### Assay—

*Mobile phase*—Dissolve 1.5 g of boric acid in about 950 mL of water, and mix. Adjust with ammonium hydroxide to a pH of 6.8, add 15 mL of acetonitrile, dilute with water to make 1000 mL, mix, filter, and degas.

*Standard preparations*—Prepare solutions of USP Gadoversetamide RS in *Mobile phase* having known concentrations of about 1.2, 1.0, and 0.8 mg per mL.

*Assay preparation*—Transfer about 100 mg of Gadoversetamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 205-nm detector and a metal-free 4.6-  $\times$  250-mm column that contains 5- $\mu\text{m}$  packing L1. The column temperature is maintained at about 50°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparations*, and record the peak responses as directed for *Procedure*. Plot the concentration of each Standard versus its peak area and perform a regression analysis to obtain a slope and intercept for the Standard response line. The correlation coefficient,  $r$ , is not less than 0.995; and the relative standard deviation for replicate injections of the 1.0 mg per mL *Standard preparation* is not more than 2%.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Assay preparation* and *Standard preparations* into the chromatograph, record the chromatograms, and measure the area of the gadoversetamide peak. Determine the quantity, in mg per mL, of gadoversetamide from the Standard response line. Calculate the quantity, in % (w/w), of  $\text{C}_{20}\text{H}_{34}\text{GdN}_5\text{O}_{10}$  in the portion of Gadoversetamide taken by the formula:

$$10,000C/W$$

in which  $C$  is the concentration, in mg per mL, obtained from the Standard response line; and  $W$  is the weight (anhydrous), in mg, of the portion of Gadoversetamide taken to prepare the *Assay preparation*.

## Gadoversetamide Injection

» Gadoversetamide Injection is a sterile solution of Gadoversetamide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of gadoversetamide ( $\text{C}_{20}\text{H}_{34}\text{GdN}_5\text{O}_{10}$ ). It may con-



tain buffers and stabilizers. It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose Type I glass containers or plastic syringes. Store at controlled room temperature.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Gadoversetamide RS

USP Gadoversetamide Related Compound A RS

Hydrogen [8, 11, 14-tris(carboxymethyl)-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(4-)]gadolinium.

**Bacterial endotoxins** (85): not more than 5 USP Endotoxin Units per mL of gadoversetamide.

**pH** (791): between 5.5 and 7.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Relaxivity** (761)—

**Standard solutions**—Dissolve an accurately weighed quantity of manganese (II) chloride tetrahydrate in water, and quantitatively dilute with water to obtain solutions having known concentrations of 0.9 mM, 2.7 mM, and 4.5 mM.

**Test stock solution**—Transfer 5.0 mL of the Injection to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Test solutions**—Transfer 1.0, 2.0, 4.0, and 6.0 mL of *Test stock solution* to individual 100-mL volumetric flasks, dilute each with water to volume, and mix. These solutions have concentrations of 0.504 mM, 1.008 mM, 2.016 mM, and 3.024 mM, respectively, based on the label claim.

**Apparatus**—Use a mini-NMR spectrometer with suitable sensitivity (see *Apparatus* under *Nuclear Magnetic Resonance* (761)).

**System suitability**—Place a portion of each of the *Standard solutions* into a separate 10-mm specimen tube. Warm to 40° for not less than 10 minutes, and measure the resonance frequency ( $T_1$ ), at 20 MHz. The average  $T_1$  for replicate measurements must be within 5% of 156 ms for the 0.9 mM *Standard solution*, 52 ms for the 2.7 mM *Standard solution*, and 32 ms for the 4.5 mM *Standard solution*.

**Procedure**—Place an accurately measured portion of each *Test solution* into a 10-mm specimen tube. Warm to 40° for not less than 10 minutes, and measure the resonance frequency ( $T_1$ ) of each *Test solution*. Plot  $1/T_1$  versus the molarities of the *Test solutions*, and perform a regression analysis. The slope of the plotted line is the relaxivity. The relaxivity is between 4.0 and 5.0 sec<sup>-1</sup> mM<sup>-1</sup>.

**Related compounds**—

**Solution A**—Proceed as directed for *Mobile phase* in the test for *Related compounds* under *Gadoversetamide*.

**Solution B**—Prepare a filtered and degassed mixture of *Solution A* and acetonitrile (475:25).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solutions**—Prepare aqueous solutions of USP Gadoversetamide Related Compound A RS having known concentrations of about 30 µg per mL, 150 µg per mL, and 360 µg per mL.

**Test solution**—Transfer 5.0 mL of the Injection to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—Proceed as directed in the test for *Related compounds* under *Gadoversetamide*. The chromatograph is programmed as follows.

Time (minutes)	Solution A %	Solution B %	Elution
0–15	97	3	equilibration
15–16	97→0	3→100	linear gradient
16–26	0	100	isocratic
26–27	0→97	100→3	linear gradient
27–45	97	3	re-equilibration

Plot the concentration, in µg per mL, of each *Standard solution* versus its peak area, and perform a regression analysis to obtain a slope and intercept for the *Standard response line*. The relative standard deviation for replicate injections of the 360 µg per mL *Standard solution* is not more than 5%; and the correlation coefficient,  $r$ , of the regression analysis is not less than 0.995.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Test Solution*, the *Standard solutions*, and water (blank) into the chromatograph, record the chromatograms, and measure the peak responses. Allow about 1 hour between injections to remove slow-eluting impurities from the column. Calculate the percentage of gadoversetamide related compound A relative to the amount of gadoversetamide (C<sub>20</sub>H<sub>34</sub>GdN<sub>5</sub>O<sub>10</sub>) in the portion of Injection taken, based on the label claim, by the formula:

$$0.003C$$

in which  $C$  is the concentration of gadoversetamide related compound A in the *Test solution*, in µg per mL, obtained from the *Standard response line*: not more than 1.0% (w/w) of gadoversetamide related compound A is found.

**Other requirements**—It meets the requirements of the *Identification tests* under *Gadoversetamide*. It also meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase, Standard preparations, and Chromatographic system**—Proceed as directed in the *Assay* under *Gadoversetamide*.

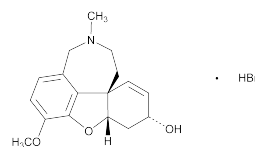
**Assay preparation**—Transfer about 3.0 mL of the Injection, accurately measured, to a 1000-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Gadoversetamide*. Calculate the quantity, in mg per mL, of gadoversetamide (C<sub>20</sub>H<sub>34</sub>GdN<sub>5</sub>O<sub>10</sub>) in the volume of Injection taken by the formula:

$$1000C/V$$

in which  $C$  is the concentration, in mg per mL, of gadoversetamide in the *Assay preparation*, obtained from the *Standard response line*; and  $V$  is the volume, in mL, of Injection taken.

## Galantamine Hydrobromide



C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub> · HBr 368.27  
 6*H*-Benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-ol, 4*a*,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-, hydrobromide, (4*a*S,6*R*,8*a*S)-;  
 (4*a*S,6*R*,8*a*S)-4*a*,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3*a*,3,2-*ef*][2]benzazepin-6-ol hydrobromide [1953-04-4].

**DEFINITION**

Galantamine Hydrobromide contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{21}NO_3 \cdot HBr$ , calculated on the dried basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)

[NOTE—Specimens are to be prepared using undried USP Galantamine Hydrobromide RS and the test article.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Bromide (191):** Meets the requirements of the silver nitrate precipitate test  
**Sample solution:** A solution of 7 mg/mL in water

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol and water (1:19)

**Buffer:** 0.79 g/L of dibasic sodium phosphate dihydrate and 2.46 g/L of anhydrous monobasic sodium phosphate in water

**Solution A:** Methanol and *Buffer* (1:19)

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6.0	100	0
20.0	95	5
35.0	85	15
50.0	80	20
51.0	40	60
55.0	40	60
56.0	100	0
60.0	100	0

**System suitability solution:** 1 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*

**Standard solution:** 1.0 mg/mL of USP Galantamine Hydrobromide RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Galantamine Hydrobromide in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L1

**Column temperature:** 55°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—For relative retention times, see *Impurity Table 1*.]

**Suitability requirements**

**Resolution:** NLT 4.5 between galantamine and 6α-hexahydrogalantamine, *System suitability solution*

**Tailing factor:** NMT 2.0 for galantamine hydrobromide, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{21}NO_3 \cdot HBr$  in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Galantamine Hydrobromide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS, Method II** (231): NMT 20 ppm

• **LIMIT OF PALLADIUM**

[NOTE— Perform this test only if palladium is a known inorganic impurity of the manufacturing process.]

**Standard stock solution:** 20 mg/L of palladium reference stock solution (NIST traceable) in water

**Aqua regia:** Under a hood, carefully mix hydrochloric acid and nitric acid (3:1).

[NOTE— To obtain each of the required *Standard solutions*, it is recommended that the required volume of *Standard stock solution* be mixed with a volume of *Aqua regia* equivalent to 5% of the final volume, followed by water.]

**Standard solution A:** 0.2 mg/L of palladium from *Standard stock solution* in water

**Standard solution B:** 1.0 mg/L of palladium from *Standard stock solution* in water

**Standard solution C:** 2.0 mg/L of palladium from *Standard stock solution* in water.

**System suitability solution:** Prepare a solution having a known concentration of 1.6 mg/L of palladium, as directed for *Standard solutions*.

**Sample solution:** Weigh 1 g of Galantamine Hydrobromide. Transfer the sample to an appropriate digestion system, and digest using appropriate acids (e.g., nitric acid or mixtures of nitric acid and sulfuric acid and mixtures of nitric acid and hydrogen peroxide). After digestion, heat to dryness. Add 0.5 mL of *Aqua regia* and 2 mL of water. Warm gently to dissolve any residue. Allow to cool. Transfer quantitatively to a 10-mL volumetric flask, and dilute with water to volume.

**Digestion blank solution:** Prepare this solution following the procedure for the *Sample solution*, without the test article.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Atomic absorption spectroscopy (flame)

**Analytical wavelength:** 247.6 nm (0.2-nm slit width)

**Lamp:** Palladium hollow-cathode

**Blank solution:** Dilute 5 mL of *Aqua regia* with water to 100 mL.

**System suitability**

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *System suitability solution*, and *Blank solution*

Using the *Standard solutions* and *Blank solution*, construct a calibration curve.

**Suitability requirements**

**Correlation coefficient:** NLT 0.99

**Recovery:** 87.5%–112.5%, *System suitability solution*.  
[NOTE— Recovery is calculated using the calibration curve.]

**Analysis**

**Samples:** *Sample solution* and *Digestion blank solution*  
Calculate the concentration of palladium in the *Sample solution*, using the calibration curve, corrected for the *Digestion blank solution* and the sample weight. Calculate the amount of palladium in the Galantamine Hydrobromide taken to prepare the *Sample solution*.

**Acceptance criteria:** NMT 10 ppm

**Organic Impurities**• **PROCEDURE**

**Diluent, Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chro-**

**matographic system, and System suitability:** Prepare as directed in the Assay.

**Standard solution:** 5.0 µg/mL of USP Galantamine Hydrobromide RS in *Diluent*

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

[NOTE—Ignore the peak due to bromide near the void volume and any peak below 0.05%.]

Calculate the percentage of each impurity in the portion of Galantamine Hydrobromide taken, on the dried basis:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (100/100 - L)$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of galantamine from the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$F$  = relative response factor from *Impurity Table 1*

$L$  = *Loss on Drying* in percent

**Acceptance criteria:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl-galantamine <sup>a</sup>	0.29	1.2	0.6
O-Desmethyl-galantamine <sup>b</sup>	0.35	1.1	0.20
6β-Hexahydrogalantamine (also known as galantamine <i>N</i> -oxide) <sup>c</sup>	0.65	0.96	0.20
6β-Octahydrogalantamine <sup>d</sup>	0.82	0.81	0.35
Galantamine hydrobromide	1.00	1.0	—
6α-Hexahydrogalantamine (also known as epigalantamine) <sup>e</sup>	1.16	0.95	0.20
Tetrahydrogalantamine <sup>f</sup>	2.05	1.2	0.40
Narwedine <sup>g</sup>	1.64	1.9	0.15

<sup>a</sup> (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>b</sup> (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-hydroxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>c</sup> [4a*S*-(4α*α*,6β,8a*R*<sup>\*</sup>)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.

<sup>d</sup> [4a*S*-(4α*α*,6β,8a*R*<sup>\*</sup>)]-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>e</sup> [4a*S*-(4α*α*,6α,8a*R*<sup>\*</sup>)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>f</sup> [4a*S*-(4a*R*<sup>\*</sup>,8a*R*<sup>\*</sup>)]-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-4a*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.

<sup>g</sup> (4a*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-one. [NOTE—This is a process impurity that may be found in galantamine hydrobromide isolated from a natural source.]

<sup>h</sup> Do not include the 4*R*,8*R*-stereoisomer.

**Impurity Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	1.0	0.10
Total impurities <sup>h</sup>	—	—	1.0

<sup>a</sup> (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>b</sup> (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-hydroxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>c</sup> [4a*S*-(4α*α*,6β,8a*R*<sup>\*</sup>)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.

<sup>d</sup> [4a*S*-(4α*α*,6β,8a*R*<sup>\*</sup>)]-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>e</sup> [4a*S*-(4α*α*,6α,8a*R*<sup>\*</sup>)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

<sup>f</sup> [4a*S*-(4a*R*<sup>\*</sup>,8a*R*<sup>\*</sup>)]-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-4a*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.

<sup>g</sup> (4a*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-one. [NOTE—This is a process impurity that may be found in galantamine hydrobromide isolated from a natural source.]

<sup>h</sup> Do not include the 4*R*,8*R*-stereoisomer.

#### SPECIFIC TESTS

• **LOSS ON DRYING (731):** Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

#### • ENANTIOMERIC PURITY

[NOTE—If Galantamine Hydrobromide is not isolated from a natural source, perform either *Procedure 1* or *Procedure 2*.]

##### Procedure 1

**Background electrolyte solution:** 8.9 g/L of dibasic sodium phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 3.0.

**Run buffer:** 19.6 g/L of α-cyclodextrin hydrate in *Background electrolyte solution*. Pass the solution through a 0.22-µm filter.

**Standard solution:** 5 µg/mL of USP Galantamine Hydrobromide Racemic RS in water. Pass the solution through a 0.22-µm filter, discarding the first 8 mL.

**Sample solution:** 0.5 mg/mL of Galantamine Hydrobromide in water. Pass the solution through a 0.22-µm filter, discarding the first 8 mL.

**Capillary rinse procedure:** Use separate *Run buffer* vials for the capillary rinse and sample analysis. Proceed as directed in the table below.

Step #	Solution/Gas	Time (min)
1	0.1 N sodium hydroxide	15
2	Water	10
3	Suitable gas	5

[NOTE—If a new or dry capillary is being used, rinse with 1 N sodium hydroxide for 30 min, followed by rinsing with water for 15 min. Dry it with air or nitrogen for 10 min.]

#### Electrophoretic system

(See *Capillary Electrophoresis (727)*, *System Suitability*.)

**Mode:** High performance CE

**Detector:** UV 214 nm

**Column:** 75-µm × 60-cm uncoated fused-silica

**Column temperature:** 20°

**Applied voltage:** 250 V/cm, positive polarity

**Run time:** 35 min

#### System suitability

**Sample:** *Standard solution*. [NOTE—For the purpose of identification, the 4*S*,8*S* stereoisomer elutes at an approximate relative migration time (RMT) of 1.00, and the 4*R*,8*R* stereoisomer elutes at an RMT of about 1.05.]

**Suitability requirements****Resolution:** NLT 2.5 between the two enantiomers**Relative standard deviation:** NMT 10% for the 4*R*, 8*R* stereoisomer peak

Measure the migration times and peak responses: the migration times for the 4*R*,8*R* stereoisomer in the electropherograms for the *Sample solution* should not deviate by more than 5% of the migration time for the same component in the electropherogram of the *Standard solution*.

**Analysis****Samples:** *Standard solution* and *Sample solution***Injection:** [NOTE—Rinse the capillary between injections as follows: water for 5 min, followed by *Run buffer* for 5 min. Rinse times are based on a rinse pressure of 1.4 bar.]**Sample solution:** 34.5 mbar for 4 s**Run buffer:** 6.9 mbar for 5 s

Calculate the corrected peak responses using the formula:

$$\text{Result} = (r/m)$$

*r* = peak response*m* = migration time of the peak, in minCalculate the limit of the 4*R*,8*R* isomer, in percent, in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = (r_{\text{CU}}/r_{\text{CS}}) \times (C_{\text{S}}/C_{\text{U}}) \times P \times 100$$

*r*<sub>CU</sub> = average corrected peak responses for the 4*R*, 8*R* isomer from the *Sample solution**r*<sub>CS</sub> = average corrected peak responses for the 4*R*, 8*R* isomer from the *Standard solution**C*<sub>S</sub> = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)*C*<sub>U</sub> = concentration of Galantamine Hydrobromide in the *Sample solution* (mg/mL)*P* = chiral purity of USP Galantamine Hydrobromide Racemic RS, 0.5**Acceptance criteria:** NMT 0.10% of the 4*R*,8*R* stereoisomer**Procedure 2**

[NOTE—Use low-actinic glassware and vials. It is recommended that precautions be taken to protect all solutions from light.]

**Buffer:** 8.2 g/L of sodium acetate in water**Mobile phase:** Acetonitrile and *Buffer* (1:49). Adjust with acetic acid to a pH of 6.5.**System suitability solution:** 1.2 mg/mL of USP Galantamine Hydrobromide RS and 3.6 µg/mL of USP Galantamine Hydrobromide Racemic RS in water.[NOTE—This solution will contain about 1.8 µg/mL of the 4*R*,8*R* stereoisomer.]**Sample solution:** 1.2 mg/mL of Galantamine Hydrobromide in water**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.0-mm × 15-cm; 5-µm packing L41.

[NOTE—Alternatively a 2.0-mm × 15.0-cm column containing 5-µm L41 packing can be used with a recommended flow rate of about 0.2 mL/min.]

**Flow rate:** 0.8 mL/min**Injection size:** 5 µL**System suitability****Sample:** *System suitability solution*. [NOTE—The 4*R*,8*R* stereoisomer elutes first as the minor peak followed by the major peak due to galantamine (which is the same as the 4*S*,8*S* stereoisomer).]**Suitability requirements****Resolution:** NLT 3.0 between the 4*R*,8*R* stereoisomer and galantamine peaks**Relative standard deviation:** NMT 5.0% for the 4*R*, 8*R* stereoisomer peak**Analysis****Sample:** *Sample solution*Calculate the percentage of 4*R*,8*R* stereoisomer in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = 100 \times [r_{4R,8R}/(r_{4R,8R} + r_{4S,8S})]$$

*r*<sub>4*R*,8*R*</sub> = peak area of the 4*R*,8*R* stereoisomer from the *Sample solution**r*<sub>4*S*,8*S*</sub> = peak area of the galantamine peak from the *Sample solution***Acceptance criteria:** NMT 0.10% of the 4*R*,8*R* stereoisomer**• OPTICAL ROTATION, Specific Rotation <781>**[NOTE—If Galantamine Hydrobromide is isolated from a natural source, perform the test for *Optical Rotation*.]**Acceptance criteria:** −90° to −100°**Sample solution:** 20 mg/mL in water**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Store at room temperature. Preserve in well-closed containers.**• LABELING:** Label it to state if the source is naturally derived or is synthetic. If the source is not natural, perform either *Procedure 1* or *Procedure 2* of the test for *Enantiomeric Purity*. If the source is natural, perform the test for *Optical Rotation* <781>, *Specific Rotation*.**• USP REFERENCE STANDARDS <11>**

USP Galantamine Hydrobromide RS

USP Galantamine Hydrobromide Racemic RS

A 50:50 mixture of 4*S*,8*S* and 4*R*,8*R* isomers.

USP Galantamine Hydrobromide Related Compounds Mixture RS

Contains galantamine hydrobromide, 6β-hexahydrogalantamine, 6β-octahydrogalantamine, 6α-hexahydrogalantamine, and tetrahydrogalantamine.

**Galantamine Tablets****DEFINITION**Galantamine Tablets contain an amount of Galantamine Hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of galantamine (C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub>).**IDENTIFICATION****• A. ULTRAVIOLET ABSORPTION <197U>** The spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Uniformity of Dosage Units*.**• B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****• PROCEDURE****Buffer solution:** 5.34 g/L of dibasic sodium phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 6.5.**Solution A:** Methanol and *Buffer solution* (1:19)**Solution B:** Acetonitrile and methanol (19:1)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40.0	75	25
45.0	60	40

Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
46.0	40	60
55.0	40	60
56.0	100	0
61.0	100	0

**Diluent:** Dissolve 35.4 g of edetate disodium in 950 mL water, and add 50 mL of methanol. [NOTE—First dissolve in water, then add methanol.]

**Standard solution:** 0.62 mg/mL of USP Galantamine Hydrobromide RS in *Diluent*

**Sample solution:** 0.48 mg/mL of galantamine from powdered Tablets (NLT 10) in *Diluent*. Pass through a PTFE filter of 0.45- $\mu$ m or finer pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  10-cm; 3- $\mu$ m L1 packing

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of galantamine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of galantamine, 287.35

$M_{r2}$  = molecular weight of galantamine hydrobromide, 368.27

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

#### Test 1

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 20 min

**Standard solution:** ( $L/400$ ) mg/mL of USP Galantamine Hydrobromide RS in *Medium*, where  $L$  is the label claim in mg

**Sample solution:** Pass portions of the solution through a suitable filter of 0.2- $\mu$ m pore size.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* <851>.)

**Mode:** UV

**Analytical wavelength:** 288 nm

**Cell:** 5-cm cell for 4-mg and 8-mg Tablets; 1-cm cell for 12-mg Tablets

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) dissolved in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of galantamine, 287.35

$M_{r2}$  = molecular weight of galantamine hydrobromide, 368.27

**Tolerances:** NLT 80% (Q) of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium, Apparatus 2, Time, Standard solution, Sample solution, and Analysis:** Proceed as directed for *Test 1*.

**Tolerances:** NLT 70% (Q) of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm, use peak vessels

**Time:** 20 min

**Buffer:** Triethylamine and 3.45 g/L of monobasic sodium phosphate in water (1:1000). Adjust with phosphoric acid to a pH of 4.5.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (10:10:80). Filtered and de-aerated.

**Standard solution:** ( $L/400$ ) mg/mL of USP Galantamine Hydrobromide RS in water, where  $L$  is the label claim in mg

**Sample solution:** Pass a portion of the solution through a suitable filter of 0.45- $\mu$ m pore size. Use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** Two times the retention time of galantamine

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of galantamine, 287.35

$M_{r2}$  = molecular weight of galantamine hydrobromide, 368.27

**Tolerances:** NLT 80% (Q) of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905)

#### Procedure for content uniformity

**Standard solution:** 0.05 mg/mL of USP Galantamine Hydrobromide RS in 0.1 N hydrochloric acid

**Sample solution:** Add 1 Tablet to each appropriately sized volumetric flask to obtain a final galantamine

concentration of 0.04 mg/mL. Add an appropriate amount of 0.1 N hydrochloric acid, equivalent to 75% of the total volume of the volumetric flask, and mechanically shake for 45 min. Dilute with 0.1 N hydrochloric acid to volume. Pass a portion of the solution through a suitable filter of 0.2- $\mu$ m pore size, and use the filtrate.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** Absorption maximum at about 289 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amount of galantamine ( $C_{17}H_{21}NO_3$ ) dissolved in filtered portions of the *Sample solution* in comparison with the *Standard solution*.

Calculate the percentage of the labeled amount of galantamine ( $C_{17}H_{21}NO_3$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of galantamine, 287.35

$M_{r2}$  = molecular weight of galantamine hydrobromide, 368.27

**Acceptance criteria:** Meet the requirements for coated Tablets

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer solution, Solution A, Solution B, Mobile phase, Diluent, Standard solution, and Sample solution:** Prepare as directed in the Assay.

**System suitability solution:** 0.6 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between 6 $\beta$ -hexahydrogalantamine and 6 $\beta$ -octahydrogalantamine, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for galantamine, *Standard solution*

[NOTE—Identify the impurities using the approximate relative retention times given in *Table 2*.]

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Ignore the peak due to bromide near the void volume.]

Calculate the percentage of each of the impurities including the unspecified degradation impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (100/F)$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of galantamine from the *Standard solution*

$C_S$  = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of galantamine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of galantamine, 287.35

$M_{r2}$  = molecular weight of galantamine hydrobromide, 368.27

$F$  = relative response factor for each of the impurities relative to galantamine (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethylgalantamine <sup>a</sup>	0.41	1.1	0.5
6 $\beta$ -Hexahydrogalantamine (also known as galantamine N-oxide) <sup>b</sup>	0.73	1.1	0.75
6 $\beta$ -Octahydrogalantamine (also known as lycoramine) <sup>c,d</sup>	0.86	—	—
Galantamine hydrobromide	1.00	1.0	—
6 $\alpha$ -Hexahydrogalantamine (also known as epigalantamine) <sup>e</sup>	1.15	1.1	0.5
Tetrahydrogalantamine <sup>f,d</sup>	2.09	—	—
Individual, unspecified degradation impurity	—	1.0	0.2
Total impurities	—	—	1.5

<sup>a</sup> [4aS,6R,8aS]-4a,5,9,10,11,12-Hexahydro-3-methoxy-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol.

<sup>b</sup> [4aS-(4 $\alpha$ ,6 $\beta$ ,8aR\*)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol, N-oxide.

<sup>c</sup> [4aS-(4 $\alpha$ ,6 $\beta$ ,8aR\*)]-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol.

<sup>d</sup> Impurities are not quantified and are intended for system suitability evaluation only.

<sup>e</sup> [4aS-(4 $\alpha$ ,6 $\alpha$ ,8aR\*)]-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol.

<sup>f</sup> [4aS-(4aR\*,8aR\*)]-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-4aH-benzofuro[3a,3,2-ef][2]benzazepine.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

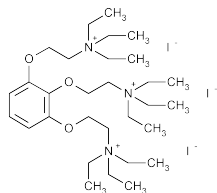
### • USP REFERENCE STANDARDS (11)

USP Galantamine Hydrobromide RS

USP Galantamine Hydrobromide Related Compounds Mixture RS

Contains galantamine hydrobromide, 6 $\beta$ -hexahydrogalantamine, 6 $\beta$ -octahydrogalantamine, 6 $\alpha$ -hexahydrogalantamine, and tetrahydrogalantamine.

## Gallamine Triethiodide



$C_{30}H_{60}I_3N_3O_3$  891.53

Ethanaminium, 2,2',2''-[1,2,3-benzenetriyltris(oxy)]tris[*N,N,N*-triethyl]-, triiodide.

[*v*-Pheneny]tris(oxyethylene)]tris[triethylammonium] triiodide [65-29-2].

» Gallamine Triethiodide contains not less than 98.0 percent and not more than 101.0 percent of  $C_{30}H_{60}I_3N_3O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** (11)—

USP Gallamine Triethiodide RS

**Clarity and color of solution**—A solution (1 in 50) is clear and colorless.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** A solution (1 in 100) responds to the tests for *Iodide* (191).

**pH** (791): between 5.3 and 7.0, in a solution (1 in 50).

**Loss on drying** (731)—Dry it at 100° for 4 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals** (231)—Dissolve 1.0 g in 25 mL of water: the limit is 0.002%.

**Assay**—

*Sodium perchlorate buffer*—Prepare a 0.14 M solution of sodium perchlorate in water, and adjust with 10 N sodium hydroxide or 0.05 M phosphoric acid to a pH of 3.0.

*Mobile phase*—Prepare a filtered and degassed mixture of *Sodium perchlorate buffer* and acetonitrile (69:31). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Gallamine Triethiodide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.

*Assay preparation*—Transfer about 25 mg of Gallamine Triethiodide, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{30}H_{60}I_3N_3O_3$  in the portion of Gallamine Triethiodide taken by the formula:

$$25C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Gallamine Triethiodide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gallamine Triethiodide Injection

» Gallamine Triethiodide Injection is a sterile solution of Gallamine Triethiodide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of gallamine triethiodide ( $C_{30}H_{60}I_3N_3O_3$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Gallamine Triethiodide RS

**Identification**—Evaporate a volume of Injection, equivalent to not less than 200 mg of gallamine triethiodide, to dryness. Take up the residue in warm alcohol, and pass through fine filter paper. Remove a portion of the filtrate, equivalent to about 100 mg of gallamine triethiodide, and evaporate to dryness: the residue responds to the *Identification* tests under *Gallamine Triethiodide*.

**Bacterial endotoxins** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of gallamine triethiodide.

**pH** (791): between 6.5 and 7.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Sodium perchlorate buffer*, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Gallamine Triethiodide*.

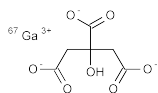
*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of gallamine triethiodide, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{30}H_{60}I_3N_3O_3$  in the portion of Injection taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Gallamine Triethiodide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gallium Citrate Ga 67 Injection



$C_6H_5^{67}GaO_7$

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, gallium- $^{67}Ga$  (1:1) salt.

Gallium- $^{67}Ga$  citrate (1:1) [41183-64-6; 52260-70-5].

» Gallium Citrate Ga 67 Injection is a sterile aqueous solution of radioactive, essentially carrier-free gallium citrate Ga 67 suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{67}Ga$  as citrate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 3.0 percent of the total radioactivity. It may contain a preservative or stabilizer.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{67}Ga$  as labeled gallium citrate expressed as total megabecquerels (MBq) (microcuries [ $\mu Ci$ ] or millicuries [ $mCi$ ]) and concentration as megabecquerels ( $\mu Ci$  or  $mCi$ ) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calibrations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{67}Ga$  is 78.26 hours.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It meets the requirements of the *Bacterial Endotoxins Test*, the limit of endotoxin content being not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 4.5 and 8.0.

**Radiochemical purity**—Place 10 to 20  $\mu L$  of Injection about 3 cm from one end of a 3- × 55-cm strip of chromatographic paper (see *Chromatography* (621)). While spots are wet, immediately develop the chromatogram at room temperature to the 14-cm mark by ascending chromatography, using a solvent system consisting of a mixture of 1.36 g of sodium acetate and 0.58 mL of glacial acetic acid in each 100 mL of water. Allow the strip to partially dry, cover with clear tape, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector: not less than 97.0% of the total radioactivity is found as gallium citrate when measured at the solvent front ( $R_f$  value equal to or greater than 0.9).

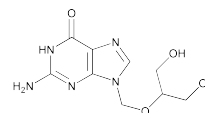
**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{67}Ga$  of known purity that exhibits major photopeaks having energies of 93.3, 184.6, and 300.2 KeV.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radionuclidic purity of the Injection: not less than 99% of the total radioactivity is present as  $^{67}Ga$  at the time of calibration.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity in MBq ( $\mu Ci$  or  $mCi$ ) per mL of Gallium Ga 67 Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Ganciclovir



$C_9H_{13}N_5O_4$  255.23  
6H-Purin-6-one, 2-amino-1,9-dihydro-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-;  
9-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine  
[82410-32-0].

### DEFINITION

Ganciclovir contains NLT 98.0% and NMT 102.0% of  $C_9H_{13}N_5O_4$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Sample solution: 10  $\mu g/mL$  in methanol

### ASSAY

#### PROCEDURE

**Solution A:** Trifluoroacetic acid and water (0.5 in 1000)  
**Mobile phase:** Acetonitrile and *Solution A* (1:1)

**System suitability solution:** 0.1 mg/mL each of USP Ganciclovir RS and USP Ganciclovir Related Compound A RS in *Mobile phase*. [NOTE—Sonicate the solution if necessary.]

**Standard solution:** 0.22 mg/mL of USP Ganciclovir RS in *Mobile phase*

**Sample solution:** 0.22 mg/mL of Ganciclovir in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L9

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu L$

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for ganciclovir related compound A and ganciclovir are 0.9 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.4 between ganciclovir and ganciclovir related compound A

**Column efficiency:** NLT 5000 theoretical plates

**Tailing factor:** NMT 1.4

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_9H_{13}N_5O_4$  in the portion of Ganciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Ganciclovir RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Ganciclovir in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **HEAVY METALS, Method II** (231): NMT 20 ppm
- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Organic Impurities****• PROCEDURE**

**Solution A, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** 0.22 mg/mL of Ganciclovir in *Mobile phase*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Ganciclovir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity in the *Sample solution*

$r_T$  = sum of the responses of all the peaks

**Acceptance criteria**

**Ganciclovir related compound A:** NMT 0.5%

**Total impurities:** NMT 1.5%

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** (921): NMT 6.0%  
[NOTE—Ganciclovir is extremely hygroscopic.]

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)  
USP Ganciclovir RS  
USP Ganciclovir Related Compound A RS

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**Ganciclovir for Injection**

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» Ganciclovir for Injection is a freeze-dried powder prepared by the neutralization of Ganciclovir with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ganciclovir ( $C_9H_{13}N_5O_4$ ), calculated on the anhydrous basis.

**Caution**—Handle Ganciclovir for Injection with great care, as it is a potent cytotoxic agent and suspected carcinogen.

**Packaging and storage**—Preserve in *Containers for Sterile Solids*, as described under *Injections* (1). Store between 15° and 30°, unless otherwise specified by the manufacturer. Protect from moisture.

**Labeling**—Label it to state that it is to be handled with great care because it is a potent cytotoxic agent and suspected carcinogen.

**USP Reference standards** (11)—

USP Endotoxin RS  
USP Ganciclovir RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.84 Endotoxin Unit per mg of Ganciclovir for Injection.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product To Be Examined*.

**pH** (791): between 10.8 and 11.4, in the solution constituted as directed in the labeling.

**Water, Method I** (921)—Proceed as directed in the chapter, except to use the following modifications. Use a mixture of anhydrous formamide and methanol (1:1) in place of methanol as the titration vessel solvent. The *Reagent* volume required in order to condition the titration vessel solvent is not greater than 10% of the initial volume of solvent. The concentration of Ganciclovir for Injection in the titration vessel is not greater than 7 mg per mL. Not more than 3.0% is found.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Assay**—

**Mobile phase**—Dissolve 1.4 g of monobasic ammonium phosphate and 2.0 g of phosphoric acid in 500 mL of water in a 1000-mL volumetric flask. Dilute with water to volume, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 75 mg of hypoxanthine to a 500-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Standard stock preparation**—Dissolve an accurately weighed amount of USP Ganciclovir RS in water to obtain a solution having a known concentration of about 250 µg per mL.

**Standard preparation**—Transfer 20.0 mL of the *Standard stock preparation* and 10.0 mL of the *Internal standard solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Assay stock preparation**—Constitute Ganciclovir for Injection in a portion of water, quantitatively transfer with water to a suitable volumetric flask, and dilute with water to volume to obtain a solution having a concentration of about 1 mg per mL.

**Assay preparation**—Transfer 5.0 mL of the *Assay stock preparation* and 10.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for hypoxanthine and 1.0 for ganciclovir; the resolution,  $R$ , between hypoxanthine and ganciclovir is not less than 3.0; the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak response ratios for the major peaks. Calculate

the quantity, in mg, of ganciclovir ( $C_9H_{13}N_5O_4$ ) in the container of Ganciclovir for Injection taken by the formula:

$$CD(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ganciclovir RS in the *Standard preparation*;  $D$  is the dilution factor, in mL, used to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of ganciclovir to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ganciclovir Oral Suspension

### DEFINITION

Ganciclovir Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ganciclovir ( $C_9H_{13}N_5O_4$ ). Prepare Ganciclovir Oral Suspension 100 mg/mL as follows (see *Pharmaceutical Compounding* (795)—Nonsterile Preparations).

Ganciclovir	10 g
Vehicle for Oral Solution (regular or sugar-free), NF, a sufficient quantity to make	100 mL

Empty the contents of the required number of capsules into a suitable mortar, or use *Ganciclovir* powder. Add sufficient *Vehicle* to wet the powder, and triturate to form a smooth paste. Add additional *Vehicle* to about half the final volume, and transfer the contents of the mortar to a calibrated bottle. Using additional *Vehicle*, rinse the mortar, and transfer the contents, stepwise and quantitatively, to bring to final volume. Mix well.

**[CAUTION]**—Avoid skin contact or inhalation of ganciclovir by using protective gloves and a fume hood or surgical mask.]

### ASSAY

#### • PROCEDURE

**Solution A:** 25-mM monobasic sodium phosphate solution. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and *Solution A* (2.5: 97.5). Filter and degas.

**Internal standard solution:** 0.4 mg/mL of hypoxanthine  
**Standard stock solution:** 1.0 mg/mL of USP Ganciclovir RS

**Standard solution:** 6 µg/mL of ganciclovir and 4 µg/mL of hypoxanthine prepared from *Standard stock solution* and *Internal standard solution*

**Sample solution:** Transfer about 1 mL of Oral Suspension from each bottle to a plastic weighing cup, and weigh to determine density. [NOTE—The exact volume of Oral Suspension taken from each bottle is calculated by the suspension density.] Transfer the Oral Suspension to a 100-mL volumetric flask, and add 50 mL of water. Place the volumetric flask on a mechanical shaker for 30 min, and dilute with water to volume. Transfer 0.6 mL of this solution and 1 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with water to volume to obtain a solution with a nominal concentration of 6 µg/mL of ganciclovir and 4 µg/mL of hypoxanthine.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 10-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for hypoxanthine and ganciclovir are 0.75 and 1.0, respectively.]

#### Suitability requirements

**Relative standard deviation:** NMT 1.5% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ganciclovir ( $C_9H_{13}N_5O_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of ganciclovir to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of ganciclovir to the internal standard from the *Standard solution*

$C_S$  = concentration of ganciclovir in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ganciclovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

- **pH (791):** 4.0–5.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded
- **USP REFERENCE STANDARDS (11)**  
USP Ganciclovir RS

## Absorbent Gauze

» Absorbent Gauze is cotton, or a mixture of cotton, and not more than 53.0 percent, by weight, of rayon, and is in the form of a plain woven cloth conforming to the standards set forth herein. Absorbent Gauze that has been rendered sterile is packaged to protect it from contamination.

NOTE—Condition all Absorbent Gauze for not less than 4 hours in a standard atmosphere of  $65 \pm 2\%$  relative humidity at  $21 \pm 1.1^\circ$  before determining the weight, thread count, and absorbency. Remove the Absorbent Gauze from its wrappings before placing it in the conditioning atmosphere, and if it is in the form of bolts or rolls, cut the quantity necessary for the various tests from the piece, excluding the first two and the last two meters when the total quantity of Gauze available so permits.

**Packaging and storage**—Preserve in well-closed containers. Absorbent Gauze that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.

**Labeling**—Its type or thread count, length, and width and the number of pieces contained are stated on the container, and the designation “non-sterilized” or “not sterilized” appears prominently thereon unless the Gauze has been rendered sterile, in which case it may be labeled to indicate that it is sterile. The package label of sterile Gauze indicates that the contents may not be sterile if the package bears evidence of damage or has been previously opened.

The name of the manufacturer, packer, or distributor is stated on the package.

**General characteristics**—Absorbent Gauze is white cloth of various thread counts and weights. It may be supplied in various lengths and widths, and in the form of rolls or folds.

The accompanying table designates for each commercial type the thread count and weight in g per square meter.

Type	Threads per 2.54 Cm		Average Count, Threads per 6.45 Sq. Cm	Weight, <sup>1</sup> g per Sq. Meter
	Warp	Filling		
I	41 to 47	33 to 39	76 to 84 <sup>2</sup>	43.8 to 55.8
II	30 to 34	26 to 30	57 to 63	32.9 to 41.9
III	26 to 30	22 to 26	49 to 55	28.4 to 36.2
IV	22 to 26	18 to 22	41 to 47	24.5 to 31.1
V	20 to 24	16 to 20	37 to 43	22.5 to 28.8
VI	18 to 22	14 to 18	33 to 39	19.8 to 25.2
VII	18 to 22	8 to 14	27 to 35	18.1 to 23.1
VIII	12 to 16	8 to 12	21 to 27	12.1 to 15.5

<sup>1</sup> For Absorbent Gauze that contains purified rayon, increase these values by 2.5%.

<sup>2</sup> For Type I rolled gauze, the range is 75 to 85 threads per 6.45 sq. cm.

**Thread count**—If the dimensions of the piece permit, count the warp and filling threads of Absorbent Gauze in three separate 76.2-mm squares, not counting threads nearer any edge than one-tenth of the dimension of the fabric and not including the same threads in any two counts. For pieces not greater than 76.2 mm in either dimension, count all the threads in three different places in that dimension of the piece.

Average the three counts for the warp and filling, respectively: the average lies within the ranges tabulated under *General characteristics*.

For Absorbent Gauze packaged in rolls, count the number of warp and filling threads in areas of 1.27 cm square at five points evenly spread along the center line of the bandage, no point being within 30.5 cm of either end of the bandage.

**Length**—Unfold or unroll it, smooth it without stretching it, and measure its length along the center line: the length is not less than 98.0% of that stated on the label.

**Width**—Measure the width at each of the points selected for the *Thread*: the average of the three measurements is within 1.6 mm of the width stated on the label.

**Weight**—Weigh a piece of gauze of stated size: the weight, expressed in terms of g per m<sup>2</sup>, meets the requirements for weight under *General characteristics*.

**Absorbency**—Fold about 0.1 m<sup>2</sup> into a 10-cm section. For Absorbent Gauze packaged in rolls, use the entire roll. Hold the folded or rolled Gauze horizontally almost in contact with the surface of water at approximately 25°, and allow it to drop lightly upon the water: complete submersion takes place in not more than 30 seconds.

**Sterility** (71)—Absorbent Gauze that has been rendered sterile meets the requirements.

**Dried and ignited residue, Acid or alkali, and Dextrin or starch, in water extract**—Place 20 ± 0.1 g in 500 mL of water, and boil the mixture for 15 minutes, adding boiling water as necessary to maintain the original vol-

ume. Pour the water through a funnel into a 1000-mL volumetric flask, transfer the Absorbent Gauze to the funnel, press out the excess water with a glass rod, and wash it with two 250-mL portions of boiling water, pressing the gauze after each washing. Cool the combined washings, dilute to volume, and mix. Then apply the following tests.

**Dried residue**—Evaporate 400 mL of the extract, filtering if necessary, in a suitable dish on a steam bath, and dry the residue at 105° to constant weight: the weight of the residue so obtained does not exceed an amount, in mg, calculated by the formula:

$$80 - 0.6C$$

in which C is the corrected percentage of cotton (50 mg maximum, or 0.6%).

**Ignited residue**—Ignite the dried residue in a muffle furnace at a dull-red heat to constant weight: the weight of the ignited residue does not exceed an amount, in mg, calculated by the formula:

$$20 - 0.14C$$

in which C is the corrected percentage of cotton (13 mg maximum, or 0.16%).

**Acid or alkali**—To separate 200-mL portions of the extract, add 3 drops of phenolphthalein TS and 1 drop of methyl orange TS, respectively: no pink color develops in either portion.

**Dextrin or starch**—To a 200-mL portion of the extract add 1 drop of iodine TS: no red, violet, or blue color develops.

**Residue on ignition**—Place about 5 g, accurately weighed, in a suitable dish, and moisten with 2 N sulfuric acid. Gently heat the mixture until it is charred, then ignite more strongly until the carbon is completely consumed: the weight of the residue corresponds to not more than the percentage of the weight of the Gauze, calculated by the formula:

$$0.002C + 0.015(100 - C)$$

in which C is the corrected percentage of cotton (0.89% maximum).

**Fatty matter**—Pack 10 ± 0.01 g in a continuous-extraction thimble with a tared flask, and extract with ether for 5 hours, adjusting the rate so that the ether siphons not less than four times per hour. The ether extract in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at 105° to constant weight: the weight of the residue does not exceed an amount, in mg, calculated by the formula:

$$0.4C + 30$$

in which C is the corrected percentage of cotton (70 mg maximum, or 0.7%).

**Alcohol-soluble dyes**—Pack 10 g in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL: when observed downward in a column 20 cm in depth, the percolate may show a yellowish color, but neither a blue nor a green tint.

**Cotton and rayon content**—

**Sulfuric acid solution** (59.5% by weight)—Add sulfuric acid slowly to water until the specific gravity, determined at 20°, is between 1.4902 and 1.4956.

**Procedure**—Place about 500 mg of Absorbent Gauze, previously bleached and dried at 110° to constant weight and accurately weighed, in a glass-stoppered, 125-mL flask, add 50.0 mL of *Sulfuric acid solution*, and shake by mechanical means for 30 minutes. Pass the mixture through a tared sintered-glass crucible, using three 10-mL portions of *Sulfuric acid solution* to rinse the flask and applying suction each time to drain the acid. Wash the residue in the crucible with

50 mL of 2 N sulfuric acid, then wash it with water until the filtrate is neutral to litmus. Add 40 mL of 6 N ammonium hydroxide to the crucible, allow the residue to soak for 10 minutes, then apply suction to remove the liquid. Similarly wash the residue with three 50-mL portions of water, allowing the residue to soak for 15 minutes each time. Dry the residue at 105° to 110° to constant weight. Calculate *C*, the corrected percentage of cotton, taken by the formula:

$$[100(1.046 / G) - 1.6]$$

in which *J* is the weight, in mg, of the residue; *G* is the weight, in mg, of the portion of Absorbent Gauze taken; and 1.046 and 1.6 are empirical correction factors. Calculate *R*, the corrected percentage of rayon, taken by the formula:

$$100 - C.$$

## Petrolatum Gauze

» Petrolatum Gauze is Absorbent Gauze saturated with White Petrolatum. The weight of the petrolatum in the gauze is not less than 70.0 percent and not more than 80.0 percent of the weight of petrolatum gauze. Petrolatum Gauze is sterile. It may be prepared by adding, under aseptic conditions, molten, sterile White Petrolatum to dry, sterile Absorbent Gauze, previously cut to size, in the ratio of 60 g of petrolatum to each 20 g of gauze.

**Packaging and storage**—Each Petrolatum Gauze unit is so packaged individually that the sterility of the unit is maintained until the package is opened for use.

**Labeling**—The package label bears a statement to the effect that the sterility of the Petrolatum Gauze cannot be guaranteed if the package bears evidence of damage or has been opened previously. The package label states the width, length, and type or thread count of the Gauze.

**Sterility** (71): meets the requirements.

**Other tests**—The petrolatum recovered by draining in the Assay has the characteristics of and meets the requirements of the tests under *White Petrolatum*. The conditioned gauze obtained in the Assay meets the requirements of the tests for *Thread count*, *Length*, *Width*, and *Weight* under *Absorbent Gauze*.

**Assay**—Weigh not less than 20 units of Petrolatum Gauze, place them in a heated glass funnel, maintaining the temperature at approximately 75°, and allow the petrolatum to melt and drain from the funnel. Draining may be facilitated by pressing the gauze with a glass rod or porcelain spatula.

Wash the gauze on the funnel with successive portions of warm methyl chloroform until it is free from petrolatum, allow the residual methyl chloroform to evaporate spontaneously, condition the gauze in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° for not less than 4 hours, and weigh. The difference between the weight of the gauze and that of the Petrolatum Gauze taken represents the weight of petrolatum.

## Absorbable Gelatin Film

» Absorbable Gelatin Film is Gelatin in the form of a sterile, absorbable, water-insoluble film.

**Packaging and storage**—Preserve in a hermetically sealed or other suitable container in such manner that the sterility of the product is maintained until the container is opened for use.

**Labeling**—The package bears a statement to the effect that the sterility of Absorbable Gelatin Film cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened.

**Sterility** (71): meets the requirements.

**Residue on ignition** (281): not more than 2.0%.

**Proteolytic digest**—Place 150 mg (±5 mg) in a glass-stoppered, 150-mL flask containing 100 mL of a 1 in 100 solution of pepsin in 0.1 N hydrochloric acid, previously warmed to 37°. Maintain at 37 ± 1°, and agitate gently every 30 minutes until digestion is complete: the average time of three proteolytic digest determinations is between 4 and 8 hours.

## Absorbable Gelatin Sponge

» Absorbable Gelatin Sponge is Gelatin in the form of a sterile, absorbable, water-insoluble sponge.

**Packaging and storage**—Preserve in a hermetically sealed or other suitable container in such manner that the sterility of the product is maintained until the container is opened for use.

**Labeling**—The package bears a statement to the effect that the sterility of Absorbable Gelatin Sponge cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened.

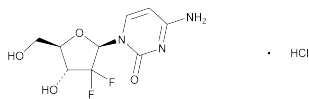
**Sterility** (71): meets the requirements.

**Residue on ignition** (281): not more than 2.0%.

**Digestibility**—Place a 50-mg piece in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all the air has been removed, taking care not to break the tissue. Lift from the water, and remove the excess water with absorbent paper. Place the wetted sample in a 150-mL flask that contains 100 mL of a 1 in 100 solution of pepsin in 0.1 N hydrochloric acid previously warmed to 37°. Maintain at a temperature of 37°, and agitate gently and continuously until digestion is complete: the average digestion time of three determinations is not more than 75 minutes.

**Water absorption**—Cut a portion of about 10 mg from 1 Absorbable Gelatin Sponge, weigh accurately, and place in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all air has been removed, taking care not to break the tissue. Lift the portion of sponge from the water, and blot twice by pressing firmly between two pieces of absorbent paper. Drop the expressed sponge into a tared weighing bottle containing about 20 mL of water, and allow to stand for 2 minutes. Lift the sponge from the water with a suitable hooked instrument, allow to drain over the weighing bottle for 5 seconds, and discard the sponge. Again weigh the weighing bottle and water: the loss in weight represents the weight of water absorbed by the sponge. Absorbable Gelatin Sponge absorbs not less than 35 times its weight of water.

## Gemcitabine Hydrochloride



$C_9H_{11}F_2N_3O_4 \cdot HCl$  299.66

Cytidine, 2'-deoxy-2',2'-difluoro-, monohydrochloride.

2'-Deoxy-2',2'-difluorocytidine monohydrochloride ( $\beta$ -isomer) [122111-03-9].

» Gemcitabine Hydrochloride contains not less than 97.5 percent and not more than 101.5 percent of  $C_9H_{11}F_2N_3O_4 \cdot HCl$ , calculated on the as-is basis.

**Caution**—*Gemcitabine Hydrochloride is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.*

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cytosine RS

USP Endotoxin RS

USP Gemcitabine Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** It meets the requirements of the tests for *Chloride* (191).

**Specific rotation** (781S): between +43° and +50°, at 20°.

*Test solution:* 10 mg per mL.

**pH** (791): between 2.0 and 3.0, in a solution containing 10 mg per mL.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method I* (231): 0.001%.

**Chromatographic purity**—

*Solution A*—Proceed as directed for *Mobile phase* in the *Assay*.

*Solution B*—Prepare filtered and degassed methanol.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system*. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Gemcitabine Hydrochloride RS and USP Cytosine RS in water, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2 µg per mL of each.

*Test solution*—Transfer about 50 mg of Gemcitabine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed under *Assay*. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–8	97	3	isocratic
8–13	97→50	3→50	linear gradient
13–20	50	50	isocratic
20–25	50→97	50→3	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for gemcitabine  $\alpha$ -anomer and 1.0 for gemcitabine; the resolution,  $R$ , between gemcitabine  $\alpha$ -anomer and gemcitabine is not less than 8.0; and the tailing factor for gemcitabine is not more than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.1 for cytosine and 1.0 for gemcitabine; the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject a volume (about 20 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of cytosine in the portion of Gemcitabine taken by the formula:

$$2.5(C_c / W)(r_t / r_s)$$

in which  $C_c$  is the concentration of USP Cytosine RS in the *Standard solution*, in µg per mL;  $W$  is the weight, in mg, of Gemcitabine taken;  $r_t$  is the peak response for cytosine in the *Test solution*; and  $r_s$  is the response for cytosine in the *Standard solution*: not more than 0.1% of cytosine is found. Calculate the percentage of each impurity other than cytosine in the portion of Gemcitabine taken by the formula:

$$2.5(C_s / W)(r_i / r_s)$$

in which  $C_s$  is the concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution*, in µg per mL;  $W$  is the weight, in mg, of Gemcitabine taken;  $r_i$  is the peak response for each impurity in the *Test solution*; and  $r_s$  is the response due to gemcitabine in the *Standard solution*: not more than 0.1% of gemcitabine  $\alpha$ -anomer or any other individual impurity is found; and the sum of all impurities is not more than 0.2%. Exclude from the sum of all impurities any peaks that are below the limit of quantitation (0.02%).

**Other requirements**—Where the label states that Gemcitabine Hydrochloride is sterile, it meets the requirements for *Bacterial endotoxins* and *Sterility* under *Gemcitabine for Injection*. Where the label states that Gemcitabine Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Gemcitabine for Injection*.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed solution containing 13.8 g of monobasic sodium phosphate and 2.5 mL of phosphoric acid in 1000 mL of water. [NOTE—The pH of this solution is between 2.4 and 2.6.]

*System suitability solution*—Transfer about 10 mg of Gemcitabine Hydrochloride to a small vial, add 4 mL of a solution containing 168 mg of potassium hydroxide per mL of methanol, cap tightly, and sonicate. Heat at 55° for 6 to 16 hours, allow to cool, and transfer the contents to a 100-mL volumetric flask with successive washes of 1% (v/v) phosphoric acid. Dilute with 1% phosphoric acid to volume, and mix. [NOTE—This solution contains about 0.02 mg per mL of gemcitabine  $\alpha$ -anomer.]

*Standard preparation*—Dissolve an accurately weighed quantity of USP Gemcitabine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Transfer about 20 mg of Gemcitabine Hydrochloride, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.2 mL per minute. Chromatograph the *System suitability solution*, and record the peak

responses as directed for *Procedure*: the resolution,  $R$ , between the gemcitabine  $\alpha$ -anomer and gemcitabine is not less than 8.0; and the tailing factor determined from gemcitabine is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_9H_{11}F_2N_3O_4 \cdot HCl$  in the portion of Gemcitabine Hydrochloride taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Gemcitabine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gemcitabine for Injection

» Gemcitabine for Injection contains an amount of Gemcitabine Hydrochloride equivalent to not less than 95 percent and not more than 105 percent of the labeled amount of gemcitabine ( $C_9H_{11}F_2N_3O_4$ ).

**Caution**—Gemcitabine Hydrochloride is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.

**Packaging and storage**—Preserve in *Containers for Sterile Solids*, as described under *Injections* (1). Store at controlled room temperature. Do not refrigerate after reconstitution.

### USP Reference standards (11)—

USP Cytosine RS

USP Endotoxin RS

USP Gemcitabine Hydrochloride RS

### Identification—

**A:** *Ultraviolet Absorption* (197U).

*Solution*: 16  $\mu$ g per mL.

**Medium**: 0.14 M phosphate buffer with a pH of 2.5, prepared as follows. Add 13.8 g of monobasic sodium phosphate and 2.5 mL of phosphoric acid to 1000 mL of Purified Water.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Clarity of solution**—Dissolve it in the solvent and at the concentration recommended in the labeling: not more than 10 NTU (see *Spectrophotometry and Light-Scattering* (851)), determined by ratio turbidimetry within 15 minutes of reconstitution, corrected for a diluent blank.

**Bacterial endotoxins** (85)—It contains not more than 0.05 USP Endotoxin Unit per mg of gemcitabine.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements for *Weight Variation*.

**pH** (791): between 2.7 and 3.3, in a solution containing 40 mg in each mL of 0.9% sodium chloride solution.

**Particulate matter** (788): meets the requirements for small-volume injections.

### Chromatographic purity—

*Mobile phase, System suitability solution, Standard solution, and Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* for *Gemcitabine Hydrochloride*.

*Test solution*—Reconstitute the vial with an appropriate amount of water to achieve a solution of 2 mg per mL, based on the labeled content of gemcitabine.

*Procedure*—Proceed as directed in *Chromatographic purity* for *Gemcitabine Hydrochloride*. Calculate the amount of cytosine, expressed as a percentage of gemcitabine hydrochloride, by the formula:

$$0.1(263.20/299.66)(C_C V/L)(r_i / r_S)$$

in which 263.20 and 299.66 are the molecular weights of gemcitabine and gemcitabine hydrochloride, respectively;  $C_C$  is the concentration of USP Cytosine RS in the *Standard solution*, in  $\mu$ g per mL;  $V$  is the volume, in mL, of water used to reconstitute the vial;  $L$  is the labeled amount of gemcitabine in the vial, in mg;  $r_i$  is the peak response for cytosine in the *Test solution*; and  $r_S$  is the response for cytosine in the *Standard solution*: not more than 0.1% of cytosine is found. Similarly, calculate the amount of each impurity other than cytosine, expressed as a percentage of gemcitabine hydrochloride, by the formula:

$$0.1(263.20/299.66)(C_S V/L)(r_i / r_S)$$

in which 263.20 and 299.66 are the molecular weights of gemcitabine and gemcitabine hydrochloride, respectively;  $C_S$  is the concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution*, in  $\mu$ g per mL;  $V$  is the volume, in mL, of water used to reconstitute the vial;  $L$  is the labeled amount of gemcitabine in the vial, in mg;  $r_i$  is the response for gemcitabine  $\alpha$ -anomer or any other individual impurity in the *Test solution*; and  $r_S$  is the peak response for gemcitabine in the *Standard solution*. Not more than 0.1% of gemcitabine  $\alpha$ -anomer is found; not more than 0.2% each of any other impurity is found; and the sum of all impurities is not more than 0.3%. Exclude from the sum of all impurities any peaks that are below the limit of quantitation (0.02%).

### Assay—

*Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Gemcitabine Hydrochloride*.

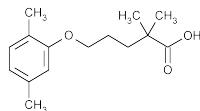
*Assay preparation*—Constitute a suitable number of vials of Gemcitabine for Injection with Purified Water to obtain a solution having a concentration of about 0.1 mg per mL, based on the labeled content of gemcitabine.

*Procedure*—Proceed as directed in the *Assay* under *Gemcitabine Hydrochloride*. Calculate the amount, in mg, of gemcitabine ( $C_9H_{11}F_2N_3O_4$ ) in each vial of Gemcitabine for Injection taken by the formula:

$$(263.20/299.66)(CV/N)(r_U / r_S)$$

in which 263.20 and 299.66 are the molecular weights of gemcitabine and gemcitabine hydrochloride, respectively;  $V$  is the total volume, in mL, of the *Assay preparation*;  $N$  is the number of vials taken; and the other terms are as defined therein.

## Gemfibrozil


$$\text{C}_{15}\text{H}_{22}\text{O}_3 \quad 250.33$$

Pentanoic acid, 5-(2,5-dimethylphenoxy)-2,2-dimethyl-  
2,2-Dimethyl-5-(2,5-xylyloxy)valeric acid [25812-30-0].

» Gemfibrozil contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{22}O_3$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards**  $\langle 11 \rangle$ —

USP Gemfibrozil RS

USP Gemfibrozil Related Compound A RS

(*E,Z*)-2,2-Dimethyl-5-[2,5-dimethyl-4-(propene-1-yl)phenoxy]valeric acid.

$C_{18}H_{26}O_3$  290.40

**Identification,** *Infrared Absorption* (197K).

**Melting range** (741): between 58° and 61°.

**Water, Method I** (921): not more than 0.25%.

**Heavy metals, Method II (231):** 0.002%.

### Related compounds—

**Mobile phase**—Add 10 mL of glacial acetic acid to 750 mL of methanol in a 1000-mL volumetric flask, dilute with water to volume, mix, and pass through a membrane filter.

**System suitability solution**—Dissolve accurately weighed quantities of USP Gemfibrozil RS, USP Gemfibrozil Related Compound A RS, and 2,5-dimethylphenol in *Mobile phase* to obtain a solution having known concentrations of about 0.2 mg per mL, 0.05 mg per mL, and 0.05 mg per mL, respectively.

**Standard solution**—Transfer 10 mg each of USP Gemfibrozil RS and USP Gemfibrozil Related Compound A RS, both accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution**—Transfer about 100 mg of Gemfibrozil, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 4.0-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.35 for 2,5-dimethylphenol, 1.0 for gemfibrozil, and 2.1 for gemfibrozil related compound A; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least three times the retention time of gemfibrozil, and measure the areas for the major peaks. Calculate the percentage of gemfibrozil related compound A in the portion of Gemfibrozil taken by the formula:

$$1000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Gemfibrozil Related Compound A RS in the *Standard solution*; W is the weight, in mg, of Gemfibrozil taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the peak areas for gemfibrozil related compound A obtained from the *Test so-*

lution and the *Standard solution*, respectively: not more than 0.1% of gemfibrozil related compound A is found. Calculate the percentage of any other impurity in the portion of Gemfibrozil taken by the formula:

$$1000(C_G / W)(r_i / r_G)$$

in which  $C_G$  is the concentration, in mg per mL, of USP Gemfibrozil RS in the *Standard solution*;  $r_i$  is the peak area of each individual impurity obtained from the *Test solution*;  $r_G$  is the gemfibrozil peak area obtained from the *Standard solution*; and  $W$  is as defined above: not more than 0.1% of any other impurity is found; and not more than 0.5% of total impurities is found.

**Assay—**

**Mobile phase**—Add 10 mL of glacial acetic acid to 800 mL of methanol in a 1000-mL volumetric flask, dilute with water to volume, mix, and pass through a membrane filter.

**Standard preparation**—Dissolve a suitable quantity of USP Gemfibrozil RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 25.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer about 100 mg of Gemfibrozil, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 25.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**System suitability preparation**—Prepare a solution in *Mobile phase* containing, in each mL, about 0.2 mg of gemfibrozil and about 0.05 mg of 2,5-xyleneol.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph about 10 µL of the *System suitability preparation*: the resolution, *R*, between gemfibrozil and 2,5-xenolol is not less than 8.0.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{15}H_{22}O_3$  in the portion of Gemfibrozil taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Gemfibrozil RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gemfibrozil Capsules

» Gemfibrozil Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gemfibrozil ( $C_{15}H_{22}O_3$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards**  $\langle 11 \rangle$ —

USP Gemfibrozil RS

**Identification**—Shake a portion of Capsule contents, equivalent to about 100 mg of gemfibrozil, with 10 mL of 0.1 N sodium hydroxide. Filter the mixture into a 50-mL centrifuge tube, and acidify the filtrate with 3 N sulfuric acid

to obtain a copious precipitate. Centrifuge, and discard the clear solution. Wash the precipitate with small portions of water, and allow it to air-dry: the IR absorption spectrum of a potassium bromide dispersion of the precipitate, previously dried over silica gel for 4 hours, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Gemfibrozil RS.

#### Dissolution <711>—

**Medium:** 0.2 M pH 7.5 phosphate buffer prepared by dissolving 545 g of monobasic potassium phosphate in 5 L of water, adding 131 g of sodium hydroxide, diluting with water to about 19.5 L, and mixing well. Adjust with either 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 20 L; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{15}H_{22}O_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with 1 N sodium hydroxide, in comparison with a Standard solution obtained as follows. Prepare a Standard stock solution of USP Gemfibrozil RS having a known concentration of about 0.33 mg per mL in **Medium**. [NOTE—Initially dissolve the USP Reference Standard in an amount of methanol not to exceed 1% of the volume of the Standard stock solution.] Quantitatively dilute the Standard stock solution with 1 N sodium hydroxide to obtain a Standard solution having a concentration estimated to correspond to that of the filtered and diluted solution under test.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{15}H_{22}O_3$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Mobile phase, Standard preparation, and System suitability preparation**—Proceed as directed in the Assay under Gemfibrozil.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of gemfibrozil, to a 100-mL volumetric flask, add about 80 mL of methanol, and shake to dissolve. Dilute with methanol to volume, mix, and filter. Transfer 5.0 mL of this clear solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under Gemfibrozil. Calculate the quantity, in mg, of  $C_{15}H_{22}O_3$  in the portion of Capsules taken by the formula:

$$500C(r_U / r_S)$$

in which the terms are as defined therein.

## Gemfibrozil Tablets

» Gemfibrozil Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gemfibrozil ( $C_{15}H_{22}O_3$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Gemfibrozil RS

**Identification**—A portion of finely ground Tablets, equivalent to about 100 mg of gemfibrozil, responds to the *Identification* test under Gemfibrozil Capsules.

#### Dissolution <711>—

**Medium:** 0.2 M pH 7.5 phosphate buffer prepared by dissolving 545 g of monobasic potassium phosphate in 5 L of water, adding 131 g of sodium hydroxide, diluting with water to about 19.5 L, and mixing well. Adjust with either 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 20 L; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_{15}H_{22}O_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with 1 N sodium hydroxide, in comparison with a Standard solution obtained as follows. Prepare a Standard stock solution of USP Gemfibrozil RS having a known concentration of about 0.33 mg per mL in **Medium**. [NOTE—Initially dissolve the USP Reference Standard in an amount of methanol not to exceed 1% of the volume of the Standard stock solution.] Quantitatively dilute the Standard stock solution with 1 N sodium hydroxide to obtain a Standard solution having a concentration estimated to correspond to that of the filtered and diluted solution under test.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{15}H_{22}O_3$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system**—Proceed as directed in the Assay under Gemfibrozil.

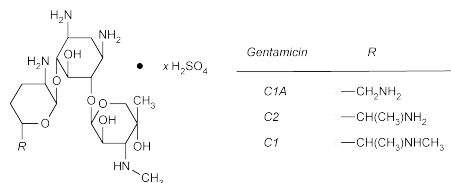
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of gemfibrozil, to a 100-mL volumetric flask, add about 80 mL of methanol, and shake to dissolve. Dilute with methanol to volume, mix, and filter. Transfer 5.0 mL of this clear solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under Gemfibrozil. Calculate the quantity, in mg, of gemfibrozil ( $C_{15}H_{22}O_3$ ) in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which the terms are as defined therein.

## Gentamicin Sulfate



Gentamicin sulfate (salt).

Gentamycin sulfate [1405-41-0].

» Gentamicin Sulfate is the sulfate salt, or a mixture of such salts, of the antibiotic substances produced by the growth of *Micromonospora purpurea*. It has a potency equivalent to not less than 590 µg of gentamicin per mg, calculated on the dried basis.



**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Gentamicin Sulfate RS

**Identification**—

A: *Infrared Absorption* (197K).

B: It responds to the tests for *Sulfate* (191).

**Specific rotation** (781S): between +107° and +121°.

*Test solution*: 10 mg per mL, in water.

**pH** (791): between 3.5 and 5.5, in a solution (1 in 25).

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 hours: it loses not more than 18.0% of its weight.

**Residue on ignition** (281): not more than 1.0%.

**Limit of methanol**—

*Internal standard solution*—Transfer 2.5 mL of *n*-propyl alcohol to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 0.50% (v/v) of *n*-propyl alcohol.

*Standard preparation*—Transfer 1.25 mL of methanol and 1.25 mL of *n*-propyl alcohol to a 500-mL volumetric flask, dilute with water to volume, and mix to obtain a *Standard preparation* containing 0.25% (v/v) of methanol and 0.25% (v/v) of *n*-propyl alcohol.

*Control solution*—Dissolve 0.50 g of Gentamicin Sulfate in 2.0 mL of water.

*Test preparation*—Dissolve 0.50 g of Gentamicin Sulfate in 1.0 mL of *Internal standard solution*, add 1.0 mL of water, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.5-m column packed with support S3. The column temperature is maintained at a constant temperature between 120° and 140°, and the injection port and detector block are maintained at a constant temperature at least 50° higher than the column temperature. Nitrogen is used as the carrier gas at a constant flow rate of between 30 and 40 mL per minute. Chromatograph the *Standard preparation*, and measure the peak responses as directed under *Procedure*: the resolution,  $R$ , between the *n*-propyl alcohol peak and the methanol peak is not less than 1.0. Chromatograph the *Control solution*, measure the peak responses as directed under *Procedure*, and examine the chromatogram: if any peak is observed at a retention time corresponding to that of *n*-propyl alcohol, use the response of that peak to correct the *n*-propyl alcohol peak response in the chromatogram obtained from the *Test preparation*.

*Procedure*—Using a syringe with a polytef-tipped plunger, separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the *n*-propyl alcohol and the methanol peak area responses. Calculate the percentage of methanol in the Gentamicin Sulfate taken by the formula:

$$1.58(P/M)(R_U/R_S)$$

in which  $P$  is the percentage (v/v) of methanol in the *Standard preparation*;  $M$  is the quantity, in g, of Gentamicin Sulfate taken to prepare the *Test preparation*;  $R_U$  is the ratio of the methanol peak area response to the *n*-propyl alcohol peak area response (corrected, if necessary, by subtracting the response of any peak at the locus of the *n*-propyl alcohol peak observed in the chromatogram of the *Control solution*) in the chromatogram obtained from the *Test preparation*; and  $R_S$  is the ratio of the methanol peak area response to the *n*-propyl alcohol peak area response in the chromato-

gram obtained from the *Standard preparation*: not more than 1.0% of methanol is found.

**Content of gentamicins**—

*o*-Phthalaldehyde solution—Dissolve 1.0 g of *o*-phthalaldehyde in 5 mL of methanol, and add 95 mL of 0.4 M boric acid, previously adjusted with 8 N potassium hydroxide to a pH of 10.4, and 2 mL of thioglycolic acid. Adjust the resulting solution with 8 N potassium hydroxide to a pH of 10.4.

*Mobile phase*—Mix 700 mL of methanol, 250 mL of water, and 50 mL of glacial acetic acid. Dissolve 5 g of sodium 1-heptanesulfonate in this solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Prepare a solution of USP Gentamicin Sulfate RS in water containing about 0.65 mg per mL. Transfer 10 mL of this solution to a suitable test tube, add 5 mL of isopropyl alcohol and 4 mL of *o*-Phthalaldehyde solution, mix, and add isopropyl alcohol to obtain 25 mL of solution. Heat at 60° in a water bath for 15 minutes, and cool.

*Test preparation*—Using Gentamicin Sulfate, proceed as directed for *Standard preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 330-nm detector and a 5-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between any two peaks is not less than 1.25, the capacity factor determined from the gentamicin  $C_1$  peak is between 2 and 7, the column efficiency determined from the gentamicin  $C_2$  peak is not less than 1200 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. The elution order is gentamicin  $C_1$ , gentamicin  $C_{1a}$ , gentamicin  $C_{2a}$ , and gentamicin  $C_2$ . Calculate the percentage contents of gentamicin  $C_1$ , gentamicin  $C_{1a}$ , gentamicin  $C_{2a}$ , and gentamicin  $C_2$  in the portion of Gentamicin Sulfate taken by the formula:

$$100r_i/r_s$$

in which  $r_i$  is the peak area response corresponding to the particular gentamicin; and  $r_s$  is the sum of the area responses of all four peaks: the content of gentamicin  $C_1$  is between 25% and 50%, the content of gentamicin  $C_{1a}$  is between 10% and 35%, and the sum of the contents of gentamicin  $C_{2a}$  and gentamicin  $C_2$  is between 25% and 55%.

**Other requirements**—Where the label states that Gentamicin Sulfate is sterile, it meets the requirements for *Sterility Tests* (71) and for *Bacterial endotoxins* in *Gentamicin Injection*. Where the label states that Gentamicin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* in *Gentamicin Injection*.

**Assay**—Proceed with Gentamicin Sulfate as directed under *Antibiotics—Microbial Assays* (81).

## Gentamicin Sulfate Cream

» Gentamicin Sulfate Cream contains the equivalent of not less than 90.0 percent and not more

than 135.0 percent of the labeled amount of gentamicin.

**Packaging and storage**—Preserve in collapsible tubes or in other tight containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—  
USP Gentamicin Sulfate RS

**Identification**—Shake a quantity of Cream, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous phase: the filtrate so obtained meets the requirements of the *Identification* test under *Gentamicin Injection*.

**Minimum fill** (755): meets the requirements.

**Assay**—Proceed with Cream as directed in the *Assay* under *Gentamicin Sulfate Ointment*.

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## Gentamicin Uterine Infusion

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» Gentamicin Uterine Infusion is a sterile solution of Gentamicin Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin. It may contain suitable buffers, preservatives, and sequestering agents.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**Labeling**—Label Uterine Infusion to indicate that it is for veterinary use only. The label states that it must be diluted with 0.9% Sodium Chloride Irrigation before aseptic uterine infusion.

**USP Reference standards** (11)—  
USP Gentamicin Sulfate RS

**Identification**—It responds to the *Identification* test under *Gentamicin Injection*, Uterine Infusion being used instead of Injection.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 3.0 and 5.5.

**Assay**—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Uterine Infusion diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (0.1 µg of gentamicin per mL).

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## Gentamicin Injection

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» Gentamicin Injection contains an amount of Gentamicin Sulfate equivalent to not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin. It may contain suitable buffers, preservatives, and sequestering agents, unless it is intended for intrathecal use, in which case it contains only suitable tonicity agents.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Gentamicin Sulfate RS

**Identification**—Apply separately a volume of Injection equivalent to 20 µg of gentamicin and the same volume of a similar preparation of USP Gentamicin Sulfate RS to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel having an average pore size of 6 nm. [NOTE—Dilute the Injection with water, if necessary, to obtain a test solution containing 1000 µg of gentamicin per mL. Where the Injection contains less than 1000 µg per mL, apply a volume of it, equivalent to 20 µg of gentamicin, to the chromatographic plate, in separate portions of not more than 20 µL each, each application being allowed to dry before the next is applied.] Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (20:13:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals: the intensities and  $R_f$  values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Bacterial endotoxins** (85)—It contains not more than 0.71 USP Endotoxin Unit per mg of gentamicin.

**pH** (791): between 3.0 and 5.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Injection diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (0.1 µg of gentamicin per mL).

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## Gentamicin Sulfate Ointment

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» Gentamicin Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

**Packaging and storage**—Preserve in collapsible tubes or other tight containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—  
USP Gentamicin Sulfate RS

**Identification**—Shake a quantity of Ointment, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer: the filtrate so obtained meets the requirements of the *Identification* test under *Gentamicin Injection*.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed with Ointment as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Ointment, equivalent to about 1 mg of gentamicin, shaken with about 50 mL of ether in a separator, and extracted with four 20-mL portions of *Buffer No. 3*. Combine the aqueous extracts, and dilute quantita-

tively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Gentamicin Sulfate Ophthalmic Ointment

» Gentamicin Sulfate Ophthalmic Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes, and avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Gentamicin Sulfate RS

**Identification**—Shake a quantity of Ophthalmic Ointment, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer: the filtrate so obtained meets the requirements of the *Identification* test under *Gentamicin Injection*.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

**Other requirements**—It meets the requirements of the test for *Water* and of the *Assay* in *Gentamicin Sulfate Ointment*.

### Gentamicin Sulfate Ophthalmic Solution

» Gentamicin Sulfate Ophthalmic Solution is a sterile, buffered solution of Gentamicin Sulfate with preservatives. It contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

**Packaging and storage**—Preserve in tight containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—

USP Gentamicin Sulfate RS

**pH** (791): between 6.5 and 7.5.

**Other requirements**—It meets the requirements of the *Identification* test under *Gentamicin Injection* and meets the requirements under *Sterility Tests* (71), when tested as directed in the section *Membrane Filtration in Test for Sterility of the Product To Be Examined*.

**Assay**—Proceed with Ophthalmic Solution as directed in the *Assay* under *Gentamicin Injection*.

### Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution

» Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution contains not less than

90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone acetate ( $C_{24}H_{31}FO_6$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Betamethasone Acetate RS

USP Gentamicin Sulfate RS

**Identification**—

**A:** Apply 10  $\mu$ L of Ophthalmic Solution and 10  $\mu$ L of a Standard solution containing 5 mg per mL of USP Gentamicin Sulfate RS in water to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and in a paper-lined tank develop the chromatogram in a solvent system consisting of the lower phase mixture of dichloromethane, methanol, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the  $R_f$  values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**B:** The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone acetate*.

**pH** (791): between 5.5 and 7.0.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration in Test for Sterility of the Product To Be Examined*.

**Other requirements**—It meets the requirements under *Antimicrobial Effectiveness Tests* (51).

**Assay for gentamicin**—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for betamethasone acetate**—

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (8:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Dissolve a quantity of *o*-phenylphenol in methanol to obtain a solution containing about 0.55 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.45 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.09 mg of USP Betamethasone Acetate RS per mL.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 2 mg of betamethasone acetate, to a 10-mL volumetric flask. Dilute with methanol to volume, and mix. Transfer a portion of this solution to a centrifuge tube, and centrifuge. Transfer 4.0 mL of the clear supernatant to a 10-mL volumetric flask. Add 1.0 mL of *Internal standard solution*, dilute with a mixture of methanol and water (1:1) to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-mm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 1.3 for o-phenylphenol and 1.0 for betamethasone acetate; the resolution,  $R$ , between the betamethasone acetate and o-phenylphenol peaks is not less than 3.9; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone acetate ( $C_{24}H_{31}FO_6$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Acetate RS, calculated on the anhydrous basis, in the *Standard preparation*;  $V$  is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the betamethasone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentamicin Sulfate and Betamethasone Valerate Ointment

» Gentamicin Sulfate and Betamethasone Valerate Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in collapsible tubes or other tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Betamethasone Valerate RS

USP Beclomethasone Dipropionate RS

USP Gentamicin Sulfate RS

**Identification**—

**A:** Transfer an amount of Ointment, equivalent to about 15 mg of gentamicin, to a centrifuge tube, and add 10 mL of a mixture of methanol and 0.1 N hydrochloric acid (4:1) and 25 mL of solvent hexane. Rotate for 30 minutes, and centrifuge. Discard the upper phase. Apply 25  $\mu$ L of the lower phase and 25  $\mu$ L of a Standard solution containing 3 mg per mL of USP Gentamicin Sulfate RS in a mixture of methanol and 0.1 N hydrochloric acid (4:1) to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crys-

als for 15 minutes: the  $R_f$  values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**B:** The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone*.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.

**Minimum fill** <755>: meets the requirements.

**Assay for gentamicin**—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* <81>, using an accurately weighed quantity of Ointment, equivalent to about 3 mg of gentamicin, shaken with about 50 mL of ether in a separator and extracted with three 25-mL portions of *Buffer No. 3*. Combine the aqueous extracts, and dilute quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for betamethasone**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (475:300). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluent**—Transfer 25 mL of water to a 500-mL volumetric flask. Add 2.5 mL of glacial acetic acid, dilute with methanol to volume, and mix.

**Internal standard solution**—Dissolve a quantity of USP Beclomethasone Dipropionate RS in *Diluent* to obtain a solution containing about 0.4 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.45 mg per mL. Transfer 5.0 mL of this solution to a stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.15 mg of USP Betamethasone Valerate RS per mL.

**Assay preparation**—Transfer an accurately weighed portion of Ointment, equivalent to about 2 mg of betamethasone, to a 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution* and 5.0 mL of *Diluent*, and shake vigorously for 10 minutes. Place the tube in an ice-methanol bath for 15 minutes, then centrifuge to separate the phases. Transfer the clear supernatant to a stoppered flask, and allow to warm to room temperature (*Assay preparation*).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.5 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution,  $R$ , between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Ointment taken by the formula:

$$(392.47 / 476.58)(15C)(R_U / R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively;  $C$  is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are

the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentamicin Sulfate and Betamethasone Valerate Otic Solution

» Gentamicin Sulfate and Betamethasone Valerate Otic Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Valerate RS

USP Gentamicin Sulfate RS

### Identification—

**A:** Transfer an amount of Otic Solution, equivalent to about 3 mg of gentamicin, to a centrifuge tube. Dissolve an accurately weighed quantity of USP Gentamicin Sulfate RS quantitatively in water to obtain a solution having a concentration of about 5 mg per mL. Transfer 1.0 mL of this solution to a centrifuge tube. To each centrifuge tube add 3 mL of water and 4 g of potassium carbonate, and mix. Add 1 mL of isopropyl alcohol to each tube, mix, and centrifuge. Use the upper phases as the test solution and Standard solution, respectively. Separately apply 20  $\mu$ L of each of these solutions to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of methanol, dichloromethane, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the  $R_f$  values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**B:** The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone*.

**pH** (791): between 3.0 and 5.0.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.

**Assay for gentamicin**—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Assay for betamethasone—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of methanol and glacial acetic acid (1000:1).

**Internal standard solution**—Dissolve a quantity of USP Beclomethasone Dipropionate RS in methanol to obtain a solution containing about 0.8 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.2 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix to obtain a solution having a known concentration of about 0.24 mg of USP Betamethasone Valerate RS per mL.

**Assay preparation**—Add 5.0 mL of *Internal standard solution* to a 10-mL volumetric flask. Transfer to the flask an accurately measured volume of Otic Solution, equivalent to about 2 mg of betamethasone, dilute with methanol to volume, and mix. Centrifuge a portion of this solution, and use the clear supernatant as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 1.6 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution,  $R$ , between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in each mL of the Otic Solution taken by the formula:

$$(392.47 / 476.58)(10C / V)(R_U / R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively;  $C$  is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Otic Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentamicin Sulfate and Betamethasone Valerate Topical Solution

» Gentamicin Sulfate and Betamethasone Valerate Topical Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** <11>—

USP Beclomethasone Dipropionate RS

USP Betamethasone Valerate RS

USP Gentamicin Sulfate RS

**Identification**—

**A:** Separately apply 50  $\mu$ L of Topical Solution and 50  $\mu$ L of a Standard solution containing 1.2 mg per mL of USP Gentamicin Sulfate RS in water to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of methanol, chloroform, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the  $R_f$  values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**B:** The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone*.

**pH** <791>: between 3.0 and 4.5.

**Microbial enumeration tests** <61> and **Tests for specified microorganisms** <62>—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.

**Assay for gentamicin**—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* <81>, using an accurately measured volume of Topical Solution diluted quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for betamethasone**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Diluent**—Prepare a mixture of methanol and glacial acetic acid (1000:1).

**Internal standard solution**—Dissolve a quantity of USP Beclomethasone Dipropionate RS in methanol to obtain a solution containing about 0.6 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.35 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with a mixture of methanol and water (4:1) to volume, and mix to obtain a solution having a known concentration of about 0.07 mg of USP Betamethasone Valerate RS per mL.

**Assay preparation**—Add 5.0 mL of *Internal standard solution* to a 25-mL volumetric flask. Transfer to the flask an accurately measured volume of Topical Solution, equivalent to about 1.4 mg of betamethasone, dilute with a mixture of methanol and water (4:1) to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 1.6 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution,  $R_s$ , between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in each mL of the Topical Solution taken by the formula:

$$(392.47 / 476.58)(25C / V)(R_U / R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively;  $C$  is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Topical Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentamicin and Prednisolone Acetate Ophthalmic Ointment

» Gentamicin and Prednisolone Acetate Ophthalmic Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of gentamicin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ( $C_{23}H_{30}O_6$ ).

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes, and avoid exposure to excessive heat.

**USP Reference standards** <11>—

USP Gentamicin Sulfate RS

USP Prednisolone Acetate RS

**Identification**—

**A:** Shake a quantity of Ophthalmic Ointment, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer: the filtrate so obtained meets the requirements of the *Identification* test under *Gentamicin Sulfate Injection*.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay for prednisolone acetate* exhibits a major peak for prednisolone acetate, the retention time of which corresponds to that obtained in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for prednisolone acetate*.

**Sterility** <71>: meets the requirements.

**Minimum fill** <755>: meets the requirements.

**Water, Method I** <921>: not more than 2.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* <751>.

**Assay for gentamicin**—Proceed with Ophthalmic Ointment as directed for gentamicin under *Antibiotics—Microbial Assays* <81>, using an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 1 mg of gentamicin, shaken with about 50 mL of ether in a separator, and extracted with four 20-mL portions of *Buffer No. 3*. Combine the aqueous extracts, and dilute quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration of gentamicin assumed to be equal to the median dose level of the Standard.

**Assay for prednisolone acetate—**

**Mobile phase**—Prepare a suitable mixture of water and acetonitrile (60:40), and pass through a suitable filter having a porosity of 1  $\mu\text{m}$  or less. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Prepare a solution in methanol containing about 2.7 mg of fluorometholone acetate per mL.

**Standard preparation**—Transfer about 38 mg of USP Prednisolone Acetate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 8.0 mL of this solution to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution*, dilute with methanol to volume, and shake vigorously for 30 seconds. Allow the layers to separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume, and mix. Centrifuge a portion of this solution, and use the clear liquid as the *Standard preparation*. This solution contains about 0.06 mg of USP Prednisolone Acetate RS per mL.

**Assay preparation**—Transfer an accurately weighed portion of Ophthalmic Ointment, equivalent to about 3 mg of prednisolone acetate, to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution*, dilute with methanol to volume, and shake vigorously for 30 seconds. Allow the layers to separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume, and mix. Centrifuge a portion of this solution, and use the clear liquid as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  20-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and measure the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; the resolution, *R*, between the prednisolone acetate peak and the fluorometholone acetate peak is not less than 2.0; the column efficiency is not less than 2500 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 30  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of prednisolone acetate ( $\text{C}_{23}\text{H}_{30}\text{O}_6$ ) in the portion of Ophthalmic Ointment taken by the formula:

$$50C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the response of the prednisolone acetate peak area to that of the fluorometholone acetate peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentamicin and Prednisolone Acetate Ophthalmic Suspension

» Gentamicin and Prednisolone Acetate Ophthalmic Suspension is a sterile aqueous suspension containing Gentamicin Sulfate and Prednisolone Acetate. It contains the equivalent of not less than 90.0 percent and not more than

130.0 percent of the labeled amount of gentamicin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ( $\text{C}_{23}\text{H}_{30}\text{O}_6$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Gentamicin Sulfate RS

USP Prednisolone Acetate RS

**Identification**—

**A:** It meets the requirements of the *Identification* test in *Gentamicin Injection*.

**B:** The retention time of the prednisolone acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for prednisolone acetate*.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* in *Test for Sterility of the Product To Be Examined*.

**pH** <791>: between 5.4 and 6.6.

**Assay for gentamicin**—Proceed with Ophthalmic Suspension as directed in the *Assay in Gentamicin Sulfate Injection*.

**Assay for prednisolone acetate—**

**Diluting solvent**—Mix 700 mL of methanol and 300 mL of water.

**Mobile phase**—Prepare a suitable mixture of water and acetonitrile (60:40), and pass through a suitable filter having a porosity of 1  $\mu\text{m}$  or less. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 60 mg of USP Prednisolone Acetate RS, accurately weighed, to a 50-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 8.0 mL of this solution to a second 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix. This solution contains about 0.2 mg of USP Prednisolone Acetate RS per mL.

**Assay preparation**—Transfer an accurately measured volume of well-mixed Ophthalmic Suspension, equivalent to about 10 mg of prednisolone acetate, to a 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

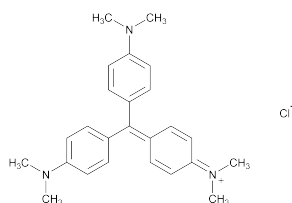
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and measure the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 1.25, the column efficiency is not less than 2000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 30  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of prednisolone acetate ( $\text{C}_{23}\text{H}_{30}\text{O}_6$ ), in each mL of the Ophthalmic Suspension taken by the formula:

$$50(C / V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Suspension taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the prednisolone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Gentian Violet



$C_{25}H_{30}ClN_3$  407.98

Methanaminium, *N*-[4-bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methyl-, chloride;

C. I. Basic violet 3;

[4-Bis[*p*-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]dimethylammonium chloride [548-62-9].

### DEFINITION

Gentian Violet contains NLT 96.0% and NMT 100.5% of gentian violet ( $C_{25}H_{30}ClN_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A.**  
**Sample:** 1 mg  
**Analysis:** Sprinkle the *Sample* on 1 mL of sulfuric acid.  
**Acceptance criteria:** The *Sample* dissolves in the acid with an orange or brown-red color. When this solution is diluted cautiously with water, the color changes to brown, then to green, and finally to blue.
- **B.**  
**Sample:** 20 mg  
**Analysis:** Dissolve the *Sample* in 10 mL of water, and add 5 drops of hydrochloric acid. To 5 mL of this solution add tannic acid TS dropwise.  
**Acceptance criteria:** A deep blue precipitate is formed.
- **C.**  
**Sample:** The remainder of the solution prepared for Identification test B  
**Analysis:** To the *Sample* add about 500 mg of zinc dust, and warm the mixture.  
**Acceptance criteria:** Rapid decolorization occurs. Place a drop of the decolorized solution adjacent to a drop of 6 N ammonium hydroxide on a filter paper: a blue color is produced at the zone of contact.

### ASSAY

#### PROCEDURE

**Sample solution:** Transfer about 400 mg of Gentian Violet to a 300-mL conical flask, add 25 mL of water and 10 mL of hydrochloric acid, displace the air in the flask with carbon dioxide, and pass a stream of carbon dioxide through the flask. Add 50.0 mL of 0.1 N titanium trichloride VS, heat to boiling, and boil gently for 10 min, swirling the liquid occasionally. Cool the solution, and add 5 mL of ammonium thiocyanate solution (1 in 10).

#### Analysis

**Sample:** *Sample solution*

Titrate with 0.1 N ferric ammonium sulfate VS until a faint red color is produced. Perform a blank determination. Each mL of 0.1 N titanium trichloride is equivalent to 20.40 mg of gentian violet ( $C_{25}H_{30}ClN_3$ ).

**Acceptance criteria:** 96.0%–100.5% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 1.5%

- **ARSENIC, Method I** (211)

**Test preparation:** Mix 300 mg with 2.5 g each of powdered potassium nitrate and anhydrous sodium carbonate, and heat the mixture in a crucible until the organic matter is completely oxidized. Dissolve the cooled residue in 15 mL of 2 N sulfuric acid, and evaporate the solution by heating until copious white fumes begin to evolve. Dissolve the residue in 35 mL of water.

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** NMT 10 ppm

#### LEAD

**Sample solution:** Place 1.0 g in a small Kjeldahl flask, add 5 mL of sulfuric acid, and insert a small funnel into the flask. Gently rotate the flask until the sulfuric acid has completely wetted the sample, then heat gently until complete carbonization has taken place. Allow to cool, and add, in small quantities, 5 mL of nitric acid. Again heat gently until copious white fumes are evolved. Allow to cool, add another 5 mL of nitric acid, and again heat until white fumes are evolved. Allow to cool, cautiously add about 25 mL of water, and boil for a few min. After cooling, neutralize to litmus paper with ammonium hydroxide, and add 5 mL of nitric acid. Transfer the solution to a 100-mL volumetric flask, and dilute with water to volume.

**Analysis:** Use 20 mL of this solution for the limit test for Lead (251). Perform a blank determination.

**Acceptance criteria:** NMT 30 ppm

#### ZINC

**Standard stock solution:** 1 g/mL of zinc prepared as follows. Transfer 1 g of zinc to a 1000-mL volumetric flask, add 50 mL of nitric acid, and dilute with water to volume.

**Standard solution:** 0.50 µg/mL of zinc, from *Standard stock solution* in water

**Sample solution:** Weigh 0.50 g of Gentian Violet in a suitable tared crucible. Place in a low-temperature plasma ashing apparatus, and ash until a constant weight is attained. Pipet 10 mL of 6 N nitric acid into the crucible, and heat to dissolve the ash. Transfer the solution to a 500-mL volumetric flask, and dilute with water to volume. Prepare a reagent blank.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Apparatus:** Atomic absorption spectrophotometer

**Analytical wavelength:** Zinc emission line, 213.9 nm

**Lamp:** Zinc

**Flame:** Air–acetylene

**Blank:** Water

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and reagent blank

**Acceptance criteria:** 0.05%; the absorbance of the *Sample solution*, corrected for that of the reagent blank, is NMT the absorbance of the *Standard solution*, similarly corrected.

#### ORGANIC IMPURITIES

**Sample solution A:** 1 mg/mL of Gentian Violet in methanol

**Sample solution B:** 0.01 mg/mL of Gentian Violet in methanol from *Sample solution A*



**Chromatographic system**(See *Chromatography* <621>, *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of octadecylsilanized chromatographic silica gel**Application volume:** 5  $\mu$ L**Developing solvent system:** Upper layer separated from a well-shaken mixture of butyl alcohol, glacial acetic acid, and water (80:20:100)**Analysis****Samples:** *Sample solution A* and *Sample solution B*

Allow the spots to dry, and develop the chromatogram in a suitable chamber with a solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and visually locate the spots on the plate.

**Acceptance criteria:** 1.0%; *Sample solution A* exhibits a principal spot and NMT one secondary spot, which, if present in the chromatogram from *Sample solution A*, is not more intense than the principal spot from *Sample solution B*.**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method I* <921>: NMT 7.5%

- **ALCOHOL-INSOLUBLE SUBSTANCES**

**Sample:** 1.0 g**Analysis:** Boil the *Sample* with 50 mL of alcohol under a reflux condenser for 15 min, pass through a tared filtering crucible, wash the residue on the filter with hot alcohol until the last washing is not colored violet, and dry the crucible at 105° for 1 h.**Acceptance criteria:** NMT 1.0% of insoluble residue remains.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

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**Gentian Violet Cream**

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**DEFINITION**Gentian Violet Cream is Gentian Violet in a suitable cream base. It contains, in each 100 g, NLT 1.20 g and NMT 1.60 g of gentian violet ( $C_{25}H_{30}ClN_3$ ).**IDENTIFICATION**

- **A.**  
**Sample:** 0.1 g  
**Analysis:** Dissolve the *Sample* in 5 mL of sulfuric acid.  
**Acceptance criteria:** An orange or brown-red color is produced.
- **B.** The visible absorption spectrum of the *Sample solution* used for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Gentian Violet RS, concomitantly measured.

**ASSAY**

- **PROCEDURE**

**Standard solution:** 13.5  $\mu$ g/mL of USP Gentian Violet RS in hydrochloric acid**Sample stock solution:** Transfer 500 mg of Cream to a 100-mL volumetric flask. Add 15 mL of hydrochloric acid, warm in a water bath to disperse the Cream thoroughly, cool, and add hydrochloric acid to volume. Pass the mixture through a sintered-glass filter of fine pore size.**Sample solution:** Pipet 2 mL of the *Sample stock solution* into a 10-mL volumetric flask, and add hydrochloric acid to volume.**Blank:** Hydrochloric acid**Instrumental conditions****Analytical wavelength:** 435 nm**Cell:** 1 cm. [NOTE—Use quartz cells having tightly fitting covers to prevent the corrosion caused by hydrochloric acid.]**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the quantity, in g/100 g, of gentian violet ( $C_{25}H_{30}ClN_3$ ) in the portion of Cream taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (1/W) \times V \times D \times (1/F) \times 100$$

 $A_U$  = absorbance from the *Sample solution* $A_S$  = absorbance from the *Standard solution* $C_S$  = concentration of USP Gentian Violet RS in the *Standard solution* ( $\mu$ g/mL) $W$  = weight of the sample in the *Sample stock solution*, 0.5 g $V$  = volume of the *Sample stock solution*, 100 mL $D$  = dilution factor, 5 $F$  = conversion factor,  $10^6 \mu$ g/g**Acceptance criteria:** It contains, in each 100 g, 1.20–1.60 g of gentian violet ( $C_{25}H_{30}ClN_3$ ).**PERFORMANCE TESTS**

- **MINIMUM FILL** <755>: Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or other tight containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** <11>  
USP Gentian Violet RS

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**Gentian Violet Topical Solution**

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**DEFINITION**Gentian Violet Topical Solution contains, in each 100 mL, NLT 0.95 g and NMT 1.05 g of gentian violet ( $C_{25}H_{30}ClN_3$ ).**IDENTIFICATION**

- **A.**  
**Sample solution:** 1 in 5 dilution in water  
**Analysis:** Add 5 drops of hydrochloric acid. To 5 mL of this solution add tannic acid TS dropwise.  
**Acceptance criteria:** A deep blue precipitate is formed.
- **B.**  
**Sample solution:** 1 in 5 dilution in water  
**Analysis:** To the remaining solution from *Identification* test A add 500 mg of zinc dust, and warm the mixture.  
**Acceptance criteria:** Rapid decolorization occurs. Place a drop of the decolorized solution adjacent to a drop of 6 N ammonium hydroxide on a filter paper. A blue color is produced at the zone of contact.

**ASSAY**

- **PROCEDURE**

**Sample solution:** Pipet 25 mL of Topical Solution into a 300-mL conical flask, add 25 mL of water and 10 mL of hydrochloric acid, displace the air in the flask with carbon dioxide, and pass a stream of carbon dioxide through the flask. Add 50.0 mL of 0.1 N titanium trichloride VS, heat to boiling, and boil gently for 10 min, swirling the liquid occasionally. Cool the solution, and add 5 mL of ammonium thiocyanate solution (1 in 10).**Analysis:** Titrate with 0.1 N ferric ammonium sulfate VS until a faint red color is produced. Perform a blank determination. Each mL of 0.1 N titanium trichloride is equivalent to 20.40 mg of gentian violet ( $C_{25}H_{30}ClN_3$ ).

**Acceptance criteria:** It contains, in each 100 mL, 0.95–1.05 g of gentian violet ( $C_{25}H_{30}ClN_3$ ).

### SPECIFIC TESTS

#### • SOLUTION OF RESIDUE IN ALCOHOL

**Sample:** 10 mL

**Analysis:** Evaporate the *Sample* on a steam bath to dryness.

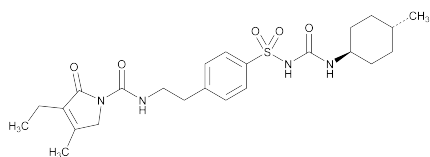
**Acceptance criteria:** The residue dissolves completely in 10 mL of alcohol.

#### • ALCOHOL DETERMINATION (611): 8.0%–10.0%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight containers.

## Glimepiride



$C_{24}H_{34}N_4O_5S$  490.62

1*H*-Pyrrole-1-carboxamide, 3-ethyl-2,5-dihydro-4-methyl-*N*-[2-[4-[[[(4-methylcyclohexyl)amino]carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-, *trans*-.

1-[[*p*-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(*trans*-4-methylcyclohexyl)urea [93479-97-1].

» Glimepiride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{24}H_{34}N_4O_5S$ , calculated on the anhydrous basis.

**Packaging and storage**—Store in well-closed containers, at a temperature not exceeding 25°.

### USP Reference standards (11)—

USP Glimepiride RS

USP Glimepiride Related Compound A RS  
Glimepiride *cis*-isomer.

USP Glimepiride Related Compound B RS  
Glimepiride sulfonamide.

USP Glimepiride Related Compound C RS  
Glimepiride urethane.

USP Glimepiride Related Compound D RS  
Glimepiride 3-isomer.

### Identification, Infrared Absorption (197K).

**Water, Method 1c (921)**—Dissolve about 0.25 g of Glimepiride, accurately weighed, in dimethylformamide previously dried over a molecular sieve (2 mm, pore size 0.4 nm), and dilute with the same solvent to 5.0 mL. Use 1.0 mL of the solution. Perform a blank determination, using 1.0 mL of the solvent. The water content is not more than 0.5%.

**Residue on ignition (281):** not more than 0.2%.

**Heavy metals, Method II (231):** 0.001%.

### Limit of *cis*-isomer (glimepiride related compound A)—

**Mobile phase**—Transfer 100 mL of isopropyl alcohol into a 1-L volumetric flask, add 1 mL of glacial acetic acid, dilute with hexane to volume, filter, and degas.

**System suitability stock solution**—Dissolve about 1 mg of USP Glimepiride Related Compound A RS in 1 mL of methylene chloride. Add 3 mL of *Mobile phase*, and mix.

**System suitability solution**—Transfer about 10 mg of USP Glimepiride RS to a 20-mL volumetric flask, and dissolve in 5 mL of methylene chloride. Dilute with *Mobile phase* to volume, and mix. Transfer 5 mL of this solution to a separate flask, add 50  $\mu$ L of the *System suitability stock solution*, and mix.

**Test solution**—Transfer about 10 mg of Glimepiride to a 20-mL volumetric flask, and dissolve in 5 mL of methylene chloride. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 228-nm detector and a 3-mm  $\times$  15-cm column containing 5- $\mu$ m packing L20. The flow rate is about 0.5 mL per minute. [NOTE—The analyses could also be performed with 4.6-mm  $\times$  15-cm, 4.6-mm  $\times$  25-cm, 4-mm  $\times$  12.5-cm, or 4-mm  $\times$  25-cm columns containing packing L20. It is recommended that the flow rate be adjusted to about 1.1 mL per minute for a 4.6-mm column and to about 0.8 mL per minute for a 4.0-mm column.] Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for glimepiride and not more than 0.9 for the glimepiride *cis*-isomer, and the signal-to-noise ratio of the glimepiride *cis*-isomer peak is not less than 15.

**Procedure**—Inject about 10  $\mu$ L of the *Test solution* into the chromatograph, and measure the peak areas for the glimepiride *cis*-isomer and glimepiride. Calculate the percentage of glimepiride *cis*-isomer in the portion of Glimepiride taken by the formula:

$$100r_{cis} / (r_{cis} + r_G)$$

in which  $r_{cis}$  and  $r_G$  are the peak areas for the glimepiride *cis*-isomer and glimepiride, respectively: not more than 0.8% of the glimepiride *cis*-isomer is found.

### Related compounds—

**Mobile phase, Diluent, System suitability solution, and Chromatographic system**—Prepare as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Diluted test solution 1**—Dilute 5.0 mL of the *Test solution* with *Diluent* to 100.0 mL. Dilute 5.0 mL of the solution obtained with *Diluent* to 50.0 mL. This solution contains about 0.001 mg of glimepiride per mL.

**Diluted test solution 2**—Dilute 1.0 mL of *Diluted test solution 1* with *Diluent* to 10.0 mL.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Test solution* and *Diluted test solutions 1* and 2 into the chromatograph, record the chromatograms, and measure the peak response for glimepiride obtained from *Diluted test solution 1* and the responses for all other peaks, except the glimepiride peak, obtained from the *Test solution*. Disregard any peak with an area less than that of the glimepiride peak in the chromatogram obtained from *Diluted test solution 2*. Continue the elution for 2.5 times the retention time of the glimepiride peak. Calculate the percentage of each related compound (see *Table 1*) and any unknown impurity in the portion of Glimepiride taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of glimepiride in *Diluted test solution 1*;  $C_T$  is the concentration, in mg per mL, of glimepiride in the *Test solution*;  $r_i$  is the peak response for each individual peak obtained from the *Test solution*; and  $r_S$  is the glimepiride peak obtained from *Diluted test solution 1*.

Table 1

Name	Relative Retention Time	Limit (%)
Glimepiride related compound B <sup>1</sup>	0.2	0.4
Glimepiride related compound C <sup>2</sup>	0.3	0.1
Glimepiride related compound D <sup>3</sup>	1.1	0.2

<sup>1</sup> Glimepiride-sulfonamide<sup>2</sup> Glimepiride-urethane<sup>3</sup> Glimepiride-3-isomer

In addition to not exceeding the limits for each impurity in Table 1, not more than 0.1% of any unspecified individual impurity is found; and not more than 0.5% of total impurities, excluding glimepiride related compound B, is found.

**Assay—**

**Mobile phase**—Dissolve 0.5 g of monobasic sodium phosphate in 500 mL of water. Adjust with phosphoric acid to a pH of 2.1 to 2.7, and add 500 mL of acetonitrile.

**Diluent**—Prepare a mixture of acetonitrile and water (4:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Glimepiride RS in *Diluent* to obtain a solution having a known concentration of about 0.2 mg per mL.

**System suitability solution**—Prepare a solution in *Diluent* containing 0.1 mg each of USP Glimepiride Related Compound B RS, USP Glimepiride Related Compound C RS, and USP Glimepiride Related Compound D RS per mL. Dilute 1 mL of this solution with the *Standard preparation* to 50 mL.

**Assay preparation**—Transfer about 20.0 mg of Glimepiride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. [NOTE—Keep the *Assay preparation* at a temperature not exceeding 12°, and store it no longer than 15 hours.]

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 228-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and identify the glimepiride peak and the peaks due to the related compounds listed in Table 1. Record the peak responses as directed for *Procedure*: the resolution, *R*, between glimepiride related compound B and glimepiride related compound C is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the percentage of C<sub>24</sub>H<sub>34</sub>N<sub>4</sub>O<sub>5</sub>S in the portion of Glimepiride taken by the formula:

$$10,000(C/W)[100/(100 - L)](r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Glimepiride RS in the *Standard preparation*; W is the weight, in mg, of Glimepiride taken to prepare the *Assay preparation*; L is the percentage of water as determined in the test for *Water*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for the glimepiride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glimepiride Tablets

**DEFINITION**

Glimepiride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of glimepiride (C<sub>24</sub>H<sub>34</sub>N<sub>4</sub>O<sub>5</sub>S).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

[NOTE—Store the solutions containing glimepiride for NMT 24 h.]

**Mobile phase:** Dissolve 0.5 g of monobasic sodium phosphate in 500 mL of water. Adjust with 10% phosphoric acid to a pH of 2.1–2.7, and add 500 mL of acetonitrile.

**Diluent:** Acetonitrile and water (9:1)

**System suitability solution:** 0.1 mg/mL of USP Glimepiride RS and 0.02 mg/mL each of USP Glimepiride Related Compound B RS and USP Glimepiride Related Compound C RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Glimepiride RS in *Diluent*

**Sample solution:** Transfer 5 whole Tablets into a suitable volumetric flask to prepare a solution of approximately 0.1 mg/mL of glimepiride, based on the label claim. Add water to 10% of the volume of the flask. Shake the flask to completely dissolve the Tablets. Add acetonitrile to about 70% of the volume of the flask, and swirl. Sonicate the samples in a water bath not to exceed 20° for NLT 5 min and NMT 10 min, with occasional shaking. Allow the solutions to come to room temperature, dilute with acetonitrile to volume, mix, and filter.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4-mm × 12.5-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for glimepiride related compound B, glimepiride related compound C, and glimepiride are 0.25, 0.35, and 1.0, respectively. Identify the glimepiride peak and the peaks due to the related compounds based on their relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between glimepiride related compound B and glimepiride related compound C, *System suitability solution*

**Tailing factor:** NMT 2.0 for the glimepiride peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of glimepiride (C<sub>24</sub>H<sub>34</sub>N<sub>4</sub>O<sub>5</sub>S) in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of glimepiride in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of glimepiride in the  
Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION <711>

#### Test 1

**Medium:** pH 7.8 phosphate buffer (0.58 g of monobasic potassium phosphate and 8.86 g of dibasic sodium phosphate, anhydrous, in 1000 mL of water, adjusted with 10% phosphoric acid or 1 N sodium hydroxide to a pH of 7.8); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 15 min

**Mobile phase:** Prepare as directed in the Assay.

**Diluting solution:** Methanol and water (1:1)

**Standard solution:** Prepare a solution of USP Glimepiride RS in a mixture of acetonitrile and water (90:10) having a known concentration of about 0.125 mg/mL of glimepiride. Transfer 4.0 mL of this solution into a 200-mL volumetric flask, dilute with *Medium* to volume, and mix. Transfer 15.0 mL of this solution into a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. The final solution contains about 0.75 µg/mL of glimepiride.

**Sample solution:** Withdraw approximately 10 mL of the solution under test, and transfer to a centrifuge tube. Centrifuge for 5 min at 2500 rpm. Pipet 3.0 mL of the supernatant into a 10-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4.0-mm × 12.5-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis:** Calculate the percentage of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** pH 7.8 phosphate buffer (add 250 mL of 0.2 M monobasic potassium phosphate to 223 mL of 0.2 M sodium hydroxide, dilute with water to 1 L, and adjust with 0.2 M sodium hydroxide or phosphoric acid to a pH of 7.8); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Buffer solution:** 4.0 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.3.

**Mobile phase:** Acetonitrile and *Buffer solution* (1:1)

**Diluent:** Methanol and acetonitrile (1:1)

**Standard stock solution:** 0.22 mg/mL of USP Glimepiride RS in *Diluent*

**Standard solution:** (L/1000) mg/mL of USP Glimepiride RS in *Medium*, from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 10-cm; 5-µm packing L1

**Flow rate:** 1.3 mL/min

**Injection size:** 100 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis:** Calculate the percentage of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** pH 7.8 phosphate buffer (prepared as indicated for *Test 1*); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 20 min

**Buffer solution:** 1.36 g/L of monobasic potassium phosphate in water. Adjust with 10% sodium hydroxide to a pH of  $7.0 \pm 0.05$ .

**Mobile phase:** *Buffer solution* and acetonitrile (675:325)

**Standard stock solution:** 0.22 mg/mL of USP Glimepiride RS in methanol

**Standard solution:** (L/1000) mg/mL of glimepiride in *Medium*, from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 100 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis:** Calculate the percentage of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of glimepiride ( $C_{24}H_{34}N_4O_5S$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

[NOTE—Store the solutions containing glimepiride for NMT 24 h.]

**Mobile phase and Diluent:** Prepare as directed in the Assay.

**System suitability solution:** 4 µg/mL of USP Glimepiride RS and 2 µg/mL each of USP Glimepiride Related Compound B RS and USP Glimepiride Related Compound C RS in Diluent

**Sensitivity solution:** Transfer 5.0 mL of the *System suitability solution* into a 100-mL volumetric flask, and dilute with Diluent to volume.

**Sample solution:** Finely powder NLT 10 Tablets, and transfer a portion of the powder to a 50-mL centrifuge tube. Add Diluent to prepare a solution containing 0.1 mg/mL of glimepiride, based on the label claim. Sonicate in a water bath at a temperature not to exceed 20° for NLT 5 min and NMT 10 min, with occasional mixing. Centrifuge the samples, and use the clear supernatant.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for glimepiride related compound B, glimepiride related compound C, and glimepiride are 0.2, 0.3, and 1.0, respectively. Identify the glimepiride peak and the peaks due to the related compounds based on their relative retention times.]

**Suitability requirements**

**Resolution:** NLT 4 between glimepiride related compound B and glimepiride related compound C, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the glimepiride peak, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

Calculate the signal-to-noise ratio, *S/N*, for the peaks of glimepiride related compounds B and C:

$$\text{Result} = (2H)/h$$

*H* = measured height of the respective related compound peak

*h* = amplitude of the average measured baseline noise

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Continue the elution for at least two times the retention time of the glimepiride peak.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

*r<sub>U</sub>* = peak response for each impurity from the *Sample solution*

*r<sub>T</sub>* = sum of all the peak responses from the *Sample solution*

*F* = relative response factor: 1.3 for glimepiride related compound B, and 1.0 for any other impurity

**Acceptance criteria**

**Glimepiride related compound B:** NMT 2.5%

**Any other individual impurity:** NMT 0.5%

**Total impurities (excluding glimepiride related compound B):** NMT 1.0%

**Total impurities (including glimepiride related compound B):** NMT 3.5%

[NOTE—Disregard any peak less than 0.1%.]

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

**• LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**• USP REFERENCE STANDARDS <11>**

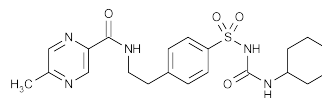
USP Glimepiride RS

USP Glimepiride Related Compound B RS

Glimepiride sulfonamide.

USP Glimepiride Related Compound C RS

Glimepiride urethane.

**Glipizide**

C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S 445.54

Pyrazinecarboxamide, *N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-5-methyl-

1-Cyclohexyl-3-[[*p*-[2-(5-methylpyrazinecarboxamido)ethyl]phenyl]sulfonyl]urea [29094-61-9].

» Glipizide contains not less than 98.0 percent and not more than 102.0 percent of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, protected from light. Store at room temperature.

**USP Reference standards <11>**

USP Glipizide RS

USP Glipizide Related Compound A RS

*N*-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide.

C<sub>14</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S 320.37

USP Glipizide Related Compound B RS

6-Methyl-*N*-[2-(4-sulfamoylphenyl)ethyl]pyrazine-2-carboxamide.

C<sub>14</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S 320.37

USP Glipizide Related Compound C RS

1-Cyclohexyl-3-[[4-[2-[(6-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]sulfonyl]urea.

C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S 445.54

**Identification—**

**A:** *Infrared Absorption* <197K>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 20 µg per mL.

*Medium:* methanol.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** <731>—Dry it in vacuum at 100° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.4%.

**Heavy metals, Method II** <231>—The limit is 0.005%.

**Related compounds**—[NOTE—Use low-actinic glassware for solutions containing glipizide and glipizide related compounds.]

**TEST 1** (limit of methyl-*N*-4-[2-(5-methylpyrazine-2-carboxamido)ethyl] benzenesulfonyl carbamate)—

**Buffer solution**—Add 4.0 mL of *n*-butylamine to 1000 mL of water. Adjust with phosphoric acid to a pH of  $3.00 \pm 0.05$ .

**Diluent**—Prepare a mixture of water, acetonitrile, and methanol (3:1:1).

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution*, acetonitrile, and methanol (3:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solution**—Prepare a solution of USP Glipizide RS in methanol containing about 0.1 mg per mL.

**Standard solution**—Prepare a solution of USP Glipizide Related Compound A RS in methanol containing about 0.1 mg per mL. Pipet 2.0 mL of this solution into a 100-mL volumetric flask, add 2.0 mL of the *Standard stock solution*, dilute with *Diluent* to volume, and mix. This solution contains about 0.002 mg of USP Glipizide RS and about 0.002 mg of USP Glipizide Related Compound A RS per mL.

**Test solution**—Transfer about 25 mg of Glipizide, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 4.0 mL of this solution into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The column temperature is maintained at 30°. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0% for each peak. [NOTE—The retention time of the glipizide peak is about 45 minutes; for the purpose of identification, the relative retention times for glipizide related compound A and glipizide are about 0.12 and 1.0, respectively.]

**Procedure**—Separately inject equal volumes (about 35  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. In the *Test solution*, identify the peaks that correspond to glipizide related compounds A, B, and C, based on their relative retention times: the relative retention time for glipizide related compound A is given above; glipizide related compound B, if present, elutes immediately after glipizide related compound A, and these two peaks are not resolved by this method; glipizide related compound C elutes at a relative retention time of approximately 1.1. Disregard these impurities when using *Test 1*, as they will be controlled using *Test 2*.

In the *Test solution*, identify the peak that corresponds to methyl-*N*-4-[2-(5-methylpyrazine-2-carboxamido)ethyl]benzenesulfonyl carbamate impurity, based on its relative retention time, which is about 0.18. Calculate the percentage of this impurity and any other individual impurity in the portion of Glipizide taken by the formula:

$$100(C_G / C_T)(r_i / r_{SG})$$

in which  $C_G$  is the concentration, in mg per mL, of glipizide in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Glipizide in the *Test solution*;  $r_i$  is the peak response for each individual impurity obtained from the *Test solution*;  $r_{SG}$  is the glipizide peak response obtained from the *Standard solution*. Disregard any impurity peak that is less than 0.05%. Not more than 0.5% of any individual impurity is found.

**TEST 2** (limit of related compounds A, B, and C)—

*0.02 M Phosphate buffer*, *Mobile phase*, *Impurity standard stock solution*, *System suitability solution*, *Sensitivity solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Impurity standard solution**—Accurately transfer 5.0 mL of the *Impurity standard stock solution* into a 100-mL volumetric

flask, and dilute with methanol to volume. Accurately transfer 10.0 mL of the solution obtained into another 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer*. Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix. This solution contains about 0.0005 mg of each of glipizide related compounds A, B, and C per mL.

**Test solution**—Use *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Impurity standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each of the glipizide related compounds A, B, or C in the portion of Glipizide taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the respective glipizide related compound in the *Impurity standard solution*;  $C_T$  is the concentration, in mg per mL, of Glipizide in the *Test solution*;  $r_U$  and  $r_S$  are the peak responses for the respective glipizide related compound obtained from the *Test solution* and the *Impurity standard solution*, respectively. Not more than 0.5% of any individual impurity is found; and not more than 1.5% of total impurities is found, the results for *Test 1* and *Test 2* being combined.

**Assay**—[NOTE—Use low-actinic glassware for solutions containing glipizide and glipizide related compounds.]

*0.02 M Phosphate buffer*—Dissolve 2.84 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of  $6.00 \pm 0.05$ .

**Mobile phase**—Prepare a filtered and degassed mixture of *0.02 M Phosphate buffer*, acetonitrile, and methanol (70:20:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock preparation**—Transfer accurately weighed amounts of USP Glipizide RS to a suitable volumetric flask, and dissolve in methanol with the aid of sonication for about 5 minutes to obtain a solution having a known concentration of about 1.0 mg of glipizide per mL.

**Standard preparation**—Accurately transfer 10.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer*. Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix. This solution contains about 0.1 mg of glipizide per mL.

**Impurity standard stock solution**—Transfer accurately weighed amounts of USP Glipizide Related Compound A RS, USP Glipizide Related Compound B RS, and USP Glipizide Related Compound C RS to a suitable volumetric flask, and dissolve in methanol with the aid of sonication for about 5 minutes to obtain a solution having known concentrations of about 0.1 mg of each of glipizide related compounds A, B, and C per mL. [NOTE—Glipizide related compound A is *N*-{2-[(4-aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide; glipizide related compound B is 6-methyl-*N*-[2-(4-sulfamoylphenyl)ethyl]pyrazine-2-carboxamide; and glipizide related compound C is 1-cyclohexyl-3-[[4-[2-[(6-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]-sulfonyl]urea.]

**System suitability stock solution**—Transfer an accurately weighed amount of USP Glipizide RS to a suitable volumetric flask, dissolve in methanol with the aid of sonication for about 5 minutes, and add an accurately measured volume of *Impurity standard stock solution* to obtain a solution containing about 1.0 mg of glipizide and about 0.005 mg of each of glipizide related compounds A, B, and C per mL.

**System suitability solution**—Accurately transfer 10.0 mL of the *System suitability stock solution* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer*. Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix.

**Sensitivity solution**—Accurately transfer 5.0 mL of the *System suitability solution* into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay stock preparation**—Transfer about 100 mg of Glipizide, accurately weighed, to a 100-mL volumetric flask, dissolve in about 50 mL of methanol with the aid of sonication for about 5 minutes, dilute with methanol to volume, and mix.

**Assay preparation**—Accurately transfer 10.0 mL of the *Assay stock preparation* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of 0.02 M *Phosphate buffer*. Sonicate the solution for about 5 minutes, dilute with 0.02 M *Phosphate buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L43. The column temperature is maintained at 40°. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the elution order is glipizide related compound A, glipizide related compound B, glipizide, and glipizide related compound C; the resolution, *R*, between glipizide related compound A and glipizide related compound B peaks is not less than 1.8, and the resolution, *R*, between glipizide and glipizide related compound C peaks is also not less than 1.8; the tailing factor for the glipizide peak is not more than 2.0; and the relative standard deviation for 5 replicate injections is not more than 1.5% for the glipizide peak and not more than 5.0% for each of the related compounds peaks. Chromatograph the *Sensitivity solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio for each of glipizide related compound A and glipizide related compound B peaks is not less than 15.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S in the portion of Glipizide taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which *C<sub>S</sub>* and *C<sub>U</sub>* are the concentrations, in mg per mL, of glipizide in the *Standard preparation* and the *Assay preparation*, respectively; and *r<sub>U</sub>* and *r<sub>S</sub>* are the glipizide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glipizide Tablets

» Glipizide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of glipizide (C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

### USP Reference standards <11>—

USP Glipizide RS

USP Glipizide Related Compound A RS

N-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazine-carboxamide.

C<sub>14</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S 320.37

### Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

B: Transfer a quantity of finely powdered Tablets, equivalent to about 10 mg of glipizide, to a glass-stoppered centrifuge tube, add 10 mL of methanol, insert a stopper into the tube, and shake. Centrifuge the mixture, and use the clear supernatant as the test solution. Separately apply, as streaks about 7 cm in length, 100 μL of the test solution and 100 μL of a Standard solution of USP Glipizide RS in methanol containing 1 mg per mL, to a thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Allow the streaks to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, ethyl acetate, and 98% formic acid (5:3:2) until the solvent front has moved to within 2.5 cm of the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 80° for 30 minutes. Cool the plate, spray it with 0.5% sodium hypochlorite solution, and allow the plate to air-dry. Spray the plate with alcohol, air-dry, and spray with a freshly prepared mixture of 1% soluble starch solution and 1% potassium iodide solution (1:1): the *R<sub>F</sub>* value of the principal band obtained from the test solution corresponds to that obtained from the Standard solution.

### Dissolution <711>—

TEST 1—

**Medium:** simulated intestinal fluid TS (without pancreatin); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amount of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Glipizide RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the label claim of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S is dissolved in 45 minutes.

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** simulated intestinal fluid TS (without pancreatin), with pH adjusted to pH 7.5 ± 0.1 with 0.2 N sodium hydroxide; 900 mL.

**Apparatus 2 and Time**—Proceed as directed under *Test 1*.

Determine the amount of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Glipizide RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the label claim of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements, the following procedure being used where the test for *Content Uniformity* is required.

**Buffer, Mobile phase, and Standard preparation**—Prepare as directed in the *Assay*.

**Test preparation**—Transfer 1 Tablet to an appropriate volumetric flask, add a volume of *Buffer* equal to one-half of the total flask volume, and shake by mechanical means for 10 minutes to allow the Tablet to disintegrate completely. Dilute with methanol to volume, and sonicate for 15 minutes to obtain a solution having a concentration of about 0.05 mg of glipizide per mL. Filter through a solvent-resistant filter.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure

the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{27}N_5O_4S$  in the Tablet taken by the formula:

$$CV(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide RS in the *Standard preparation*; V is the volume, in mL, of the *Test preparation* taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

#### Related compounds—

*Buffer and Mobile phase*—Prepare as directed in the *Assay*.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Glipizide Related Compound A RS in methanol to obtain a solution having a known concentration of about 50 µg per mL.

*Standard solution*—Dissolve an accurately weighed quantity of USP Glipizide RS in methanol. To this solution add sufficient *Standard stock solution*, and dilute quantitatively with methanol to obtain a solution having known concentrations of about 100 µg per mL of USP Glipizide RS and about 0.5 µg per mL of USP Glipizide Related Compound A RS. Transfer 25 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix.

*Test solution*—Use the *Assay preparation*.

*Chromatographic system*—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.2 for glipizide related compound A and 1.0 for glipizide; the resolution, *R*, between glipizide related compound A and glipizide is not less than 1.5; and the relative standard deviation for replicate injections is not more than 5% for glipizide related compound A.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of N-[2-[(4-aminosulfonyl)phenyl]ethyl]-5-methyl-pyrazine-carboxamide (glipizide related compound A) in the Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide Related Compound A RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of glipizide related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 2.0% of glipizide related compound A relative to the glipizide content, as determined in the *Assay*, is found.

#### Assay—

*Buffer*—Dissolve 13.8 g of monobasic sodium phosphate in water, and dilute with water to 1000 mL. Adjust with 2.0 N sodium hydroxide to a pH of  $6.00 \pm 0.05$ .

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer* and methanol (55 : 45). Make adjustments if necessary (see *System suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Glipizide RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix to obtain a solution having a known concentration of about 0.05 mg per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of glipizide, to a 100-mL volumetric flask. Add 50 mL of methanol, and place in an ultrasonic bath for 15 minutes. Dilute with *Buffer* to volume, and place in the ultrasonic bath for an additional 15 minutes. Filter through a solvent-resistant filter.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 225-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{27}N_5O_4S$  in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glipizide and Metformin Hydrochloride Tablets

### DEFINITION

Glipizide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glipizide ( $C_{21}H_{27}N_5O_4S$ ) and metformin hydrochloride ( $C_4H_{11}N_5 \cdot HCl$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION <197A>

**Sample:** Transfer NLT 10 Tablets to a suitable container, add 10 mL of methanol, and shake to remove any Tablet coating. Drain the methanol, add 20 mL of water, and stir until the Tablets dissolve (1 h). Transfer the solution to a separatory funnel, and extract twice with 10-mL portions of chloroform, shaking for approximately 5 min. Transfer the lower organic layer into a beaker containing 3 to 4 g of anhydrous magnesium sulfate. Repeat the extraction of the solution in the separatory funnel two more times, each time using 20-mL portions of chloroform. Swirl the mixture in the beaker for 1 min. Filter, and collect the filtrate. Evaporate the solvent under vacuum, and dry the residue under vacuum for 4 h at 105°. Mound the residue onto a diamond cell.

**Acceptance criteria:** The IR spectrum exhibits maxima only at the same wavelengths as a similarly obtained spectrum of USP Glipizide RS.

- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Glipizide*.
- **C.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Metformin Hydrochloride*.

### ASSAY

#### • GLIPIZIDE

**Solution A:** 2.6 g/L of dibasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 8.0.

**Solution B:** Acetonitrile, water, and *Solution A* (1:14:5)

**Solution C:** Acetonitrile, water, and *Solution A* (2:1:1)

**Diluent:** Acetonitrile and water (60:40)

**Standard stock solution:** Transfer a weighed quantity of USP Glipizide RS to a suitable low-actinic volumetric flask. Dissolve first in acetonitrile, using 60% of the final volume, by sonicating for 20 min, then dilute with water to volume to obtain a solution having a concen-



tration of 0.1 mg/mL of glipizide. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

**Standard solution:** Transfer 25.0 mL of *Standard stock solution* to a 200-mL low-actinic volumetric flask. Add 75 mL of *Diluent*, and dilute with water to volume to obtain a solution having a known glipizide concentration of 0.0125 mg/mL. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

**System suitability solution:** Transfer approximately 5 mg of USP Glipizide Related Compound A RS to a 500-mL volumetric flask, and fill halfway with acetonitrile. Sonicate for 30 min to dissolve, and dilute with acetonitrile to volume. Transfer 1 mL of this solution to a 50-mL low-actinic volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** Transfer NLT 5 Tablets to a suitable volumetric flask, and fill halfway with *Diluent*. Sonicate for 30 min, and shake vigorously for another 30 min to dissolve. Dilute with water to volume, and mix to obtain a solution with a final glipizide concentration of 0.0125 mg/mL. Pass a portion of this solution through a nylon or PVDF filter of 0.2-μm pore size, and use the filtrate. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
3	100	0
18	0	100
20	0	100
22	100	0
30	100	0

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 223 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Flow rate:** 1 mL/min

**Injection size:** 50 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for glipizide related compound A and glipizide are 0.92 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** Greater than 1.2 between glipizide related compound A and glipizide, *System suitability solution*

**Relative standard deviation:** Less than 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Glipizide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of glipizide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### • METFORMIN HYDROCHLORIDE

**Solution A:** 9.41 g/L of sodium 1-hexanesulfonate in water, and adjust with trifluoroacetic acid to a pH of 2.0 (50 mM hexanesulfonic acid solution)

**Solution B:** Acetonitrile and water (40:60)

**Mobile phase:** *Solution A*, *Solution B*, and water (3:2:5)

**Diluent pH 2.0:** Acetonitrile, *Solution A*, and water (7:30:63)

**Standard solution:** 0.1 mg/mL of USP Metformin Hydrochloride RS in *Diluent pH 2.0*

**System suitability solution:** 5 μg/mL of USP Metformin Related Compound A RS in water. Pipet 0.5 mL of this solution into a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** Dilute a portion of the *Sample solution*, obtained as directed in the *Assay for Glipizide*, with *Diluent pH 2.0*, to obtain a solution having a concentration of 0.1 mg/mL of metformin hydrochloride.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 218 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L11

**Flow rate:** 1 mL/min

**Injection size:** 25 μL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for metformin related compound A and metformin are 0.26 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between metformin related compound A and metformin, *System suitability solution*

**Relative standard deviation:** Less than 2.0%, determined from the metformin peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>4</sub>H<sub>11</sub>N<sub>5</sub> · HCl in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

###### Test 1

**Medium:** 0.05 M phosphate buffer, pH 6.8 ± 0.05. (Dissolve 12.96 g of monobasic potassium phosphate and 1.66 g of sodium hydroxide in approximately 400 mL of water, and dilute with water to 2000 mL. Adjust the pH, if necessary, with diluted sodium hydroxide); 1000 mL. [NOTE—Tight control of the pH is critical.]

**Apparatus 2:** 50 rpm

**Time:** 45 min for glipizide, and 30 min for metformin hydrochloride.

**Determination of glipizide:** Determine the amount of glipizide (C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>S) dissolved as follows.

**Solution A:** Dissolve approximately 3.4 g of monobasic potassium phosphate in approximately 800 mL of water. Adjust with 10 N sodium hydroxide solution to a pH of 6.0 ± 0.1. Dilute with water to 1000 mL.

**Mobile phase:** Methanol and *Solution A* (13:12)

**Standard stock solution:** Transfer 50 mg of USP Glipizide RS to a 1000-mL low-actinic volumetric flask, and dissolve in 100 mL of methanol. Dilute with *Medium* to volume, and sonicate for 5 min. [NOTE—This solution is stable for 7 days at 5° when protected from light.]

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a solution containing (L/1000) mg/mL, with L being the glipizide Tablet label claim, in mg.

**Sample solution:** After the specified time, withdraw 10 mL of the solution under test. Pass the solution through a suitable PVDF filter of 0.45- $\mu$ m pore size or a glass fiber filter of 1.0- $\mu$ m pore size, discarding the first mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms running for 8 min and measure the peak response for glipizide.

Calculate the percentage of  $C_{21}H_{27}N_5O_4S$  dissolved:

$$\text{Result} = (r_U \times C_S \times V \times 100) / (r_S \times L)$$

$r_U$  = peak response from the *Sample solution*

$C_S$  = concentration of glipizide in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$r_S$  = peak response from the *Standard solution*

$L$  = label claim for glipizide (mg/Tablet)

#### Determination of metformin hydrochloride

**Test 1:** Determine the amount of metformin hydrochloride dissolved by employing UV absorption at the wavelength of maximum absorbance at 233 nm on portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*. Calculate the amount of  $C_4H_{11}N_5 \cdot HCl$  dissolved:

$$\text{Result} = (A_U \times C_S \times V \times 100) / (A_S \times L)$$

$A_U$  = absorbance from the *Sample solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$A_S$  = absorbance from the *Standard solution*

$L$  = label claim for metformin hydrochloride (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{21}H_{27}N_5O_4S$  is dissolved in 45 min. NLT 80% (Q) of the labeled amount of  $C_4H_{11}N_5 \cdot HCl$  is dissolved in 30 min.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** 0.05 M phosphate buffer, pH 6.8; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 60 min for both metformin hydrochloride and glipizide

**pH 6.0 buffer solution:** 4.3 g/L of octanesulfonic acid sodium salt and 6.9 g/L of monobasic monohydrate sodium phosphate in water, adjusted with diluted sodium hydroxide to a pH of  $6.00 \pm 0.05$

**Glipizide standard stock solution:** 0.05 mg/mL of USP Glipizide RS in methanol

**Standard solution:** Transfer a quantity of USP Metformin Hydrochloride RS to a volumetric flask, add a suitable aliquot of *Glipizide standard stock solution*, and dilute with *Medium* to obtain a final concentration

of (L/1000) mg/mL, where L is the Tablet label claim for both metformin hydrochloride and glipizide, in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable polyethersulfone filter of 0.45- $\mu$ m pore size, discarding the first few mL.

**Mobile phase:** Methanol and pH 6.0 buffer solution (1:1)

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Sample compartment chiller:** 4°

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2 between the glipizide and metformin hydrochloride peaks

**Relative standard deviation:** NMT 2.0% for both glipizide and metformin hydrochloride

**Analysis:** Calculate the percentages of  $C_{21}H_{27}N_5O_4S$  and  $C_4H_{11}N_5 \cdot HCl$  dissolved:

$$\text{Result} = (r_U / r_S) \times (C_S / L) \times V \times 100$$

$r_U$  = peak response for glipizide or metformin hydrochloride from the *Sample solution*

$r_S$  = peak response for glipizide or metformin hydrochloride from the *Standard solution*

$C_S$  = concentration of glipizide or metformin hydrochloride in the *Standard solution* (mg/mL)

$L$  = label claim for glipizide or metformin hydrochloride (mg/Tablet)

$V$  = volume of *Medium*, 1000 mL

**Tolerances:** NLT 80% (Q) of the labeled amounts of  $C_{21}H_{27}N_5O_4S$  and  $C_4H_{11}N_5 \cdot HCl$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE 1: GLIPIZIDE

**Solution A, Solution B, Solution C, Sample solution, and Chromatographic system:** Prepare as directed in the Assay for *Glipizide*.

##### Analysis

**Sample:** *Sample solution*

Calculate the percentage of glipizide related compound A (approximate relative retention time 0.92) and other individual impurities in the portion of Tablets taken:

$$\text{Result} = (r_U / r_T) \times (1 / F) \times 100$$

$r_U$  = peak response of each impurity

$r_T$  = sum of all the peak responses

$F$  = relative response factor for each impurity equal to 1.4 for glipizide related compound A and 1.0 for all other peaks

##### Acceptance criteria

**Glipizide related compound A:** NMT 2.0%

**Individual impurities:** NMT 0.5% of any other individual glipizide related impurity (eluting after approximately 8 min)

**Total impurities:** NMT 1.0%, excluding glipizide related compound A. [NOTE— Disregard the broad peak due to metformin that elutes before 8 min. Disregard any peak observed in the blank, and disregard any peak less than 0.05%.]

• **PROCEDURE 2: METFORMIN HYDROCHLORIDE**

**Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system:** Prepare as directed in the Assay for Metformin Hydrochloride.

**Analysis**

**Sample:** Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of all the peak responses

**Acceptance criteria**

**Individual impurities:** NMT 0.1%

**Total impurities:** NMT 0.5%

[NOTE— Disregard any peak less than 0.05%. Disregard any peak observed in the blank.]

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** (11)

USP Glipizide RS

USP Glipizide Related Compound A RS

*N*-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methylpyrazinecarboxamide.

$C_{14}H_{16}N_4O_3S$  320.37

USP Metformin Hydrochloride RS

USP Metformin Related Compound A RS

1-Cyanoguanidine.

## Immune Globulin

» Immune Globulin conforms to the regulations of the FDA concerning biologics (640.100 to 640.104) (see *Biologics* (1041)). It is a sterile, nonpyrogenic solution of globulins that contains many antibodies normally present in adult human blood, prepared by pooling approximately equal amounts of material (source blood, plasma, serum, or placentas) from not less than 1000 donors. It contains not less than 15 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It contains 0.3 M glycine as a stabilizing agent and contains a suitable preservative. It has a potency of component antibodies of diphtheria antitoxin based on the U.S. Standard Diphtheria Antitoxin and a diphtheria test toxin, tested in guinea pigs (not less than 2 antitoxin units per mL), and antibodies for measles and poliovirus. It meets the requirements of the tests for heat stability in absence of gelation on heating, and for pH.

**Packaging and storage**—Preserve at a temperature between 2° and 8°.

**Expiration date**—The expiration date is not later than 3 years after date of issue from manufacturer's cold storage (5°, 3 years).

**Labeling**—Label it to state that passive immunization with Immune Globulin modifies hepatitis A, prevents or modifies

measles, and provides replacement therapy in persons having hypo- or agammaglobulinemia, that it is not standardized with respect to antibody titers against hepatitis B surface antigen and that it should be used for prophylaxis of viral hepatitis type B only when the specific Immune Globulin is not available, that it may be of benefit in women who have been exposed to rubella in the first trimester of pregnancy but who would not consider a therapeutic abortion, and that it may be used in immunosuppressed patients for passive immunization against varicella if the specific Immune Globulin is not available. Label it also to state that it is not indicated for routine prophylaxis or treatment of rubella, poliomyelitis, or mumps, or for allergy or asthma in patients who have normal levels of immunoglobulin, that the plasma units from which it has been derived have been tested and found non-reactive for hepatitis B surface antigen, and that it should not be administered intravenously but be given intramuscularly, preferably in the gluteal region.

## Rh<sub>0</sub> (D) Immune Globulin

» Rh<sub>0</sub> (D) Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* (1041)). It is a sterile, nonpyrogenic solution of globulins derived from human blood plasma containing antibody to the erythrocyte factor Rh<sub>0</sub> (D). It contains not less than 10 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It has a potency, determined by a suitable method, not less than that of the U.S. Reference Rh<sub>0</sub> (D) Immune Globulin. It contains 0.3 M glycine as a stabilizing agent and contains a suitable preservative.

**Packaging and storage**—Preserve at a temperature between 2° and 8°.

**Expiration date**—The expiration date is not later than 6 months from the date of issue from manufacturer's cold storage, or not later than 1 year from the date of manufacture, as indicated on the label.

## Glucagon

HSQGTFTSDY SKYLSRRRAQ DFLVQALMNT

$C_{153}H_{225}N_{43}O_{49}S$

Glucagon (human) [16941-32-5].

3482.75

### DEFINITION

Glucagon is a polypeptide hormone that has the property of increasing the concentration of glucose in the blood. It has the same structure (29 amino acids) as the hormone produced by the  $\alpha$ -cells of the human pancreas. Glucagon is produced by microbial processes using recombinant DNA (rDNA) technology. The host cell-derived protein content and/or the host cell-derived or vector-derived DNA content are determined by validated methods. During the course of product development, it must be demonstrated that the manufacturing process produces Glucagon having a biological activity of NLT 0.80 USP unit/mg, using a validated bioassay approved by a competent authority. It contains NLT 90% and NMT 105% of  $C_{153}H_{225}N_{43}O_{49}S$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B.** Determine the peptide fragments, using the following peptide mapping procedure.

**Ammonium bicarbonate buffer:** Prepare a 1 M solution of ammonium bicarbonate, and adjust with ammonia to a pH of 10.3. Prepare a mixture of 1 M ammonium bicarbonate and water (1:9).

**Enzyme solution:** 2 mg/mL of  $\alpha$ -chymotrypsin (peptide mapping grade) in *Ammonium bicarbonate buffer*.

**Solution A:** Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid and 1000 mL of water.

**Solution B:** Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid, 600 mL of ethanol, and 400 mL of water.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	53	47
45	0	100
46	100	0
75	100	0

**Standard digest solution:** Prepare a 5-mg/mL solution of USP rGlucagon RS in 0.01 M hydrochloric acid. Mix 200  $\mu$ L of this solution with 800  $\mu$ L of *Ammonium bicarbonate buffer*. To this solution add 25  $\mu$ L of *Enzyme solution*, and place in a closed vial at about 37° for 2 h. Remove the vial, and stop the reaction immediately by adding 120  $\mu$ L of glacial acetic acid.

**Sample digest solution:** Prepare a 5-mg/mL solution of Glucagon in 0.01 M hydrochloric acid. Proceed as directed for *Standard digest solution*, beginning with "Mix 200  $\mu$ L of this solution".

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.0-mm  $\times$  5-cm; 5- $\mu$ m or less packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard digest solution*

**Suitability requirements**

**Chromatogram similarity:** The chromatogram from the *Standard digest solution* is similar to the reference chromatogram provided with the lot of USP rGlucagon RS being used.

**Analysis**

**Samples:** *Standard digest solution* and *Sample digest solution*

**Acceptance criteria:** The chromatographic profile of the *Sample digest solution* corresponds to that of the *Standard digest solution*.

**ASSAY****PROCEDURE**

**Solution A:** Dissolve 16.3 g of monobasic potassium phosphate in 750 mL of water, adjust with phosphoric acid to a pH of 2.7 ( $\pm 0.05$ ), add water to 800 mL, add 200 mL of acetonitrile, and degas.

**Solution B:** Prepare a degassed solution of acetonitrile and water (4:6).

**Mobile phase:** See *Table 2*. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25 <sup>a</sup>	61	39
29	12	88
30	12	88
31	61	39
70	61	39

<sup>a</sup> The end time of the isocratic elution can be adjusted so that the gradient begins after the fourth desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

**System suitability solution:** Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

**Standard solution:** Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

**Sample solution:** 0.5 mg/mL of Glucagon in 0.01 N hydrochloric acid

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Column temperature:** 45°

**Flow rate:** 0.5 mL/min

**Injection volume:** 15  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible. The resolution between the main peak and the first eluting desamido peak is NLT 1.5, *System suitability solution*

**Tailing factor:** NMT 1.8 for the glucagon peak, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glucagon ( $C_{153}H_{225}N_{43}O_{49}S$ ) in the portion of Glucagon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90%–105% on the anhydrous basis

**IMPURITIES****ORGANIC IMPURITIES**

**Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all peaks

**Acceptance criteria:** NMT 2.0% total of all four desamido glucagons is found, and NMT 6.0% of total impurities and related compounds is found.

### SPECIFIC TESTS

- **WATER DETERMINATION, Method 1c (921):** NMT 10%, determined on a 20- to 50-mg sample
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 10 USP Endotoxin Units/mg

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in airtight containers, protected from light, and store in a freezer.
- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS  
USP rGlucagon RS

## Glucagon for Injection

### DEFINITION

Glucagon for Injection is a sterile lyophilized mixture of the hydrochloride of glucagon with one or more suitable buffering and stabilizing agents. It contains NLT 65% and NMT 110% of the labeled amount of glucagon.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. GLUCOGEN BIOIDENTITY TESTS (123):** Meets the requirements

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 16.3 g of monobasic potassium phosphate in 750 mL of water, adjust with phosphoric acid to a pH of 2.7 ( $\pm 0.05$ ), add water to 800 mL, add 200 mL of acetonitrile, and degas.

**Solution B:** Prepare a degassed solution of acetonitrile and water (4:6).

**Mobile phase:** See *Table 1*. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25 <sup>a</sup>	61	39
29	12	88
30	12	88
31	61	39
70	61	39

<sup>a</sup> The end time of the isocratic elution can be adjusted so that the gradient begins after the 4th desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

**System suitability solution:** Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

**Standard solution:** Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

**Sample solution:** Dissolve an adequate amount of Glucagon for Injection in order to obtain a 0.5-mg/mL concentration of glucagon in 0.01 N hydrochloric acid.

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3-mm  $\times$  15-cm; 3- $\mu$ m or less packing L1

**Column temperature:** 45°

**Flow rate:** 0.5 mL/min

**Injection volume:** 15  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the main peak and the first eluting desamido peak. Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible, *System suitability solution*

**Tailing factor:** NMT 1.8 for the glucagon peak, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glucagon ( $C_{153}H_{225}N_{43}O_{49}S$ ) in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 65%–110%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Dissolve the substance to be examined in water in order to obtain a concentration of 0.5 mg/mL of glucagon.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all peaks

**Acceptance criteria:** NMT 14% total of all four desamido glucagons is found, and NMT 31% of total impurities and related compounds is found.

### SPECIFIC TESTS

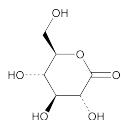
- **WATER DETERMINATION, Method 1c (921):** NMT 4.0%
- **PH AND CLARITY OF SOLUTION:** Dissolve it in the solvent and in the concentration recommended in the labeling; the pH of the solution is between 1.7 and 3.5, and the solution is clear.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 10 USP Endotoxin Units/mg.
- **STERILITY TESTS (71):** Meets the requirements
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections (1)*, *Constituted Solutions*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Injections (1)*, *Containers for Sterile Solids*.

- **LABELING:** The labeling states that the material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS** (11)
  - USP Endotoxin RS
  - USP rGlucagon RS

## Gluconolactone



$C_6H_{10}O_6$

178.14

D-Gluconic acid  $\delta$ -lactone;  
Glucono delta-lactone [90-80-2].

### DEFINITION

Gluconolactone contains NLT 99.0% and NMT 101.0% of gluconolactone ( $C_6H_{10}O_6$ ).

### IDENTIFICATION

- **A.**

**Sample solution:** Dissolve 0.5 g of Gluconolactone in 5 mL of warm water in a test tube.

**Analysis:** To the *Sample solution* add 1 mL of freshly distilled phenylhydrazine, and heat on a steam bath for 30 min. Cool the solution, induce crystallization by scratching the inner surface of the test tube with a glass rod, and collect the crystals of the phenylhydrazine of gluconic acid. Dissolve the crystals in 10 mL of hot water to which a small amount of activated charcoal has been added, filter, and recrystallize. Wash the crystals with 2 mL of cold water, and dry the crystals at 105° for 1 h.

**Acceptance criteria:** The crystals obtained melt between 195° and 200°.

### ASSAY

- **PROCEDURE**

**Sample:** 600 mg

**Titrimetric system**  
(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N sodium hydroxide VS

**Back-titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 100 mL of water in a 300-mL conical flask, add 50.0 mL of *Titrant*, and allow to stand for 15 min. Add phenolphthalein TS. Titrate excess alkali with *Back-titrant*. Perform a blank determination. Each mL of *Back-titrant* is equivalent to 17.81 mg of gluconolactone ( $C_6H_{10}O_6$ ).

**Acceptance criteria:** 99.0%–101.0%

### IMPURITIES

- **LEAD** (251)
 

**Test preparation:** Prepare as directed in the chapter.

**Analysis:** Use 10 mL of *Diluted Standard Lead Solution* (10  $\mu$ g of lead) for the procedure.

**Acceptance criteria:** NMT 10 ppm
- **HEAVY METALS** (231)
 

**Test preparation:** 40 mg/mL

**Acceptance criteria:** NMT 20 ppm

### SPECIFIC TESTS

- **REDUCING SUBSTANCES**

**Sample solution:** Transfer 10.0 g to a 400-mL beaker, add 40 mL of water, and swirl to dissolve. Add 2 drops

of phenolphthalein TS, and neutralize with sodium hydroxide solution (1 in 2). Dilute with water to 50 mL, and add 50 mL of alkaline cupric tartrate TS. Heat so that the solution begins to boil in 4 min, and allow boiling to continue for 120 s. Pass the suspension through a medium pore size filtering crucible, and wash the filter with three 5-mL portions of water. Place the crucible in an upright position in the original beaker, add 5 mL of water and 3 mL of nitric acid to the crucible, mix with a glass rod to ensure complete solution of the cuprous oxide, and wash the solution from the crucible into a beaker with the aid of 5 mL of water. Add bromine TS, usually 5–10 mL, until the solution becomes yellow in color, and dilute with water to 75 mL. Add a few glass beads, boil until the bromine has been driven off, and cool. Slowly add ammonium hydroxide until a deep blue color appears, then adjust with glacial acetic acid to a pH of 4, and add water to make 100 mL.

**Analysis:** Add 4 g of potassium iodide to the *Sample solution*, and titrate with 0.1 N sodium thiosulfate VS, adding starch TS just before the endpoint is reached.

**Acceptance criteria:** NMT 16.1 mL of 0.1 N sodium thiosulfate is consumed.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Glucose Enzymatic Test Strip

» Glucose Enzymatic Test Strip consists of the enzymes glucose oxidase and horseradish peroxidase, a suitable substrate for the reaction of hydrogen peroxide catalyzed by peroxidase, and other inactive ingredients impregnated and dried on filter paper. When tested in human urine containing known glucose concentrations, it reacts in the specified times to produce colors corresponding to the color chart provided.

**Packaging and storage**—Preserve in the original container, in a dry place, at controlled room temperature.

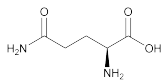
**Identification**—Remove the Strips from the container, and test them as directed in the instructions provided by the manufacturer, first in dextrose solution (1 in 50) and then in sucrose solution (1 in 50): color develops from the dextrose solution, but not from the sucrose solution.

### Calibration

**Glucose standard solutions**—Dissolve anhydrous dextrose in separate portions of freshly voided, normal, glucose-free human urine, previously adjusted to a pH of 6.0 with dilute formic acid (1 in 5), and with sodium hydroxide solution (1 in 2), respectively, to obtain separate solutions of the final concentrations corresponding to the color chart calibrations provided. Allow the standard solutions to stand for 1 hour prior to use.

**Procedure**—Remove the Strips from the container, and test each *Glucose standard solution* as directed in the instructions provided by the manufacturer: the colors formed on each of the Strips during the specified times match the colors on the color chart provided.

## Glutamine



$C_5H_{10}N_2O_3$  146.14  
L-Glutamine;  
2-Aminoglutaric acid [56-85-9].

### DEFINITION

Glutamine contains NLT 98.5% and NMT 101.5% of L-glutamine ( $C_5H_{10}N_2O_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Sample:** 150 mg of Glutamine

**Blank:** Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*. Perform a *Blank* determination.

Calculate the percentage of glutamine ( $C_5H_{10}N_2O_3$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F/W\} \times 100$$

$V_S$  = volume of *Titrant* consumed by the *Sample* (mL)

$V_B$  = volume of *Titrant* consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 146.1 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

- **CHLORIDE AND SULFATE, Chloride** (221)

**Standard solution:** 0.50 mL of 0.020 N hydrochloric acid

**Sample:** 0.7 g of Glutamine

**Acceptance criteria:** NMT 0.05%

- **CHLORIDE AND SULFATE, Sulfate** (221)

**Standard solution:** 0.25 mL of 0.020 N sulfuric acid

**Sample:** 0.8 g of Glutamine

**Acceptance criteria:** NMT 0.03%

- **IRON** (241): NMT 30 ppm

- **HEAVY METALS, Method I** (231): NMT 15 ppm

#### • RELATED COMPOUNDS

**Standard solution:** 0.05 mg/mL of USP Glutamine RS in water

**Sample solution:** 10 mg/mL of Glutamine in water

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

**Analysis:** Dry the plate at 80° for 30 min. Spray the plate with *Spray reagent*, heat at 80° for 10 min, and examine under white light.

**Acceptance criteria:** No secondary spot of the *Sample solution* is larger or more intense than the principal spot of the *Standard solution* (NMT 0.5%).

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 40 mg/mL in water. Warm at 40° to dissolve.

**Analysis:** Proceed as directed in the chapter, except measure at 20°.

**Acceptance criteria:** +6.3° to +7.3°

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

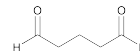
### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Glutamine RS

## Glutaryl Concentrate



$C_5H_8O_2$  100.12  
Pentanedial;  
Glutarylaldehyde [111-30-8].

### DEFINITION

Glutaryl Concentrate is a solution of glutarylaldehyde in Purified Water. It contains NLT 100.0% and NMT 104.0% of the labeled amount of glutaryl ( $C_5H_8O_2$ ). The labeled amount is 50.0 g of  $C_5H_8O_2$  per 100 g of Concentrate.

### IDENTIFICATION

#### • A.

**Solution A:** Add 4 mL of sulfuric acid to 0.8 g of 2,4-dinitrophenylhydrazine, then add 6 mL of water, dropwise, with swirling. When dissolution is essentially complete, add 20 mL of alcohol, and filter. The filtrate is the 2,4-dinitrophenylhydrazine reagent.

**Analysis:** Add 0.4 mL of Concentrate to 20 mL of *Solution A*, mix by swirling, and allow to stand for 5 min. Collect the precipitate on a filter, and rinse thoroughly with alcohol. Dissolve the precipitate in 20 mL of hot ethylene dichloride, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter. Redissolve the precipitate by refluxing with 30 mL of acetone, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter.

**Acceptance criteria:** The 2,4-dinitrophenylhydrazone so obtained melts at 185°–195°, within a 3° range (see *Melting Range or Temperature* (741)).

### ASSAY

#### • PROCEDURE

**Solution A:** 35 g/L of hydroxylamine hydrochloride prepared as follows. Dissolve 35 g of hydroxylamine hydrochloride in 150 mL of water in a 1000-mL volumetric flask. Add isopropyl alcohol to volume.

**Solution B:** Transfer 65 mL of triethanolamine to a glass-stoppered, 1000-mL volumetric flask, and add water to volume.

**Analysis:** To 500 mL of *Solution A* add 15 mL of a solution of bromophenol blue in alcohol (1 in 2500), and add *Solution B* from a buret to obtain a neutralized solution that appears greenish-blue by transmitted light. Transfer 65.0 mL of the neutralized solution to a glass-stoppered, 500-mL conical flask, add 50.0 mL of *Solution B*, purge with nitrogen, and insert the stopper. Add

a weighed quantity of Concentrate containing 1.2 g of glutaral by means of a suitable weighing pipet. Insert the stopper, and allow to stand for 60 min, swirling the flask occasionally. Titrate with 0.5 N sulfuric acid VS to a greenish-blue endpoint, and perform a blank determination (see *Titrimetry* (541), *Residual Titrations*). Calculate the percentage of the labeled amount of glutaral ( $C_5H_8O_2$ ) in the Concentrate taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F] / W\} \times 100$$

$V_B$  = volume of 0.5 N sulfuric acid VS consumed by the blank (mL)

$V_S$  = volume of 0.5 N sulfuric acid VS consumed by the Sample solution (mL)

$N$  = normality of the sulfuric acid

$F$  = equivalency factor for glutaraldehyde, 0.05006 g/mEq

$W$  = weight of Concentrate taken (g)

Acceptance criteria: 100.0%–104.0%

## IMPURITIES

- **HEAVY METALS** (231): NMT 10 ppm

## SPECIFIC TESTS

- **SPECIFIC GRAVITY** (841): 1.126–1.135 at 20°

## ACIDITY

**Analysis:** Transfer 60.0 g to a conical flask, add phenolphthalein TS, and titrate with 0.10 N alcoholic potassium hydroxide to a pink endpoint that is permanent for NLT 15 s.

**Acceptance criteria:** NMT 40 mL of 0.10 N potassium hydroxide is consumed, corresponding to NMT 0.4% (w/w) of acid, calculated as acetic acid.

- **pH** (791): 3.7–4.5

## CLARITY OF SOLUTION

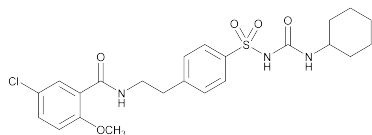
**Analysis:** Transfer 5.0 mL of Concentrate to a glass-stoppered, 100-mL graduated cylinder, add water to obtain 100 mL of mixture, insert the stopper, and mix by inverting the graduated cylinder several times. Allow the bubbles to rise, and view downward through the solution against a dark background.

**Acceptance criteria:** The solution is clear.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid exposure to excessive heat.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

## Glyburide



$C_{23}H_{28}ClN_3O_5S$  494.00

Benzamide, 5-chloro-N-[2-[4-[[[(cyclohexylamino)carbonyl]amino]-sulfonyl]phenyl]ethyl]-2-methoxy-

1-[[p-[2-(5-Chloro-o-anisamido)ethyl]phenyl]sulfonyl]-3-cyclohexylurea [10238-21-8].

» Glyburide contains not less than 98.0 percent and not more than 102.0 percent of  $C_{23}H_{28}ClN_3O_5S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

## USP Reference standards (11)—

USP Glyburide RS

## Identification—

**A: Infrared Absorption** (197M).

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for glyburide, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Loss on drying**—Dry it at 105° for 6 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** (231): 0.002%.

## Chromatographic purity—

**Mobile phase**—Prepare as directed in the *Assay*.

**Test solution**—To about 10 mg of Glyburide, accurately weighed, add 10 mL of acetonitrile, and shake to dissolve. Add 4 mL of water, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3500 theoretical plates.

**Procedure**—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Glyburide taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 1.5% of any impurity, which elutes before glyburide, is found; not more than 0.5% of any other individual impurity is found; and not more than 2.0% of total impurities is found.

## Assay—

**Mobile phase**—Dissolve 2.6 g of monobasic ammonium phosphate in 450 mL of water. Add 550 mL of acetonitrile, filter, and degas. Adjust, if necessary, with phosphoric acid or sodium hydroxide to a pH of  $5.25 \pm 0.30$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve progesterone in acetonitrile to obtain a solution containing about 0.2 mg per mL.

**Standard preparation**—To about 10 mg of USP Glyburide RS, accurately weighed, add 20.0 mL of *Internal standard solution*, and shake vigorously to dissolve. Add 4.0 mL of water, and mix.

**Assay preparation**—To about 10 mg of Glyburide, accurately weighed, add 20.0 mL of *Internal standard solution*, and shake vigorously to dissolve. Add 4.0 mL of water, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for glyburide and 1.0 for progesterone; the resolution,  $R$ , between glyburide and progesterone is not less than 5.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the heights for the major peaks. Calculate the quantity,



in mg, of  $C_{23}H_{28}ClN_3O_5S$  in the portion of Glyburide taken by the formula:

$$W_5 (R_U / R_S)$$

in which  $W_5$  is the weight, in mg, of USP Glyburide RS taken to prepare the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glyburide Tablets

### DEFINITION

Glyburide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ).

### IDENTIFICATION

#### • INFRARED ABSORPTION <197K>

**Sample:** Grind to a fine powder a number of Tablets, equivalent to 15 mg of glyburide. Add 30 mL of acetonitrile, and shake. Filter the mixture, evaporate the filtrate to dryness, and dry the residue in a vacuum at 60° for 3 h.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Dissolve 2.6 g of monobasic ammonium phosphate in 450 mL of water. Add 550 mL of acetonitrile, filter, and degas. Adjust, if necessary, with phosphoric acid or sodium hydroxide to a pH of 5.25 ± 0.30.

**Progesterone solution:** 0.2 mg/mL of progesterone in acetonitrile

**System suitability solution:** Dissolve 10 mg of USP Glyburide RS in 20 mL of *Progesterone solution*. Add 4.0 mL of water.

**Standard solution:** To 10 mg of USP Glyburide RS add 20.0 mL of acetonitrile, and shake vigorously to dissolve. Add 4.0 mL of water.

**Sample solution:** Transfer NLT 20 Tablets to a suitable container. Add water equivalent to 0.4 mL of water per mg of glyburide, and swirl to disperse and wet Tablet material. Then add acetonitrile equivalent to 2.0 mL of acetonitrile per mg of glyburide, and shake for 30 min. Centrifuge a portion of the suspension, and use the clear supernatant.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for glyburide and progesterone are about 0.4 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between glyburide and progesterone

**Relative standard deviation:** NMT 2.0% for glyburide

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of glyburide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

**Test 1** (nonmicronized glyburide)

**Medium:** 0.05 M borate buffer, pH 9.5 (381.5 g of sodium borate and 19.1 g of sodium hydroxide in 20 L of water, and adjust with phosphoric acid to a pH of 9.5 ± 0.1); 500 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min. [NOTE—Use low-actinic volumetric flasks.]

**Mobile phase:** Acetonitrile and water (1:1), containing 4.0 mL of phosphoric acid per L of solution

**Standard stock solution:** 0.15 mg/mL of USP Glyburide RS in *Medium*. [NOTE—Sonicate for about 25 min to dissolve, and dilute with *Medium* to volume.]

**Standard solutions:** Dilute the *Standard stock solution* with *Medium* to obtain 0.003 mg/mL (for Tablets labeled to contain 1.5 mg), 0.006 mg/mL (for Tablets labeled to contain 3.0 mg), 0.009 mg/mL (for Tablets labeled to contain 4.5 mg), and 0.012 mg/mL (for Tablets labeled to contain 6.0 mg).

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 30-cm; 10-µm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 4000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 500 mL

**Tolerances:** NLT 70% (Q) of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) is dissolved.

**Test 2** (micronized glyburide): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.05 M phosphate buffer, pH 8.5 (6.8 g of monobasic potassium phosphate and 1.99 g of sodium hydroxide in 1 L of water, and adjust with diluted phosphoric acid or diluted sodium hydroxide to a pH of 8.5 ± 0.05); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Mobile phase:** Acetonitrile and water containing 5 g/L of monobasic ammonium phosphate (480:520)

**Standard stock solution:** Transfer 67 mg of USP Glyburide RS to a 500-mL volumetric flask, dissolve in 40 mL of methanol with sonication for 5 min, and dilute with *Medium* to volume.

**Standard solutions:** Dilute the *Standard stock solution* with *Medium* to obtain solutions having known concentrations of 0.0017 mg/mL (for Tablets labeled to contain 1.5 mg), 0.0034 mg/mL (for Tablets labeled to

contain 3 mg), 0.0047 mg/mL (for Tablets labeled to contain 4.5 mg), and 0.0067 mg/mL (for Tablets labeled to contain 6 mg).

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.5- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.0-mm  $\times$  25-cm; 10- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) is dissolved.

**Test 3 (micronized glyburide):** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** 0.05 M phosphate buffer, pH 7.5 (40.8 g of monobasic potassium phosphate and 9.4 g of sodium hydroxide, in 6 L of water, and adjust with diluted sodium hydroxide to a pH of  $7.5 \pm 0.1$ ); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Mobile phase:** Proceed as directed in the *Assay*.

**Diluent:** Acetonitrile and water (5:1)

**Standard stock solution:** 0.67 mg/mL of USP Glyburide RS in *Diluent*

**Standard solution:** 6.7  $\mu$ g/mL of USP Glyburide RS in *Medium* from *Standard stock solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection size:** 75  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) is dissolved.

**Test 4 (nonmicronized glyburide):** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** 0.05 M borate buffer, pH 8.0, with 0.014 M hexadecyltrimethylammonium bromide (prepared by dissolving about 180.0 g of hexadecyltrimethylammonium bromide, 55.6 g of boric acid, 67.1 g of potassium chloride, and 2.8 g of sodium hydroxide in 1500 mL of water at 50° under vigorous stirring for several hours, cooling to room temperature, diluting with water to 2000 mL, adjusting with diluted hydrochloric acid or diluted sodium hydroxide to a pH of  $8.0 \pm 0.05$ , and diluting 50 mL of this solution with water to 900 mL); 900 mL

**Apparatus 1:** 50 rpm

**Time:** 45 min

**Mobile phase:** Acetonitrile and water (11:9) containing 5.2 g of monobasic ammonium phosphate for each 2 L

**Standard stock solution:** 0.27 mg/mL of USP Glyburide RS in alcohol. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

**Standard solution:** 2.8  $\mu$ g/mL of USP Glyburide RS in *Medium*, from the *Standard stock solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 5- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection size:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim (mg)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) is dissolved.

**Test 5 (micronized glyburide):** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** 0.05 M phosphate buffer, pH 7.5 (40.8 g of monobasic potassium phosphate and 9.4 g of sodium hydroxide, in 6 L of water, and adjust with diluted sodium hydroxide to a pH of  $7.5 \pm 0.1$ ); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** USP Glyburide RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Mobile phase, Diluent, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 3*.

**Tolerances:** NLT 75% (Q) of the labeled amount of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) is dissolved.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** <11>  
USP Glyburide RS

## Glyburide and Metformin Hydrochloride Tablets

**DEFINITION**

Glyburide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glyburide ( $C_{23}H_{28}ClN_3O_5S$ ) and metformin hydrochloride ( $C_4H_{11}N_5 \cdot HCl$ ).

**IDENTIFICATION**

- **A. GLYBURIDE:** The retention time of the glyburide peak of the *Sample solution* corresponds to that of the major peak of the *Standard solution*, as obtained in the Assay for Glyburide.
- **B. METFORMIN HYDROCHLORIDE:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Metformin Hydrochloride.

**ASSAY**• **GLYBURIDE**

**Solution A:** 28.8 g/L of monobasic ammonium phosphate

**Mobile phase:** Acetonitrile and *Solution A* (40:60). Adjust with 1 N sodium hydroxide to a pH of 5.3.

**Diluent:** Acetonitrile and water (50:50)

**Standard stock solution:** 0.25 mg/mL of USP Glyburide RS prepared as follows: Dissolve first in the acetonitrile, using 50% of the final volume, and then dilute with water to volume.

**Standard solution:** 0.025 mg/mL of USP Glyburide RS in *Diluent*, from the *Standard stock solution*

**System suitability solution 1:** Prepare a solution containing 0.025 mg/mL of USP Glyburide Related Compound A RS in *Diluent*. Transfer 50  $\mu$ L of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

**System suitability solution 2:** 5.0 mg/mL of USP Metformin Hydrochloride RS in *System suitability solution 1*

**Sample solution:** Dissolve NLT 5 Tablets in *Diluent* by stirring with a magnetic stirring bar for a least 1 h. Dilute to obtain a solution containing 0.025 mg/mL of glyburide, based on the label claim. Centrifuge a portion of this solution at 3000 rpm for 10 min and use the clear supernatant. [NOTE—Retain a portion of this solution for the Assay for Metformin Hydrochloride.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection size:** 100  $\mu$ L

**System suitability**

**Sample:** *System suitability solution 2*

[NOTE—The relative retention time for the peak due to glyburide related compound A is about 0.30 with respect to glyburide.]

**Suitability requirements**

**Capacity factor, k':** NLT 7 for the peak due to glyburide

**Column efficiency:** NLT 3000 theoretical plates for the peak due to glyburide

**Relative standard deviation:** NMT 1.5% for the peak due to glyburide

**Relative standard deviation:** NMT 10% for the peak due to glyburide related compound A

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms for about 1.25 times the retention time of the glyburide peak. Calculate the percentage of  $C_{23}H_{28}ClN_3O_5S$  in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of glyburide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of glyburide label claim

• **METFORMIN HYDROCHLORIDE**

**Solution A:** Transfer 1.0 g each of sodium heptanesulfonate and sodium chloride to a 2000-mL volumetric flask. Add 1800 mL of water, and adjust with 0.06 M phosphoric acid to a pH of 3.85. Dilute with water to volume.

**Mobile phase:** Acetonitrile and *Solution A* (10:90)

**Diluent:** Acetonitrile and water (1:40)

**Standard solution:** 0.25 mg/mL of USP Metformin Hydrochloride RS in *Diluent*. [NOTE—Sonicate to achieve complete dissolution, if necessary.]

**System suitability stock solution:** 25  $\mu$ g/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*

**System suitability solution:** Transfer 0.5 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** Dilute with water a portion of the retained *Sample solution* from the Assay for Glyburide to obtain 0.25 mg/mL of metformin hydrochloride based on the label claim.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 218 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 5  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for metformin related compound B, metformin, and metformin related compound C are about 0.86, 1.0, and 2.1–2.3, respectively. (Metformin related compound C can have a variable retention time.)]

**Suitability requirements**

**Resolution:** NLT 1.5 between metformin related compound B and metformin

**Tailing factor:** 0.8–2.0 for the metformin peak

**Relative standard deviation:** NMT 1.5% for the metformin peak and NMT 10% for each of the peaks due to metformin related compound B and metformin related compound C.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_4H_{11}N_5 \cdot HCl$  in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

- $C_s$  = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0% of metformin label claim

**PERFORMANCE TESTS****• DISSOLUTION <711>****Glyburide**

**Medium:** 0.05 M boric acid and 0.05 M potassium chloride solution. Prepare by dissolving 3.09 g of boric acid and 3.73 g of potassium chloride in 250 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.5, and dilute with water to 1 L; 500 mL.

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Standard solution:** Transfer 10 mg of USP Glyburide RS to a 100-mL volumetric flask. Dissolve in 20 mL of acetonitrile, and dilute with *Medium* to volume. Dilute further with *Medium* to obtain a solution having a glyburide concentration, in mg/mL, of L/500 where L is the label claim, in mg, of glyburide.

**Sample solution:** Sample per *Dissolution <711>*. Pass a portion of the solution under test through a 0.45- $\mu$ m polypropylene filter or a 1- $\mu$ m glass fiber filter. Dilute with *Medium*, if necessary.

**Solution A:** 28.7 mg/mL of monobasic ammonium phosphate in water

**Mobile phase:** *Solution A* and acetonitrile (1:1). Adjust with 1 N sodium hydroxide to a pH of 5.3.

**Chromatographic system**

(See *Chromatography <621>*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 200  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 5000

**Tailing factor:** 0.8–2.0

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of  $C_{23}H_{28}ClN_3O_5S$  dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of glyburide in the *Sample solution* (mg/mL)

**Tolerances:** NLT 85% (Q) of the labeled amount of glyburide is dissolved.

**Metformin hydrochloride**

**Medium:** 0.05 M phosphate buffer, pH 6.8. Prepare by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 0.2 N sodium hydroxide to a pH of  $6.8 \pm 0.1$ ; 1000 mL.

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** Dissolve a quantity of USP Metformin Hydrochloride RS in *Medium*. Dilute further, if necessary, with *Medium* to obtain a solution having a metformin hydrochloride concentration, in mg/mL, of L/1000 where L is the label claim, in mg, of metformin hydrochloride.

**Sample solution:** Sample per *Dissolution <711>*. Pass a portion of the solution under test through a 0.45- $\mu$ m polypropylene filter or a 1- $\mu$ m glass fiber filter. Dilute with *Medium*, if necessary.

**Spectrometric conditions**

(See *Spectrophotometry and Light-Scattering <851>*.)

**Mode:** UV-Vis

**Analytical wavelength:** 232 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_4H_{11}N_5 \cdot HCl$  dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

**Tolerances:** NLT 85% (Q) of the labeled amount of metformin hydrochloride is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* for metformin hydrochloride and for *Content Uniformity* for glyburide

**IMPURITIES****Organic Impurities****• PROCEDURE 1: GLYBURIDE**

**Solution A, Mobile phase, Diluent, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Glyburide*.

**Standard solution:** Dilute 1.0 mL of the *Standard solution* from the *Assay for Glyburide* with *Diluent* to 100 mL.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each glyburide impurity in the Tablets:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of glyburide in the *Sample solution* (mg/mL)  
 $F$  = relative response factor, use 0.8 for glyburide related compound A, and use 1.0 for all other impurities

**Acceptance criteria**

[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

**Glyburide related compound A:** NMT 1.0%

**Any other individual impurities:** NMT 0.2%

**Total impurities:** NMT 0.50%, excluding glyburide related compound A

**• PROCEDURE 2: METFORMIN HYDROCHLORIDE**

**Solution A, Mobile phase, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Metformin Hydrochloride*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- $r_U$  = peak response for each impurity from the *Sample solution*  
 $r_T$  = sum of the responses of all peaks from the *Sample solution*

**Acceptance criteria**

[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

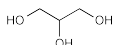
**Individual impurities:** NMT 0.1%

**Total impurities:** NMT 0.5 %

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Glyburide RS
  - USP Glyburide Related Compound A RS
  - (4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonamide.
  - USP Metformin Hydrochloride RS
  - USP Metformin Related Compound B RS
  - 1-Methylbiguanide.
  - C<sub>3</sub>H<sub>9</sub>N<sub>5</sub> 115.14
  - USP Metformin Related Compound C RS
  - Dimethylmelamine, or *N,N*-dimethyl-[1,3,5]triazine-2,4,6-triamine.
  - C<sub>5</sub>H<sub>10</sub>N<sub>6</sub> 154.17

## Glycerin



C<sub>3</sub>H<sub>8</sub>O<sub>3</sub> 92.09  
1,2,3-Propanetriol;  
Glycerol [56-81-5].

**DEFINITION**

Glycerin contains NLT 99.0% and NMT 101.0% of C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>, calculated on the anhydrous basis.

**IDENTIFICATION** [NOTE—Compliance is determined by meeting the requirements for *Identification* tests A, B, and C.]

- **A. INFRARED ABSORPTION** (197F)

- **B. LIMIT OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL**

**Standard solution:** 2.0 mg/mL of USP Glycerin RS, 0.050 mg/mL of USP Ethylene Glycol RS, 0.050 mg/mL of USP Diethylene Glycol RS, and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

**Sample solution:** 50 mg/mL of Glycerin and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 30-m fused-silica analytical column coated with 3.0-μm G43 stationary phase, and a deactivated split liner with glass wool

**Temperature**

**Injector:** 220°

**Detector:** 250°

**Column:** See the temperature program table.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
120	50	220	6

**Carrier gas:** Helium

**Injection size:** 1.0 μL

**Flow rate:** 4.5 mL/min

**Injection type:** Split ratio, about 10:1

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethylene glycol, 2,2,2-trichloroethanol, diethylene glycol, and glycerin are about 0.3, 0.6, 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between diethylene glycol and glycerin

**Analysis**

**Sample:** *Sample solution*

**Acceptance criteria:** If a peak at the retention times for the diethylene glycol or ethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is NMT the peak response ratio for diethylene glycol or ethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*; NMT 0.10% each for diethylene glycol and ethylene glycol is found.

- **C.** Examine the chromatograms obtained in *Identification* test B. The retention time of the glycerin peak of the *Sample solution* corresponds to that obtained in the *Standard solution*.

**ASSAY**

- **PROCEDURE**

**Sodium periodate solution:** Dissolve 60 g of sodium metaperiodate in sufficient water containing 120 mL of 0.1 N sulfuric acid to make 1000 mL. Do not heat to dissolve the periodate. If the solution is not clear, pass through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container. Test the suitability of this solution as follows. Pipet 10 mL into a 250-mL volumetric flask, and dilute with water to volume. To 550 mg of Glycerin dissolved in 50 mL of water, add 50 mL of the diluted periodate solution with a pipet. For a blank, pipet 50 mL of the solution into a flask containing 50 mL of water. Allow the solutions to stand for 30 min, then to each add 5 mL of hydrochloric acid and 10 mL of potassium iodide TS, and rotate to mix. Allow to stand for 5 min, add 100 mL of water, and titrate with 0.1 N sodium thiosulfate, shaking continuously and adding 3 mL of starch TS as the endpoint is approached. The ratio of the volume of 0.1 N sodium thiosulfate required for the glycerin–periodate mixture to that required for the blank should be between 0.750 and 0.765.

**Analysis:** Transfer 400 mg of Glycerin to a 600-mL beaker, dilute with 50 mL of water, add bromothymol blue TS, and acidify with 0.2 N sulfuric acid to a definite green or greenish yellow color. Neutralize with 0.05 N sodium hydroxide to a definite blue endpoint, free from green color. Prepare a blank containing 50 mL of water, and neutralize in the same manner. Pipet 50 mL of the *Sodium periodate solution* into each beaker, mix by swirling gently, cover with a watch glass, and allow to stand for 30 min at room temperature (not exceeding 35°) in the dark or in subdued light. Add 10 mL of a mixture of equal volumes of ethylene glycol and water, and allow to stand for 20 min. Dilute each solution with water to 300 mL, and titrate with 0.1 N sodium hydroxide VS to a pH of 8.1 ± 0.1 for the specimen under assay and 6.5 ± 0.1 for the blank, using a pH meter. Each mL of 0.1 N sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>.

**Acceptance criteria:** 99.0%–101.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **CHLORIDE AND SULFATE**, *Chloride* <221>: A 7.0-g portion shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (NMT 10 ppm).
- **CHLORIDE AND SULFATE**, *Sulfate* <221>: A 10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (NMT 20 ppm).
- **HEAVY METALS** <231>  
**Analysis:** Mix 4.0 g with 2 mL of 0.1 N hydrochloric acid, and dilute with water to 25 mL.  
**Acceptance criteria:** NMT 5 ppm
- **RESIDUE ON IGNITION** <281>: Heat 50 g in an open, shallow 100-mL porcelain dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 0.5 mL of sulfuric acid, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.01%).

**Organic Impurities****• PROCEDURE 1: RELATED COMPOUNDS**

**System suitability solution:** 0.5 mg/mL each of USP Diethylene Glycol RS and USP Glycerin RS

**Sample solution:** 50 mg/mL of Glycerin

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 30-m fused-silica analytical column coated with 3.0-μm G43 stationary phase, and an inlet liner having an inverted cup or spiral structure

**Temperature**

**Injector:** 220°

**Detector:** 250°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	—
100	7.5	220	4

**Carrier gas:** Helium

**Injection size:** 0.5 μL

**Linear velocity:** 38 cm/s

**Injection type:** Split ratio, about 10:1

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 7.0 between diethylene glycol and glycerin

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity, excluding any solvent peaks and diethylene glycol, in the portion of Glycerin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of the responses of all the peaks from the *Sample solution*

**Acceptance criteria**

**Individual impurities:** NMT 0.1%

**Total impurities:** NMT 1.0%

**• PROCEDURE 2: LIMIT OF CHLORINATED COMPOUNDS**

**Sample:** 5 g of Glycerin

**Analysis:** Transfer the *Sample* into a dry, round-bottom, 100-mL flask. Add 15 mL of morpholine, and connect the flask by a ground joint to a reflux condenser. Reflux gently for 3 h. Rinse the condenser with 10 mL of water, receiving the washings in the flask, and cau-

tiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.50 mL of silver nitrate TS, and dilute with water to 50.0 mL.

**Acceptance criteria:** The turbidity is not greater than that of a blank to which 0.20 mL of 0.020 N hydrochloric acid has been added, the refluxing being omitted (NMT 30 ppm of Cl).

**• PROCEDURE 3: FATTY ACIDS AND ESTERS**

**Sample solution:** Mix 50 g of Glycerin with 50 mL of freshly boiled water and 5 mL of 0.5 N sodium hydroxide VS. Boil the mixture for 5 min, cool, and add phenolphthalein TS.

**Analysis:** Titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Titrimetry* <541>, *Residual Titrations*).

**Acceptance criteria:** NMT 1 mL of 0.5 N sodium hydroxide is consumed.

**SPECIFIC TESTS**

- **COLOR:** When viewed downward against a white surface in a 50-mL color-comparison tube, the color is not darker than the color of a standard made by diluting 0.40 mL of ferric chloride CS with water to 50 mL and similarly viewed in a color-comparison tube of approximately the same diameter and color as that containing the Glycerin.
- **SPECIFIC GRAVITY** <841>: NLT 1.249
- **WATER DETERMINATION**, *Method I* <921>: NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

**• USP REFERENCE STANDARDS <11>**

USP Diethylene Glycol RS

USP Ethylene Glycol RS

USP Glycerin RS

1,2,3-Propanetriol.

C<sub>3</sub>H<sub>8</sub>O<sub>3</sub> 92.10

**Glycerin Ophthalmic Solution**

» Glycerin Ophthalmic Solution is a sterile, anhydrous solution of Glycerin, containing not less than 98.5 percent of glycerin (C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>). It may contain one or more suitable antimicrobial preservatives. [NOTE—In the preparation of this Ophthalmic Solution, use Glycerin that has a low water content, in order that the Ophthalmic Solution may comply with the *Water* limit. This may be ensured by using Glycerin having a specific gravity of not less than 1.2607, corresponding to a concentration of 99.5 percent.]

NOTE—Do not use the Ophthalmic Solution if it contains crystals, or is cloudy or discolored, or contains a precipitate.

**Packaging and storage**—Preserve in tight containers of glass or plastic, containing not more than 15 mL, protected from light. The container or individual carton is sealed and tamper-proof so that sterility is ensured at time of first use.

**USP Reference standards <11>—**

USP Glycerin RS

**Identification**—It responds to the *Identification* test under *Glycerin*.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 4.5 and 7.5, determined potentiometrically in a solution prepared by the addition of 5 mL of Sodium Chloride Injection to 5 mL of Ophthalmic Solution.

**Water, Method I** (921): not more than 1.0%.

**Assay**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 3 g of glycerin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 3-mL portion to a conical flask, add 100.0 mL of a solution of potassium periodate (prepared by dissolving 3 g of potassium periodate in about 500 mL of warm water, cooling to room temperature, and then diluting with water to 1000 mL), swirl, and allow to stand at room temperature for 10 minutes. Add 4 g of sodium bicarbonate and 2 g of potassium iodide, and titrate immediately with 0.1 N potassium arsenite VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of the Ophthalmic Solution, and note the difference in volumes required. Each mL of 0.1 N potassium arsenite is equivalent to 2.303 mg of glycerin ( $C_3H_8O_3$ ).

## Glycerin Oral Solution

» Glycerin Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of glycerin ( $C_3H_8O_3$ ).

**Packaging and storage**—Preserve in tight containers.

**Identification**—Heat a few drops with about 500 mg of potassium bisulfate in a test tube: pungent vapors of acrolein are evolved.

**pH** (791): between 5.5 and 7.5.

**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to about 3 g of glycerin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 3-mL portion to a conical flask, add 100.0 mL of a solution of potassium periodate (prepared by dissolving 3 g of potassium periodate in about 500 mL of warm water, cooling to room temperature, and then diluting with water to 1000 mL), swirl, and allow to stand at room temperature for 10 minutes. Add 4 g of sodium bicarbonate and 2 g of potassium iodide, and titrate immediately with 0.1 N potassium arsenite VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of the Oral Solution, and note the difference in volumes required. Each mL of 0.1 N potassium arsenite is equivalent to 2.303 mg of glycerin ( $C_3H_8O_3$ ).

## Glycerin Suppositories

» Glycerin Suppositories contain Glycerin solidified with Sodium Stearate. [NOTE—If preferred, the Sodium Stearate for Glycerin Suppositories may be prepared during the making of the Suppositories by the direct reaction between Stearic Acid and Sodium Bicarbonate, Sodium Carbonate, or Sodium Hydroxide, these being taken in the correct proportion.] Glycerin Suppositories contain not less than 75.0 percent and not more than 90.0 percent, by weight, of glycerin ( $C_3H_8O_3$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Stearic Acid RS

### Identification—

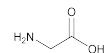
**A:** Dissolve 1 g of sodium borate in 100 mL of water, add 25 drops of phenolphthalein TS, and mix. To a test tube containing 0.5 mL of this solution add 2 drops of 1 Suppository that has been melted: the pink solution becomes colorless, and when it is heated the pink color reappears.

**B:** Disperse 12 Suppositories in about 125 mL of water in a 250-mL beaker on a hot plate. Cool, add 1.5 mL of hydrochloric acid, and pour the mixture into a 250-mL separator. Extract with 75 mL of hexanes, discarding the lower aqueous layer and collecting the organic layer in a beaker. Evaporate with the aid of a steam bath to near dryness: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a mineral oil dispersion of USP Stearic Acid RS.

**Water, Method I** (921): not more than 15.0%.

**Assay**—Transfer an accurately weighed quantity of Suppositories, equivalent to about 250 mg of glycerin, to a 250-mL volumetric flask. Dissolve in water, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 250-mL conical flask, and add 50.0 mL of a reagent prepared by mixing 40 mL of dilute sulfuric acid (1 in 20) with 60 mL of potassium periodate solution (1 in 1000) acidified with 3 to 5 drops of sulfuric acid. Heat the solution on a steam bath for 15 minutes, cool to room temperature, and add 1 g of potassium iodide. Allow the flask to stand for 5 minutes, and titrate with 0.02 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of Suppositories, and note the difference in volumes required. Each mL of the difference in volume of 0.02 N sodium thiosulfate consumed is equivalent to 0.4604 mg of glycerin ( $C_3H_8O_3$ ).

## Glycine



$C_2H_5NO_2$   
Glycine [56-40-6].

75.07

### DEFINITION

Glycine contains NLT 98.5% and NMT 101.5% of glycine ( $C_2H_5NO_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)

### ASSAY

- **PROCEDURE**

**Sample:** 150 mg of Glycine

**Blank:** 100 mL of glacial acetic acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 100 mL of glacial acetic acid, and add 1 drop of crystal violet TS. Titrate with the *Titrant* to a green endpoint. Perform the *Blank* determination.

Calculate the percentage of glycine ( $C_2H_5NO_2$ ) in the *Sample* taken:

$$\text{Result} = \{(V_s - V_b) \times N \times F/W\} \times 100$$

$V_s$  = Titrant volume consumed by the *Sample* (mL)

$V_b$  = Titrant volume consumed by the *Blank* (mL)

*N* = actual normality of the *Titrant* (mEq/mL)  
*F* = equivalency factor, 75.07 mg/mEq  
*W* = *Sample weight* (mg)  
**Acceptance criteria:** 98.5%–101.5% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **CHLORIDE AND SULFATE, Chloride** (221)  
*Standard solution:* 0.10 mL of 0.020 N hydrochloric acid  
*Sample:* 1 g of Glycine  
*Acceptance criteria:* NMT 0.007%
- **CHLORIDE AND SULFATE, Sulfate** (221)  
*Standard solution:* 0.20 mL of 0.020 N sulfuric acid  
*Sample:* 3 g of Glycine  
*Acceptance criteria:* NMT 0.0065%
- **HEAVY METALS, Method I** (231): NMT 20 ppm
- **HYDROLYZABLE SUBSTANCES**  
*Sample solution:* 100 mg/mL of Glycine  
*Analysis:* Boil 10 mL of the *Sample solution* for 1 min, and set aside for 2 h.  
*Acceptance criteria:* The solution appears as clear and as mobile as 10 mL of the same solution that has not been boiled.

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.2% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Glycine RS

## Glycine Irrigation

» Glycine Irrigation is a sterile solution of Glycine in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of glycine (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>).

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

**USP Reference standards** (11)—

USP Endotoxin RS  
 USP Glycine RS

**Identification**—Evaporate a portion of Irrigation to dryness, and obtain the IR absorption spectrum of a mineral oil dispersion of the residue: it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Glycine RS.

**Bacterial endotoxins** (85)—It contains not more than 0.5 Endotoxin Unit per mL.

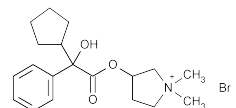
**pH** (791): between 4.5 and 6.5, determined potentiometrically on a portion to which 0.3 mL of saturated potassium chloride has been added for each 100 mL.

**Other requirements**—It meets the requirements under *Injections* (1), except that the container in which the solution is packaged may be designed to empty rapidly and may exceed 1000 mL in capacity.

**Assay**—Dilute an accurately measured volume of Irrigation, equivalent to about 150 mg of glycine, with water to 25 mL, and add 10 mL of formaldehyde TS, previously adjusted to a pH of 9.0, and 5 drops of mixed indicator solution (prepared by dissolving 75 mg of phenolphthalein and 25 mg of thymol blue in a mixture of equal volumes of alcohol and water to make 100 mL). Titrate with 0.1 N sodium hydroxide VS until the yellow color disappears and a

faint violet color appears. Each mL of 0.1 N sodium hydroxide is equivalent to 7.507 mg of glycine (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>).

## Glycopyrrolate



C<sub>19</sub>H<sub>28</sub>BrNO<sub>3</sub> 398.33  
 Pyrrolidinium, 3-[(*SR*)-(cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethyl-, [*RS*-] bromide;  
 (*RS*)-[3-(*SR*)-Hydroxy-1,1-dimethylpyrrolidinium bromide] α-cyclopentylmandelate [596-51-0].

**DEFINITION**

Glycopyrrolate contains NLT 98.0% and NMT 102.0% of C<sub>19</sub>H<sub>28</sub>BrNO<sub>3</sub>, calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Bromide** (191)  
*Sample solution:* 25 mg/mL  
*Acceptance criteria:* Meets the requirements

**ASSAY****PROCEDURE**

**Buffer:** Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (20:15:65)

**Standard solution:** 0.1 mg/mL of USP Glycopyrrolate RS in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Glycopyrrolate in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 222 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection size:** 50 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glycopyrrolate

(C<sub>19</sub>H<sub>28</sub>BrNO<sub>3</sub>) in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of Glycopyrrolate from the *Sample solution*

*r<sub>S</sub>* = peak response of glycopyrrolate from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)



Acceptance criteria: 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

- **ORGANIC IMPURITIES**

**Buffer:** Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

**Diluent:** Prepare a solution of 1.0 g of anhydrous sodium sulfate, 6.8 g of monobasic potassium phosphate, and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, 150 mL of methanol, and 200 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.8.

**Solution A:** Acetonitrile, methanol, and *Buffer* (20:15:65)

**Solution B:** Acetonitrile, methanol, and *Buffer* (50:15:35)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

**Standard solution:** 1.5 µg/mL each of USP Glycopyrrolate RS, USP Glycopyrrolate Related Compound A RS, USP Glycopyrrolate Related Compound B RS, and USP Glycopyrrolate Related Compound C RS in *Diluent*. Sonicate, if necessary, to facilitate dissolution.

**Sample solution:** 1.0 mg/mL of Glycopyrrolate in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 222 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between glycopyrrolate and glycopyrrolate related compound B

**Tailing factor:** NMT 2.0 for the glycopyrrolate peak

**Relative standard deviation:** NMT 6.0% for each peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glycopyrrolate related compounds A, B, and C in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each related compound from the *Sample solution*

$r_S$  = peak response of the corresponding related compound from the *Standard solution*

$C_S$  = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

$C_U$  = concentration of glycopyrrolate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of glycopyrrolate from the *Standard solution*

$C_S$  = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Nitroisophthalic acid <sup>a</sup>	0.45	0.15
Glycopyrrolate	1.00	—
Glycopyrrolate base <sup>b</sup>	1.14	0.15
Cyclopentylmandelic acid <sup>c</sup>	2.68	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.50

<sup>a</sup> Glycopyrrolate related compound A.

<sup>b</sup> Glycopyrrolate related compound B.

<sup>c</sup> Glycopyrrolate related compound C.

- **LIMIT OF ERYTHRO ISOMER**

**Buffer:** 2.8 g/L of monobasic sodium phosphate in water. Adjust with a sodium hydroxide solution (1 in 10) to a pH of 6.50 ± 0.05.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (50:10:40)

**System suitability solution:** 40 µg/mL each of USP Glycopyrrolate Erythro Isomer RS and USP Glycopyrrolate RS in *Mobile phase*

**Standard solution:** 10 µg/mL of USP Glycopyrrolate RS in *Mobile phase*

**Sample solution:** 500 µg/mL of Glycopyrrolate in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 222 nm

**Column:** 4.0-mm × 25-cm; 5-µm packing L45

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.2 between the erythro isomer and glycopyrrolate, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 6.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of erythro isomer in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the erythro isomer from the *Sample solution*

$r_S$  = peak response of glycopyrrolate from the *Standard solution*

$C_S$  = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythro isomer (R,R/S,S-glycopyrrolate) <sup>a</sup>	0.89	0.4
Glycopyrrolate	1.00	—

<sup>a</sup> USP Glycopyrrolate Erythro Isomer RS.

#### SPECIFIC TESTS

- **Loss on Drying** (731): Dry at 105° for 3 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Glycopyrrolate RS
  - USP Glycopyrrolate Related Compound A RS  
5-Nitrobenzene-1,3-dicarboxylic acid.  
 $C_8H_5NO_6$  211.13
  - USP Glycopyrrolate Related Compound B RS  
1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate.  
 $C_{18}H_{25}NO_3$  303.40
  - USP Glycopyrrolate Related Compound C RS  
2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.  
 $C_{13}H_{16}O_3$  220.26
  - USP Glycopyrrolate Erythro Isomer RS  
(R,S)-3-[(R,S)-2-cyclopentyl-2-hydroxy-2-phenylacetoxyl]-1,1-dimethylpyrrolidinium bromide.  
 $C_{19}H_{28}BrNO_3$  398.33

## Glycopyrrolate Injection

» Glycopyrrolate Injection is a sterile solution of Glycopyrrolate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of glycopyrrolate ( $C_{19}H_{28}BrNO_3$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Glycopyrrolate RS

**Identification**—

**Spray reagent**—Dissolve 2 g of bismuth subnitrate in a solution consisting of 100 mL of water and 25 mL of glacial acetic acid (Solution A). Dissolve 40 g of potassium iodide in 100 mL of water (Solution B). Add 10 mL of Solution A and 10 mL of Solution B to a solution consisting of 100 mL of water and 20 mL of glacial acetic acid, and mix.

**Procedure**—Pipet an amount of Injection equivalent to about 1 mg of glycopyrrolate into a 10-mL volumetric flask, dilute with water to volume, and mix to obtain the test solution. Prepare a Standard solution of USP Glycopyrrolate RS in water containing about 0.1 mg of glycopyrrolate per mL. Apply 30  $\mu$ L of the test solution and 30  $\mu$ L of the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and

water (3:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to air-dry. Spray the plate with *Spray reagent*, and allow to air-dry: the  $R_f$  value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

**Bacterial endotoxins** (85)—It contains not more than 555.5 USP Endotoxin Units per mg of glycopyrrolate.

**pH** (791): between 2.0 and 3.0.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay—

**Mobile phase**—Dissolve 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-pentanesulfonate in 615 mL of water in a 1000-mL volumetric flask. Add 3.0 mL of 1 N sulfuric acid, 235 mL of acetonitrile, and 150 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Glycopyrrolate RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Resolution solution**—Prepare a solution of benzaldehyde in *Mobile phase* containing about 0.5 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.

**Assay preparation**—Dilute a volume of Injection, quantitatively if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.2 mg of glycopyrrolate per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 222-nm detector and a 3.9-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the benzaldehyde and glycopyrrolate peaks is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 35  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of glycopyrrolate ( $C_{19}H_{28}BrNO_3$ ) in each mL of the Injection taken by the formula:

$$C(L/D)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Glycopyrrolate RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg per mL, of glycopyrrolate in the Injection;  $D$  is the concentration, in mg per mL, of glycopyrrolate in the *Assay preparation*, on the basis of the labeled quantity and the extent of dilution; and  $r_U$  and  $r_S$  are the glycopyrrolate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Glycopyrrolate Tablets

#### DEFINITION

Glycopyrrolate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of glycopyrrolate ( $C_{19}H_{28}BrNO_3$ ).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (20:15:65)

**Standard solution:** 0.1 mg/mL of USP Glycopyrrolate RS in *Mobile phase*

**Sample solution:** 0.1 mg/mL of glycopyrrolate in *Mobile phase*. Prepare by transferring 10 Tablets to a suitable volumetric flask. Add *Mobile phase* to 50% of the volume of the flask, and sonicate for 10 min or until the Tablets disintegrate completely. Add *Mobile phase* to 75% of the volume of the flask, shake mechanically for 30 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of the solution, and pass the supernatant through a suitable filter, discarding the first few mL of the filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 222 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection size:** 50 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of glycopyrrolate (C<sub>19</sub>H<sub>28</sub>BrNO<sub>3</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of glycopyrrolate from the *Sample solution*

$r_S$  = peak response of glycopyrrolate from the *Standard solution*

$C_S$  = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS****• DISSOLUTION <711>**

**Medium:** Water; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Buffer:** 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-pentanesulfonate in 620 mL of water

**Mobile phase:** Acetonitrile, methanol, *Buffer*, and 1 N sulfuric acid (200:180:620:3)

**Standard stock solution:** 0.2 mg/mL of USP Glycopyrrolate RS in *Medium*. A small volume of methanol, not exceeding 20% of the final volume, can be used to solubilize glycopyrrolate.

**Standard solution:** (L/500) mg/mL of glycopyrrolate in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet. Prepare this solution fresh, and refrigerate immediately at 5°.

**Sample solution:** Pass a portion of the solution under test through a suitable filter, discarding the first few mL of the filtrate. Refrigerate the samples at 5°.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Temperature**

**Column:** 40°

**Sampler:** 5°

**Flow rate:** 1.2 mL/min

**Injection size:** 80 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

Calculate the average percentage of the labeled amount of glycopyrrolate dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of glycopyrrolate in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of glycopyrrolate (C<sub>19</sub>H<sub>28</sub>BrNO<sub>3</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

**Buffer:** Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

**Diluent:** Prepare a solution of 1.0 g of anhydrous sodium sulfate, 6.8 g of monobasic potassium phosphate, and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, 150 mL of methanol, and 200 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.8.

**Solution A:** Acetonitrile, methanol, and *Buffer* (20:15:65)

**Solution B:** Acetonitrile, methanol, and *Buffer* (50:15:35)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

**Standard solution:** 1.5 μg/mL each of USP Glycopyrrolate RS, USP Glycopyrrolate Related Compound B RS, and USP Glycopyrrolate Related Compound C RS in *Diluent*. Sonicate, if necessary, to facilitate dissolution.

**Sample solution:** 500 μg/mL of glycopyrrolate in *Diluent*. Prepare by transferring the equivalent of 25 mg of glycopyrrolate from a portion of NLT 20 powdered Tablets to a 50-mL volumetric flask. Add 30 mL of *Diluent*, sonicate for 10 min, shake mechanically for 30 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution, and pass the supernatant through a suitable filter, discarding the first few mL of the filtrate.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 222 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 1 mL/min**Injection size:** 50 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between glycopyrrolate and glycopyrrolate related compound B**Tailing factor:** NMT 2.0 for the glycopyrrolate peak**Relative standard deviation:** NMT 6.0% for the glycopyrrolate and glycopyrrolate related compound C peaks**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of glycopyrrolate related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of glycopyrrolate related compound C from the *Sample solution* $r_S$  = peak response of glycopyrrolate related compound C from the *Standard solution* $C_S$  = concentration of USP Glycopyrrolate Related Compound C RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of glycopyrrolate from the *Standard solution* $C_S$  = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 2*.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Nitroisophthalic acid <sup>a</sup>	0.45	— <sup>d</sup>
Glycopyrrolate	1.00	—
Glycopyrrolate base <sup>b</sup>	1.14	— <sup>d</sup>
Cyclopentylmandelic acid <sup>c</sup>	2.68	0.5
Any other individual impurity	—	0.2
Total impurities	—	1.2

<sup>a</sup> Glycopyrrolate related compound A.<sup>b</sup> Glycopyrrolate related compound B.<sup>c</sup> Glycopyrrolate related compound C.<sup>d</sup> Disregard the peaks due to 5-nitroisophthalic acid and glycopyrrolate base, because these are process impurities and are controlled in the drug substance monograph.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

- USP REFERENCE STANDARDS** <11>

USP Glycopyrrolate RS

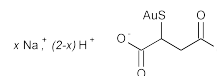
USP Glycopyrrolate Related Compound B RS

1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate.

C<sub>18</sub>H<sub>25</sub>NO<sub>3</sub> 303.40

USP Glycopyrrolate Related Compound C RS

2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.

C<sub>13</sub>H<sub>16</sub>O<sub>3</sub> 220.26**Gold Sodium Thiomalate**C<sub>4</sub>H<sub>3</sub>AuNa<sub>2</sub>O<sub>4</sub>S plus C<sub>4</sub>H<sub>4</sub>AuNaO<sub>4</sub>S 368.09

Butanedioic acid, mercapto-, monogold(1+) sodium salt.

Mercaptosuccinic acid, monogold(1+) sodium salt

[12244-57-4].

» Gold Sodium Thiomalate is a mixture of the mono- and di-sodium salts of gold thiomalic acid. It contains not less than 44.8 percent and not more than 49.6 percent of Au. It contains not less than 49.0 percent and not more than 52.5 percent of Au on a dry, alcohol- and glycerin-free basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Identification**—

**A:** To 2 mL of a solution (1 in 10) add 1 mL of calcium nitrate solution (1 in 10): a white precipitate is formed, and it dissolves in 2 N nitric acid and reappears upon the addition of ammonium acetate TS.

**B:** To 2 mL of a solution (1 in 10) add 4 mL of silver nitrate TS: a yellowish precipitate is formed, and it dissolves completely in an excess of 6 N ammonium hydroxide.

**C:** To 2 mL of a solution (1 in 10) add 1 mL of 6 N ammonium hydroxide and 1 mL of 30 percent hydrogen peroxide, evaporate in a porcelain dish, and ignite. Add 20 mL of water to the ignited residue, and filter: particles of gold remain on the filter. Separate portions of the filtrate meet the requirements of the tests for *Sodium* <191> and for *Sulfate* <191>.

**pH** <791>: between 5.8 and 6.5, in a solution (1 in 10).

**Loss on drying** <731>—Dry it at 60° and at a pressure not exceeding 5 mm of mercury for 2 hours: it loses not more than 8.0% of its weight.

**Limit of alcohol**—

**Standard solution**—Transfer 50 mg of dehydrated alcohol to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL to a 50-mL volumetric flask, and dilute with water to volume. This solution contains about 0.025 mg of alcohol per mL.

**Test solution**—Transfer about 50 mg of Gold Sodium Thiomalate, accurately weighed, to a 10-mL volumetric flask, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m fused-silica capillary column coated with a 3.0-μm film of phase G43. The column temperature is maintained at 40° for 8.5 minutes, then the temperature is increased at 30° per minute to 240°. The total chromatographic time is about 15 minutes. The injection

port and detector block temperatures are maintained at 150°. The carrier gas is helium, flowing at a rate of about 2 mL per minute, and the split flow rate is about 20 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*; the relative standard deviation for replicate injections is not more than 4.6%.

**Procedure**—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the portion of Gold Sodium Thiomalate taken by the formula:

$$100(C_a / C_b)(r_U / r_S)$$

in which  $C_a$  is the concentration, in mg per mL, of C<sub>2</sub>H<sub>5</sub>OH in the *Standard solution*;  $C_b$  is the concentration, in mg per mL, of Gold Sodium Thiomalate in the *Test solution*; and  $r_U$  and  $r_S$  are the alcohol peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 4.0% is found.

**Limit of glycerin**—[NOTE—This procedure is based on the absorption characteristics of a sodium–copper–glycerin complex. The stability of this complex prepared as directed herein is such that all measurements are to be taken within 1 hour. Thoroughly rinse all glassware used in this procedure with water to avoid large blank errors.]

**Sodium hydroxide solution**—Dissolve 23.6 g of sodium hydroxide in water to obtain 100 mL of solution.

**Cupric chloride solution**—Dissolve 3.8 g of cupric chloride in water to obtain 100 mL of solution.

**Glycerin standard solutions**—Dissolve an accurately weighed quantity of glycerin in water to obtain a solution having a known concentration of about 8 mg per mL. Pipet 1.0, 2.0, and 3.0 mL of this solution into a series of 10-mL volumetric flasks, followed by 4.0, 3.0, and 2.0 mL of water, respectively.

**Reagent blank**—Pipet 5.0 mL of water into a 10-mL volumetric flask.

**Test solution**—Dissolve about 400 mg Gold Sodium Thiomalate, accurately weighed, in 5.0 mL of water in a 10-mL volumetric flask.

**Procedure**—To each of the *Glycerin standard solutions*, the *Reagent blank*, and the *Test solution* add 1.0 mL of *Sodium hydroxide solution*, and mix. With vigorous shaking, and in increments of 0.1 mL, add *Cupric chloride solution*, checking for turbidity after each addition. After the solutions turn slightly turbid, add an excess of 0.1 mL of *Cupric chloride solution*, insert the stopper, and shake for 1 minute. Dilute with water to volume, and mix. Centrifuge the solutions in tapered, graduated 15-mL centrifuge tubes. The presence of 1 mm to 4 mm of copper hydroxide precipitate is observed. Using a suitable spectrophotometer equipped with 1-cm cells, and using water as a reference, measure the absorbance of the clear supernatant at a wavelength of 635 nm. Subtract the absorbance value of the *Reagent blank*, which is 0.040 or less, from the absorbance values of the *Glycerin standard solutions* and the *Test solution*. Plot the corrected absorbance readings of the *Glycerin standard solutions* against the corresponding weight of glycerin. From the standard curve so obtained, and the corrected absorbance of the *Test solution*, determine the weight of glycerin in the test specimen: not more than 5.5% is found.

**Assay**—Dissolve about 600 mg of Gold Sodium Thiomalate, accurately weighed, in water in a 25-mL volumetric flask, dilute with water to volume, and mix. Pass the entire solution through a clean, dry 0.5-µm filter into a clean, dry receiver. Pipet 20.0 mL of the filtrate into a 300-mL Kjeldahl flask, add 20 mL of nitric acid, and mix. To this solution add

15 mL of sulfuric acid slowly, with mixing. Heat over a low flame, gently at first, and then increase the heat until fumes of sulfur trioxide are evolved. Allow the flask and contents to cool to room temperature, add 30 mL of water slowly, with mixing, and 20 mL of hydrogen peroxide TS, again heat to fumes of sulfur trioxide, cool, and dilute with 30 mL of water. Pass the mixture through an ignited, tared filtering crucible, wash with water, heat the crucible and contents over a low flame to dry the precipitate, and ignite at 650 ± 50° to constant weight. The weight of the residue so obtained, multiplied by 1.25, is the weight of Au in the Gold Sodium Thiomalate taken.

## Gold Sodium Thiomalate Injection

» Gold Sodium Thiomalate Injection is a sterile solution of Gold Sodium Thiomalate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of Gold Sodium Thiomalate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store protected from light.

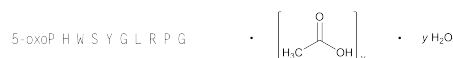
**Identification**—It responds to *Identification tests A and B* under *Gold Sodium Thiomalate*.

**pH** (791): between 5.8 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of gold sodium thiomalate, to a 300-mL Kjeldahl flask, and proceed as directed in the *Assay* under *Gold Sodium Thiomalate*, beginning with “add 20 mL of nitric acid.” The weight of the gold so obtained, multiplied by 2.116, represents the weight of Gold Sodium Thiomalate in the portion of Injection taken.

## Gonadorelin Acetate



C<sub>55</sub>H<sub>75</sub>N<sub>17</sub>O<sub>13</sub> · xC<sub>2</sub>H<sub>4</sub>O<sub>2</sub> · yH<sub>2</sub>O 1374.46  
Luteinizing hormone-releasing factor acetate (salt) hydrate.  
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycyl-L-leucyl-L-arginyl-L-prolylglycinamide acetate (salt) hydrate  
[52699-48-6; 33515-09-2].

» Gonadorelin Acetate is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains not less than 80 percent by weight of C<sub>55</sub>H<sub>75</sub>N<sub>17</sub>O<sub>13</sub>, the remainder being acetic acid and water.

**NOTE**—Gonadorelin Acetate is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.

**Packaging and storage**—Preserve in tight, well-sealed containers, protected from moisture. Store at a temperature of not more than 8°.

**Labeling**—Label it to indicate it is for veterinary use only.

**USP Reference standards** (11)—

USP Gonadorelin Acetate RS

$C_{55}H_{75}N_{17}O_{13} \cdot xC_2H_4O_2 \cdot yH_2O$  1182.3 (acetate free)

USP Gonadorelin Acetate Related Compound A RS

Gonadorelin free acid.

$C_{55}H_{74}N_{16}O_{14}$  1183.3

**Identification**—

**A:** The monoisotopic mass by *Mass Spectrometry* (736) is  $1181.6 \pm 1$  mass units.

**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Related compounds*.

**Specific rotation** (781S): between  $-54^\circ$  and  $-66^\circ$ , at  $20^\circ$ , calculated with reference to the peptide content determined in the *Assay*.

*Test solution:* 10 mg per mL, in 1% (v/v) acetic acid.

**Water**, *Method 1c* (921): not more than 7.0%, determined by directly introducing not less than 2 mg of the solid substance into the titrator.

**Limit of fluoride**—[NOTE—Use polypropylene vessels for preparation of solutions and standards.]

*Standard solutions*—Prepare a series of calibration standards containing 10, 1, 0.1, and 0.05 ppm fluoride dissolved in an ionic strength adjustment buffer suitable for the electrode in use (pH about 5).

*Test solution*—Dissolve between 3 and 5 mg of Gonadorelin Acetate in 1.375 mL of the same buffer as that used for the preparation of the *Standard solutions*.

*Procedure*—Using a fluoride ion-selective electrode connected to a pH/ion meter, measure the potential of each *Standard solution*, and plot the response versus the logarithm of the concentration. Determine the regression line using the least squares method. The test is considered valid if the slope of the curve is in the range of  $-54$  to  $-60$  mV per decade and the regression curve has a square of the correlation coefficient,  $r^2$ , not less than 0.995. From the calibration curve and the concentration of the *Test solution*, determine the amount of fluoride in the sample: not more than 0.1% (w/w) is found.

**Acetic acid and trifluoroacetic acid**—

*Solution A*—To 900 mL of water add 7.0 mL of phosphoric acid and 5.0 mL of concentrated ammonia. Mix, and dilute with water to 1000 mL, pass through a  $0.45\text{-}\mu\text{m}$  filter, and degas. Add 20 mL of methanol, mix, and degas for an additional 2 minutes.

*Solution B*—Prepare a degassed mixture of acetonitrile and water (1:1).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluent*—Dilute 5.0 mL of phosphoric acid with water to 1000 mL, and mix thoroughly.

*Trifluoroacetic acid stock solution*—Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Carefully add 670  $\mu\text{L}$  of trifluoroacetic acid to the flask, stopper immediately, and weigh. Dilute with water to volume.

*Standard solutions*—Accurately weigh out 150, 75, and 10 mg of sodium acetate trihydrate into three separate 100-mL volumetric flasks. Add 10 mL, 2 mL, and 100  $\mu\text{L}$ , respectively, of the *Trifluoroacetic acid stock solution* to the flasks, and dilute each with *Diluent* to the 100-mL mark.

Calculate the concentration, in mg per mL, of acetic acid in each *Standard solution* using the following equation:

$$0.00434W_A$$

in which  $W_A$  is the weight, in mg, of sodium acetate trihydrate taken. Calculate the concentration, in mg per mL, of trifluoroacetic acid in each *Standard solution* using the following equation:

$$0.0001(W_TV)$$

in which  $W_T$  is the weight, in mg, of trifluoroacetic acid used for preparation of the *Trifluoroacetic acid stock solution*; and  $V$  is the volume, in mL, of *Trifluoroacetic acid stock solution* used to prepare the *Standard solution*.

*Test solution*—Prepare duplicate samples by accurately weighing out two separate aliquots of about 4.0 mg of Gonadorelin Acetate and dissolving each with 1 mL of the *Diluent*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a  $4.6\text{-mm} \times 25\text{-cm}$  column containing  $5\text{-}\mu\text{m}$  packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	100	0	isocratic
5–6	100→0	0→100	linear
6–14	0	100	isocratic
14–15	0→100	100→0	return to initial
15–25	100	0	re-equilibration

Chromatograph the *Standard solutions*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates for the trifluoroacetic acid peak and not less than 10,000 for the acetic acid peak; and the relative standard deviation for six replicate injections of the most concentrated *Standard solution* is not more than 2.0%.

*Procedure*—Inject in duplicate equal volumes (about 20  $\mu\text{L}$ ) of each of the *Standard solutions* followed by the duplicate *Test solutions*. Plot the peak areas of each of the components in the *Standard solutions* versus concentration, in mg per mL, and determine the regression line using the least squares method. The test is considered valid if the regression curves for both acetic acid and trifluoroacetic acid have a square of the correlation coefficient,  $r^2$ , not less than 0.995. From the resulting graph, determine the percentages of acetic acid and trifluoroacetic acid in the *Test solution*: between 8% and 12.5% of acetic acid is found, and not more than 0.25% of trifluoroacetic acid is found.

**Related compounds**—

*Standard solution*—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

*System suitability solution*—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate Related Compound A RS in water to obtain a solution having a known concentration of about 0.5 mg per mL. Mix equal volumes of this solution and the *Standard solution*.

*Test solution*—Dissolve an accurately weighed quantity of Gonadorelin Acetate in water to obtain a solution having a known concentration of about 0.5 mg per mL.

**SYSTEM 1**—

*Solvent 1*—Mix 1 mL of trifluoroacetic acid with 1 L of water. Pass through a  $0.45\text{-}\mu\text{m}$  filter, and degas.

*Solvent 2*—Mix 1 mL of trifluoroacetic acid with 1 L of acetonitrile.

**Solution A**—Prepare a mixture of *Solvent 1* and *Solvent 2* (95:5).

**Solution B**—Prepare a mixture of *Solvent 2* and *Solvent 1* (60:40).

**Chromatographic system** (see *Chromatography* <621>)—The HPLC is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	91	9	initial
0–25	91→45	9→55	linear
25	45→91	55→9	return to initial
25–30	91	9	re-equilibrium

#### SYSTEM 2—

**Mobile phase**—Add 47 mL of phosphoric acid and 55 mL of triethylamine to 4 L of water, and adjust with phosphoric acid or triethylamine to a pH of 2.5, as appropriate. Pass through a 0.45-μm filter, and degas. Add acetonitrile to obtain a 13% (v/v) concentration of acetonitrile.

**Chromatographic system** (see *Chromatography* <621>)—The HPLC is equipped with a 215-nm detector and a 4.6-mm × 10-cm column that contains 5-μm packing L1. The flow rate is approximately 1.5 mL per minute using isocratic elution and having a run time of 50 minutes.

Using both *System 1* and *System 2* chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*. The *Standard solution* is used only to identify the gonadorelin acetate peak. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between gonadorelin acetate and gonadorelin acetate related compound A is not less than 2.0; the column efficiency is not less than 75,000 theoretical plates for *System 1* and not less than 3000 theoretical plates for *System 2*; the tailing factor is not more than 2.0 for both *System 1* and *System 2*; and the relative standard deviation for five replicate injections is not more than 2.0%.

**Procedure**—Inject equal volumes (about 20 μL) of each of the *Standard solution*, the *System suitability solution*, and the *Test solution*, followed by a co-injection of the *Test solution* with the *Standard solution*, into both *System 1* and *System 2*. Include blank injections between the different solutions. Integrate all peaks in order to obtain a baseline similar to that in the blank chromatograms, disregarding any peaks due to the solvent, counter-ion, and baseline artifacts. Using peak areas, and including all peaks greater than 0.05%, calculate the percentage of each impurity in the portion of Gonadorelin Acetate taken: not more than 1% of any single impurity is found, and not more than 2% of total impurities is found.

**Amino acid analysis**—Proceed as directed in the *Assay*. Express the content of each amino acid in μmoles, and calculate the total number of μmoles of Gonadorelin Acetate in the test sample as directed in the *Assay*. By dividing the number of μmoles of each amino acid by the total number of μmoles of Gonadorelin Acetate in the test sample, the relative proportions of amino acids are found: serine, 0.7 to 1.05; glutamic acid, 0.95 to 1.05; proline, 0.95 to 1.05; glycine, 1.9 to 2.1; leucine, 0.9 to 1.1; tyrosine, 0.7 to 1.05; histidine, 0.95 to 1.05; and arginine, 0.95 to 1.05. Isoleucine and lysine are absent; not more than traces of other amino acids except tryptophan are detected.

**Assay**—(see *Biotechnology-Derived Articles—Amino Acid Analysis* <1052>). [NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used.]

Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the

following amino acids: lysine, threonine, alanine, leucine, histidine, serine, valine, tyrosine, arginine, glutamic acid, methionine, phenylalanine, aspartic acid, proline, isoleucine, tryptophan, and cystine.

**Assay preparation** (see *Protein Hydrolysis, Method 1* <1052>)—Accurately weigh out between 0.4 and 1.0 mg of Gonadorelin Acetate in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis Solution* containing 4% phenol, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 hours. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter having a 0.45-μm porosity.

**Procedure**—Prepare a co-injection of the *Standard solution* and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak. Express the content of each amino acid in μmoles. The total number of μmoles of gonadorelin acetate in the test sample is calculated by summing the number of μmoles for glutamic acid, proline, glycine, leucine, tyrosine, histidine, and arginine, and dividing by eight. Calculate the percentage of C<sub>55</sub>H<sub>75</sub>N<sub>17</sub>O<sub>13</sub> in the portion of Gonadorelin Acetate taken by the formula:

$$118.23(N/W)$$

in which *N* is the total number of μmoles of gonadorelin acetate; and *W* is the weight of the sample in mg.

## Gonadorelin Hydrochloride

C<sub>55</sub>H<sub>75</sub>N<sub>17</sub>O<sub>13</sub> · 2HCl 1255.21  
Gonadorelin dihydrochloride [51952-41-1].

» Gonadorelin Hydrochloride is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains not less than 94.0 percent and not more than 104.0 percent of C<sub>55</sub>H<sub>75</sub>N<sub>17</sub>O<sub>13</sub> · 2HCl, calculated on the anhydrous basis.

**NOTE**—Gonadorelin Hydrochloride is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.

**Packaging and storage**—Preserve in tight, well-sealed containers.

**USP Reference standards** <11>—  
USP Gonadorelin Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between −57° and −63°, calculated on the anhydrous and chloride-free basis.

**Test solution**: 10 mg per mL, in water.

**Water**—[NOTE—Dry all glassware used in the following procedure at 105° for a minimum of 1 hour, and cool in a desiccator at room temperature. Store in a desiccator. Perform as many operations as possible in a low-humidity glove box.]

**Anhydrous methanol**—Wash about 150 g of 8- to 17-mesh type 3A molecular sieve with several 100-mL portions of methanol to remove the fine particles. Place the washed molecular sieve in a shallow glass dish, heat in an oven at 350° for 2 hours, and cool in a desiccator. Transfer the dry molecular sieve to a 1-L glass container, add about 700 mL

of methanol, insert a stopper, mix, and allow to stand in a desiccator for not less than 48 hours before using.

**Standard solutions**—Prepare solutions in *Anhydrous methanol* containing 0.4, 0.8, and 1.2 mg of distilled water per mL.

**Test solution**—[NOTE—Prepare immediately prior to use.] Transfer about 20 mg of Gonadorelin Hydrochloride, accurately weighed, to a vial, place a cap on the vial, add 800  $\mu$ L of *Anhydrous methanol* by means of a 1000- $\mu$ L gas-tight syringe, and swirl to mix.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a thermal conductivity detector and a 2-mm  $\times$  180-cm glass column packed with 80- to 100-mesh support S3. The column temperature is maintained at about 100°, and the injection port and detector temperatures are maintained at 130°. Helium is used as the carrier gas at a flow rate of about 30 mL per minute. Chromatograph the *Standard solution* containing 1.2 mg per mL, record the chromatograms, and measure the peak responses as directed for *Procedure*: the elution order is water, followed by a broad methanol peak; the retention time of the water peak is between 0.5 and 3 minutes; and the relative standard deviation for not less than three replicate injections is not more than 2.5%.

**Procedure**—Separately inject equal volumes (1 to 3  $\mu$ L) of each of the *Standard solutions*, *Test solution*, and *Anhydrous methanol* into the chromatograph, and measure the responses for the first (water) and second (methanol) major peaks, correcting the peak areas obtained from the *Test solution* and the *Standard solutions* against the *Anhydrous methanol* blank. Plot the responses of the water peaks versus concentration, in mg per mL, of water in each of the *Standard solutions*, and determine the regression line using the least-squares method. The coefficient of variation from the regression line is not more than 3.0%. From the graph so obtained, determine the concentration, *C*, in mg per mL, of water in the *Test solution*. Calculate the percentage of water in the portion of Gonadorelin Hydrochloride taken by the formula:

$$80C/W$$

in which *W* is the weight, in mg, of Gonadorelin Hydrochloride in the *Test solution*: not more than 7.0% is found.

#### Limit of acetate—

**Mobile phase**—To 500 mL of water in a 1-L volumetric flask, add 1 mL of sulfuric acid. Dilute with water to volume, mix, and pass through a membrane filter having a 0.45- $\mu$ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Acetate standard solutions**—Dissolve an accurately weighed quantity of sodium acetate trihydrate in *Mobile phase* to obtain a stock solution having a known concentration of about 0.5 mg per mL. Quantitatively dilute accurately measured volumes of this stock solution with *Mobile phase* to obtain *Standard solutions* having known concentrations of about 100, 10, and 1  $\mu$ g of sodium acetate trihydrate per mL.

**Test solution**—Transfer about 50 mg of Gonadorelin Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 205-nm detector and a 6.5-mm  $\times$  30-cm column that contains packing L17. The flow rate is about 0.5 mL per minute. [NOTE—Do not allow the flow rate to exceed 0.6 mL per minute. Condition the column for about 60 minutes until a stable baseline is obtained.] Chromatograph the *Acetate standard solution* containing 100  $\mu$ g of sodium acetate trihydrate per mL, and record the peak responses as directed for *Procedure*: the retention time of the acetate peak is between 10 and 16 minutes; the column efficiency is not less than 2000 theoretical

plates; the tailing factor is not more than 2; and the relative standard deviation for not less than three replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of each *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—If more than five specimens are analyzed, reinject the *Standard solutions* before injecting further specimens of the *Test solution*.] Plot the responses of the *Standard solutions* versus concentration, in  $\mu$ g of sodium acetate trihydrate per mL, and determine the regression line, using the least-squares method. The coefficient of variation from the regression line is not more than 3.0%. From the graph so obtained, determine the concentration, *C*, in  $\mu$ g per mL, of sodium acetate trihydrate in the *Test solution*. Calculate the percentage of acetate ( $C_2H_3O_2$ ) in the portion of Gonadorelin Hydrochloride taken by the formula:

$$(59.03/136.08)(5C/W)$$

in which 59.03 and 136.08 are the molecular weights of acetate and sodium acetate trihydrate, respectively; and *W* is the weight, in mg, of Gonadorelin Hydrochloride in the *Test solution*: not more than 1.0% is found.

**Chromatographic purity**—[NOTE—Perform all procedures in a low-humidity glove box. The *Test solution* may be stored at room temperature for up to 20 minutes, or at 4° for up to 8 hours.]

**Solution A**—Dissolve 13.6 g of monobasic potassium phosphate in water, dilute with water to 2000 mL, and mix. Filter and degas. Adjust with 1 N potassium hydroxide to a pH of 6.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

**Standard solutions**—Dissolve an accurately weighed quantity of USP Gonadorelin Hydrochloride RS in and dilute quantitatively with *Solution A* to obtain a stock solution having a known concentration of about 1 mg per mL. Quantitatively dilute accurately measured volumes of this stock solution with *Solution A* to obtain *Standard solutions* having known concentrations of about 40, 5, and 1.5  $\mu$ g per mL.

**Test solution**—Dissolve an accurately weighed quantity of Gonadorelin Hydrochloride in *Solution A* to obtain a solution having a concentration of 1 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm UV detector and a 4.6-mm  $\times$  15-cm column containing 5- $\mu$ m packing L1, and is programmed to provide variable mixtures of *Solution A* and *Solution B*, beginning with 100% of *Solution A*, changing after 3 minutes to a mixture of 82% *Solution A* and 18% *Solution B*, maintained at that composition for the next 17 minutes, then changed linearly over the next 10 minutes so that it consists of a mixture of 30% *Solution A* and 70% *Solution B* at 30 minutes, and maintained at that composition for the next 5 minutes, then changed linearly over the next 3 minutes so that the composition at 38 minutes is again 100% *Solution A*. Pump *Solution A* through the column at a flow rate of about 1 mL per minute for about 30 minutes or until a stable baseline is obtained, then inject 100  $\mu$ L of *Solution A*, and run the gradient elution program to completion to condition the column. Again inject 100  $\mu$ L of *Solution A*, and run the gradient elution program to completion. Chromatograph the *Standard solution* containing 40  $\mu$ g per mL, and record the peak responses as directed for *Procedure*: the retention time for gonadorelin is between 24 and 30 minutes; the column efficiency is not less than 5000 theoretical plates; and the tailing factor is not more than 2.0. If necessary, adjust the flow rate (to between 0.8 and 2 mL per minute) or, alternatively, change (by not more



than 3%) the percentages of *Solution A* and *Solution B* at 3 minutes and at 20 minutes.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of *Solution A*, each of the *Standard solutions*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Correct the peak responses, using the chromatogram of *Solution A* as a blank. Plot the corrected responses of the *Standard solutions*, and determine the regression line, using the least-squares method. The coefficient of variation from the regression line is not more than 3.0%. From the graph so obtained, determine the concentration of each impurity in the *Test solution*: not more than 3.0% of any individual impurity is found, and not more than 5.0% of total impurities is found.

**Content of chloride**—Dissolve 25 mg of Gonadorelin Hydrochloride, accurately weighed, in 1 mL of methanol. Add 10 mL of water and 1 drop of glacial acetic acid, and titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N silver nitrate is equivalent to 0.3545 mg of chloride: the chloride content is between 4.0% and 6.0%.

**Assay**—[NOTE—Perform all manipulations involving the weighing of the Gonadorelin Hydrochloride and the Reference Standard in a low-humidity glove box.]

**Buffer solution**—Dissolve 6.8 g of monobasic potassium phosphate in water, and dilute with water to 1000 mL. Adjust with 1 N potassium hydroxide to a pH of 6.5.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (82:18). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparations**—Quantitatively dissolve accurately weighed quantities of USP Gonadorelin Hydrochloride RS in *Mobile phase* to obtain solutions having known concentrations of about 0.08 mg per mL, 0.10 mg per mL, and 0.12 mg per mL. [NOTE—These *Standard preparations* may be stored in a refrigerator for 2 months. Remove suitable portions and warm to room temperature before use.]

**Assay preparation**—Dissolve an accurately weighed quantity of Gonadorelin Hydrochloride in *Mobile phase* to obtain a solution containing 0.10 mg per mL.

**Identification solution**—Mix equal volumes of the *Assay preparation* and the *Standard preparation* containing 0.10 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. [NOTE—Condition the column with *Mobile phase* until a stable baseline is obtained.] Chromatograph about 20  $\mu$ L of the *Identification solution*: the ratio,  $R_r$ , of the retention times of the gonadorelin peaks obtained from the *Assay preparation* and the *Standard preparation* is  $1.00 \pm 0.05$ . Chromatograph the *Standard preparation* containing 0.08 mg per mL, and record the peak responses as directed for *Procedure*: the retention time for gonadorelin is between 8 and 11 minutes; the column efficiency is not less than 900 theoretical plates; the tailing factor is not more than 2.5; and the relative standard deviation for not fewer than three replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of each of the *Standard preparations* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—If more than five specimens are analyzed, reinject the *Standard preparations* before injecting further specimens of the *Assay preparation*.] Plot the responses of the gonadorelin peaks versus concentration, in mg per mL, of gonadorelin in each of the *Standard preparations*, and determine the regression line, using the least-squares method. The coefficient of vari-

ation from the regression line is not more than 3.0%. From the graph so obtained, determine the concentration,  $C$ , of gonadorelin in the *Assay preparation*. Calculate the percentage of  $C_{55}H_{75}N_{17}O_{13} \cdot 2HCl$  in the portion of Gonadorelin Hydrochloride taken by the formula:

$$100C.$$

## Gonadorelin for Injection

» Gonadorelin for Injection is a sterile mixture of Gonadorelin Hydrochloride with suitable diluents. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of gonadorelin ( $C_{55}H_{75}N_{17}O_{13}$ ).

**Packaging and storage**—Preserve in tight, well-sealed containers.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Gonadorelin Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Bacterial endotoxins** <85>—It contains not more than 3.60 USP Endotoxin Units per  $\mu$ g.

**pH** <791>: between 4.0 and 8.0, in a solution constituted as directed in the labeling.

**Other requirements**—It meets the requirements for *Sterility Tests* <71> and for *Labeling* under *Injections* <1>.

**Assay**—

*Buffer solution*, *Mobile phase*, *Standard preparations*, *Chromatographic system*, and *Identification solution*—Proceed as directed in the *Assay* under *Gonadorelin Hydrochloride*.

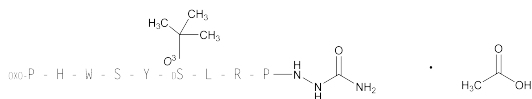
**Assay preparation**—Separately dissolve the contents of not less than 5 vials of Gonadorelin for Injection in *Mobile phase* to obtain a concentration of 100  $\mu$ g of gonadorelin per mL. Place the vials in an ultrasonic bath for 5 minutes, and allow to cool at room temperature. Combine the solutions in the vials to obtain the *Assay preparation*.

**Procedure**—Proceed as directed in the *Assay* under *Gonadorelin Hydrochloride*. Plot the responses of the gonadorelin peaks versus concentration, in mg per mL, of gonadorelin in each of the *Standard preparation*. Calculate the quantity of gonadorelin ( $C_{55}H_{75}N_{17}O_{13}$ ) in the Gonadorelin for Injection taken for the *Assay preparation* by the formula:

$$(1182.33 / 1255.4)(C)(V + 0.06)$$

in which 1182.33 and 1255.4 are the molecular weights of gonadorelin and gonadorelin hydrochloride, respectively;  $C$  is the concentration of gonadorelin in the *Assay preparation* as obtained from the regression line;  $V$  is the volume, in mL, of *Mobile phase* used to prepare the *Assay preparation*; and 0.06 is the correction factor for the volume created by the dissolved test specimen.

## Goserelin Acetate



$C_{59}H_{84}N_{18}O_{14} \cdot xC_2H_4O_2$

Luteinizing hormone-releasing factor (pig), 6-[O-(1,1-dimethylethyl)-D-serine]-10-deglycinamide-, 2-(amino-carbonyl)hydrazide, acetate (salt) [145781-92-6].  
1-(5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-O-tert-butyl-D-seryl-L-leucyl-L-arginyl-L-prolyl)semicarbazide (Goserelin) 1269.41  
[65807-02-5].

### DEFINITION

Goserelin Acetate is a synthetic nonapeptide analog of the hypothalamic decapeptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate salt. It contains NLT 94.5% and NMT 103.0% of goserelin ( $C_{59}H_{84}N_{18}O_{14}$ ), calculated on the anhydrous and acetic acid-free basis.

### IDENTIFICATION

- A.** The retention time of the goserelin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Prepare a filtered and degassed mixture of water, acetonitrile, and trifluoroacetic acid (1600:400:1).

**Standard solution:** 1 mg/mL of USP Goserelin Acetate RS in water

**Diluted standard solution:** Transfer 1 mL of the *Standard solution* to a 10-mL volumetric flask, and dilute with water to volume.

**Resolution solution:** Dissolve the contents of a vial of USP Goserelin Related Compound A RS in water to obtain a concentration of 0.1 mg/mL, and mix with an equal volume of *Diluted standard solution*.

**System suitability solution:** Dissolve the contents of a vial of USP Goserelin System Suitability Mixture RS with 1 mL of water.

**Sample solution:** 1 mg/mL of Goserelin Acetate in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L1

**Column temperature:** 50°–55°

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

#### System suitability

**Samples:** *Standard solution*, *Resolution solution*, and *System suitability solution*.

#### Suitability requirements

[NOTE—Two minor peaks are visible prior to the elution of the principal peak, *System suitability solution*.]

**Resolution:** NLT 7.0 between the goserelin and goserelin related compound A (4-D-ser-goserelin) peaks, *Resolution solution*

**Retention time:** 40–50 min for the goserelin peak, *Resolution solution*

**Relative standard deviation:** NMT 2.0% for the goserelin peak, from replicate injections, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of goserelin ( $C_{59}H_{84}N_{18}O_{14}$ ) in the portion of Goserelin Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 94.5%–103.0% of goserelin ( $C_{59}H_{84}N_{18}O_{14}$ ) on the anhydrous, acetic acid-free basis

### OTHER COMPONENTS

#### LIMIT OF ACETIC ACID

**Mobile phase:** Transfer 49.04 g of sulfuric acid to a 1000-mL volumetric flask, dilute with water to volume, and mix. Accurately transfer 20 mL of this solution to a 2000-mL volumetric flask, dilute with water to volume, mix, filter, and degas.

**Standard stock solution:** Transfer 2.0 mL of glacial acetic acid to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Sample solution:** Dissolve about 20 mg of Goserelin Acetate, accurately weighed, in 2–3 mL of *Mobile phase*. Connect a 1-mL cartridge containing packing L44 to a 1-mL cartridge containing packing L2, which is then attached to a suitable vacuum apparatus. With the vacuum applied, wash the cartridge combination with 2 mL of methanol followed by 15 mL of *Mobile phase*, and discard the washings. Quantitatively apply the solution containing the sample to the cartridge combination, and wash through the cartridge system with several small volumes of *Mobile phase*. Collect the solution and washings in a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

[NOTE—Condition the column for about 24 h until a stable baseline is obtained.]

**Mode:** LC

**Detector:** UV 206 nm

**Column:** 7.8-mm × 30-cm; packing L17

**Column temperature:** 65°

**Flow rate:** 0.8 mL/min

**Injection size:** 100 μL

#### System suitability:

**Sample:** *Standard solution*

[NOTE—The retention time of the acetic acid peak is about 11 min.]

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.1%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetic acid in the portion of Goserelin Acetate taken by the formula:

$$\text{Result} = (r_U/r_S) \times (1/W) \times (1.049/5)$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$W$  = sample weight of Goserelin Acetate taken to prepare the *Sample solution* (g) and corrected (for the purposes of the calculation) to eliminate the water content, which is determined immediately prior to the test

1.049 = weight of glacial acetic acid (g/mL)

Acceptance criteria: 4.5%–15.0%

## IMPURITIES

### • ORGANIC IMPURITIES: RELATED COMPOUNDS

Mobile phase, Standard solution, Diluted standard solution, Resolution solution, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the Assay.

**Diluted sample solution:** Transfer 1 mL of the Sample solution into a 100-mL volumetric flask, and dilute with water to volume.

#### System suitability

**Samples:** Resolution solution and System suitability solution

[NOTES—The retention time for the goserelin peak is between 40 and 50 min, Resolution solution. See Table 1 for the relative retention times, Resolution solution. Two peaks, corresponding to decarbamoylgoserelin and 2-D-his-goserelin, eluting prior to the principal peak, are visible, System suitability solution.]

Table 1

Name	Relative Retention Time
4-D-ser-Goserelin	0.67
Decarbamoylgoserelin	0.89
5-D-tyr-Goserelin	0.92
2-D-his-Goserelin	0.94
Goserelinare	1.0

**Resolution:** NLT 7.0, Resolution solution

**Column efficiency:** NLT 2000 theoretical plates, System suitability solution

**Tailing factor:** NMT 2.0, System suitability solution

**Relative standard deviation:** NMT 2.0%, System suitability solution

#### Analysis

**Samples:** Sample solution and Diluted sample solution  
Calculate the percentage of goserelin-related impurities in the portion of Goserelin Acetate taken:

$$\text{Result} = r_i/r_u$$

$r_i$  = peak response for any individual impurity in the Sample solution

$r_u$  = peak response of the main goserelin peak in the Diluted sample solution

**Acceptance criteria:** NMT 1.0% of decarbamoylgoserelin is found, NMT 0.5% of any other impurity is found, and NMT 2.5% of total impurities is found.

## SPECIFIC TESTS

### • AMINO ACID CONTENT, Nuclear Magnetic Resonance (761)

[NOTE—Concentrations of goserelin in both the Standard solution and the Sample solution must be the same (within 5% of each other) but can be adjusted based on the quality of the  $^{13}\text{C}$  spectrum obtained. The spectra must be acquired under the same conditions for both the Standard solution and the Sample solution. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the Standard solution and the Sample solution can be repeated and averaged.]

**Standard solution:** Dissolve USP Goserelin Acetate RS in deuterium oxide to obtain a solution having a known concentration of about 10% w/v, and adjust with deuterated acetic acid-d4 to a pH of 4.

**Sample solution:** Prepare a 10% w/v solution of Goserelin Acetate in deuterium oxide, and adjust with deuterated acetic acid-d4 to a pH of 4.

**Analysis:** Obtain a  $^{13}\text{C}$ , proton-decoupled NMR spectrum of both the Standard solution and the Sample solution. The spectra from both solutions are qualitatively similar, and all the resonances from the spectrum of the

Standard solution are present in the spectrum of the Sample solution and have the same chemical shift values ( $\pm 0.1$  ppm for goserelin,  $\pm 0.5$  ppm for acetate). Identify any other resonances in the spectrum of the Sample solution. The relative amino acid ratio between the Standard solution and the Sample solution can be calculated as follows. Integrate the resonances at the approximate ppm corresponding to each amino acid in Table 2.

Table 2

Amino Acids	Resonances (ppm)
Azo-glycine	162.2
Histidine	118.4
Tyrosine	116.7
<i>tert</i> -Butyl serine	62.5
Serine	62.2
Tryptophan	55.7
Arginine	41.8
Pyroglutamic acid	26.3
Proline	26.0
Leucine	23.5

Calculate the ratio of each of the amino acids from the integrals of the Standard solution and the Sample solution by the formula:

$$\text{Result} = r_u/r_s$$

$r_u$  = integral of the resonance of a designated amino acid from the Sample solution

$r_s$  = integral of the resonance of a designated amino acid from the Standard solution

**Acceptance criteria:** The resulting ratios fall within the following limits: histidine, tyrosine, *tert*-butyl serine, serine, tryptophan, arginine, pyroglutamic acid, proline, and leucine 0.9–1.1; azo-glycine 0.8–1.2.

### • OPTICAL ROTATION, Specific Rotation (781S)

**Sample solution:** 2 mg/mL, in water, calculated on the anhydrous and acetic acid-free basis

**Acceptance criteria:** Between  $-52^\circ$  and  $-56^\circ$

### • BACTERIAL ENDOTOXINS TEST (85):

It contains NMT 16 USP Endotoxin Units/mg of goserelin, if intended for use in the manufacture of parenteral dosage forms without a further, appropriate procedure for the removal of bacterial endotoxins.

### • WATER DETERMINATION, Method I (921):

NMT 10.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.

### • USP REFERENCE STANDARDS (11)

USP Goserelin Acetate RS

USP Goserelin Related Compound A RS

USP Goserelin System Suitability Mixture RS

## Chorionic Gonadotropin

» Chorionic Gonadotropin is a gonad-stimulating polypeptide hormone obtained from the urine of pregnant women. Its potency is not less than 1500 USP Chorionic Gonadotropin Units in each mg, and not less than 80.0 percent and not more than 125.0 percent of the potency stated on the label.

**Packaging and storage—**Preserve in tight containers, preferably of Type I glass, in a refrigerator.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Human Chorionic Gonadotropin RS

**Bacterial endotoxins** <85>—It contains not more than 0.03 USP Endotoxin Unit per USP Chorionic Gonadotropin Unit.**Sterility** <71> (Where it is labeled as sterile): meets the requirements.**Acute toxicity**—Select five healthy mice, weighing between 18 g and 22 g. Prepare a test solution as directed in the test for *Pyrogen*, but containing 2000 USP Chorionic Gonadotropin Units per mL. Inject intravenously a dose of 0.5 mL of the test solution into each of the mice. Observe the animals over the 48 hours following the injection. If, at the end of 48 hours, all of the animals survive and not more than one of the animals shows outward symptoms of a toxic reaction, the requirements of the test are met. If more than one of the animals show outward signs of a toxic reaction or if not more than two of the animals die, repeat the test on ten additional, similar animals: if all of the animals of the repeat test survive for 48 hours and show no symptoms of a toxic reaction, the requirements of the test are met.**Water, Method I** <921>: not more than 5.0%.**Estrogenic activity**—Dissolve a suitable quantity in saline TS to obtain a test solution containing the equivalent of 1000 USP Chorionic Gonadotropin Units per mL. Into each of five rats that have been ovariectomized not less than 2 weeks previously, inject subcutaneously 0.25 mL of the test solution in the forenoon and in the afternoon of two successive days. On each of the three following days, take a vaginal smear from each animal: the requirements of the test are met if the cellular elements in the smears consist mainly of leucocytes, and a few nucleated epithelial cells, but no cornified epithelial cells.**Assay**—**Standard preparations**—Dissolve a suitable quantity of USP Human Chorionic Gonadotropin RS in a diluent consisting of saline TS, freshly prepared to contain 1 mg per mL of bovine serum albumin and adjusted with sodium hydroxide TS to a pH between 6.9 and 8.0, to obtain a solution having a known concentration of 10 USP Chorionic Gonadotropin Units in each mL. Using the same diluent, prepare three *Standard preparations* such that the respective concentrations of chorionic gonadotropin constitute a geometric series such as 1:1.2:1.44 or 1:2:4 and such that the activity in each mL lies within the range of 0.1 to 1.0 Unit.**Assay preparations**—Following the procedure outlined for the *Standard preparations*, prepare solutions of Chorionic Gonadotropin to obtain three *Assay preparations* corresponding to those of the *Standard*.**The animals**—Select 20- to 23-day-old female rats, but restrict the selection so that no rat is more than 30% heavier than the lightest. House the animals under uniform conditions of temperature, lighting, feeding, and watering. Mark the animals for identification, and divide them at random into groups of the same number but not fewer than 10 animals. Assign one group to each of the three *Standard preparations* and three *Assay preparations*, respectively.**Procedure**—Inject each rat subcutaneously in the dorsal area with 0.20 mL of the solution to which it was assigned, at approximately the same time on each of three consecutive days. On the afternoon of the fifth day, sacrifice the animals, and excise the uterus from each animal by cutting through the cervix, stripping off the surrounding tissue, and severing at the utero-tubal junction. Gently press out the uterine fluid on moistened absorbent paper, and weigh the uterus to the nearest 0.2 mg, using a suitable balance.**Calculation**—Tabulate the observed uterine weight for each rat, designated by the symbol  $y$ , for each dosage group of  $f$  rats. Proceed as directed in the *Assay* under Cor-*ticotropin Injection*, beginning with "If the data from one or more rats." Compute the log confidence interval  $L$  (see *Confidence Intervals for Individual Assay* <111>). If the confidence interval is more than 0.1938, which corresponds at  $P = 0.95$  to confidence limits of 80% and 125% of the computed potency, repeat the assay until the combined data of two or more assays, redetermined as described under *Combination of Independent Assays* <111>, meet this limit.

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**Chorionic Gonadotropin for Injection**

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» Chorionic Gonadotropin for Injection is a sterile, dry mixture of Chorionic Gonadotropin with suitable diluents and buffers. Its potency is not less than 80.0 percent and not more than 125.0 percent of the potency stated on the label in USP Chorionic Gonadotropin Units. It may contain an antimicrobial agent.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>.**Labeling**—Label it to indicate the expiration date.**USP Reference standards** <11>—

USP Endotoxin RS

USP Human Chorionic Gonadotropin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.**Bacterial endotoxins** <85>—It contains not more than 0.03 USP Endotoxin Unit per USP Chorionic Gonadotropin Unit.**Uniformity of dosage units** <905>—Open 10 containers and weigh accurately each individual container and its contents, taking care to preserve the identity of each container. Remove the contents of each container by rinsing thoroughly with water, dry at 105° to constant weight, and reweigh. Calculate for each container the net weight of its contents by subtracting the weight of the dry, empty container from its initial gross weight. Determine the average weight of the contents and the relative standard deviation (see *Calculation of the Relative Standard Deviation* under *Uniformity of Dosage Units* <905>). The requirements are met if the weight of the contents of each container does not deviate from the average weight by more than 5.0% and the relative standard deviation of the 10 containers is not greater than 3.0%. If the requirements of the test are not met, test 20 additional containers. The requirements are met if the net weight of not more than 1 container of the 30 deviates by more than 7.5% from the average weight of the contents of the 30 containers and the relative standard deviation of the 30 containers is not greater than 3.3%.**pH** <791>—The pH of the solution prepared for the test for *Estrogenic activity* is between 6.0 and 8.0.**Estrogenic activity**—When constituted as directed in the labeling, it meets the requirements of the test for *Estrogenic activity* under *Chorionic Gonadotropin*.**Other requirements**—It meets the requirements for *Sterility Tests* <71> and *Labeling* under *Injections* <1>.**Assay**—Proceed with Chorionic Gonadotropin for Injection as directed in the *Assay* under *Chorionic Gonadotropin*, using an *Assay preparation* obtained by diluting a portion of the solution prepared for the test for *Estrogenic activity* quantitatively and stepwise with the specified diluent.

## Graftskin

» Graftskin<sup>1</sup> is a living, bilayered skin substitute derived from neonatal foreskins manufactured under Class 100 sterile conditions. The upper, epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum. The inner, dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. Graftskin does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, hair follicles, or any other epidermally derived components. The fibroblast and keratinocyte cell banks from which Graftskin is derived test negative for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, and tumorigenicity. The cell banks are also tested for normal human karyology and isoenzymes. The final product is tested for morphology, cell viability, and physical container integrity. Used tissue culture media are tested for mycoplasma and sterility. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy.

**Packaging and storage**—Graftskin is aseptically packaged in a Class 100 environment in single-use containers that preserve cell viability and product integrity. Store at controlled room temperature for no longer than 5 days, and do not subject to freezing temperatures. The atmosphere within the package contains air enriched with 10% carbon dioxide. The device is translucent and off-white in color. The upper, epidermal surface is dull with small irregularities resulting from the cornification of keratinocytes, while the bottom surface is smooth and shiny in appearance. The device is packaged so that the dermal layer (glossy layer) is closest to the agarose-based nutrient medium. The packaging permits easy observation of the medium and provides ready access to the Graftskin when needed. The medium contains all of the required nutrients for the living cell components of Graftskin, plus an appropriate, nontoxic, pH-sensitive dye to indicate package breaches or microbial contamination. The medium should appear pink (pH 6.8–7.7) when compared to the enclosed pH color chart.

**Labeling**—Label it to indicate the dimensions of the enclosed Graftskin, the expiry date, the required storage conditions, and the lot number. The label indicates that the enclosed Graftskin and surrounding medium are to be examined for signs of contamination or deterioration. The label also contains a pH color code to be used for determination of the acceptability of the pH of the Graftskin medium. The label cautions that Graftskin is not to be used if the package shows signs of damage or microbial contamination.

<sup>1</sup>Four tests for Graftskin are specified: *Histological characterization*, *Gene expression profile*, *Barrier integrity assessment*, and *Metabolic activity assessment*. The histological examination of the 3-dimensional organotypic structure demonstrates control of the Graftskin manufacturing process and shows a bilayered construct with a dermal matrix, differentiated epidermis, and developed stratum corneum. Reference photomicrographs, representing examples of both passing and failing Graftskin units, are specified to assist in ascertaining quality. PCR analysis of the gene expression profile of a finished Graftskin unit demonstrates that its keratinocytes and fibroblasts are producing cytokines that have been documented to influence wound healing. The test also demonstrates cell purity. Graftskin is positive for the gene expression of *Interleukin-1α*, *Platelet-derived growth factor*, and *Transforming growth factor-β1*. Graftskin is negative for the gene expression of *Interleukin-4*, which is produced neither by fibroblasts nor by keratinocytes. *Glyceraldehyde-3-phosphate dehydrogenase* is run with this assay as a housekeeping gene, and Graftskin is positive for the expression of this gene. The *Barrier integrity assessment* demonstrates the presence of a stratum corneum and the functionality of the epithelium in Graftskin. The purpose of the *Metabolic activity assessment* is to demonstrate cellular viability of the article.

Label it to indicate that sterile techniques are to be used in handling Graftskin and that cytotoxic agents are not to be used. Label it to indicate the time frame for use after package opening.

**USP Authentic Visual References** (11)—*USP Graftskin Reference Photomicrographs*. [NOTE—These 10 photomicrographs represent examples of both passing and failing Graftskin units. They are specified to assist in ascertaining histological quality.]

### Histological characterization—

#### SOLUTION PREPARATIONS—

**2.0 M Monobasic potassium phosphate**—Dissolve 13.61 g of anhydrous monobasic potassium phosphate in 50 mL of water.

**2.0 M Dibasic potassium phosphate**—Dissolve 17.42 g of anhydrous dibasic potassium phosphate in 50 mL of water.

**Phosphate-buffered saline solution (pH 7.1–7.5)**—Combine 3.6 mL of 2.0 M Monobasic potassium phosphate, 16.4 mL of 2.0 M Dibasic potassium phosphate, 8 g of sodium chloride, and 1 L of water. Mix thoroughly.

**0.3% Acid alcohol**—To 100 mL of 70% alcohol, add 0.3 mL of hydrochloric acid, and mix.

**Hematoxylin-alcohol solution**—Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol, with heating.

**Potassium alum solution**—Dissolve 50.0 g of potassium alum in 500 mL of water, with heating.

**Hematoxylin staining solution**—Mix Hematoxylin-alcohol solution and Potassium alum solution, and heat to boiling as rapidly as possible with constant stirring. Do not heat for more than 1 minute. Slowly add 0.185 g of sodium iodate, and reheal to a simmer until the solution becomes a deep purple. Remove from the heat, and quickly cool. Filter daily before use.

**Bluing agent**—Dissolve 200 mg of sodium bicarbonate and 40 mg of lithium carbonate in 63 mL of water and 37 mL of methanol, and mix.

**Eosin solution**—Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

**TISSUE PREPARATION**—Remove three 2-cm diameter circular sections from every 30-cm<sup>2</sup> section of Graftskin (not less than 30% of the total unit area), using the appropriate size biopsy punch. Cut with a circular rocking motion to prevent crushing the tissue. Immerse the sections in 3.7% dimethoxymethane for 30 minutes, using a gentle rocking motion. Remove the sections, and lay on a cutting surface, dermal side (glossy side) down. Cut an approximately 3-mm-wide strip through the center of the specimen, using a new, single-edged razor blade. Place the strips in a histological microwave cassette, using suitable biopsy pads premoistened with Phosphate-buffered saline solution (pH 7.1–7.5) to hold the strips in place. Insert the cassette into a histological microwave processing rack, place the rack inside a suitable microwave container, and add sufficient Phosphate-buffered saline solution (pH 7.1–7.5) to completely cover the rack. Place the container in a microwave oven suitable for histological work,<sup>2</sup> and heat for 4 minutes at 55°. Remove the Phosphate-buffered saline solution (pH 7.1–7.5), and add enough dehydrated alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 minutes at 67°. Remove the alcohol, and add enough dehydrated isopropyl alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 minutes at 74°. Remove the isopropyl alcohol, and add enough suitable grade paraffin<sup>3</sup> that has been melted and held at 84° prior to use, to completely cover the rack. Return the container to the microwave oven, and heat for 7 minutes at 84°. Remove the histological microwave cassette from the container and

<sup>2</sup>A microwave oven suitable for histological preparation can be obtained from Energy Beam Sciences, Inc., 11 Bowles Road, P.O. Box 468, Agawam, MA.

<sup>3</sup>A suitable paraffin for use is Accumate™ Tissue Embedding/Infiltration Medium, which can be obtained from Sigma Diagnostics, 545 S. Ewing Ave., St. Louis, MO 63103.

rack while the paraffin is still melted, and disassemble, discarding the biopsy pads. Fill preheated embedding molds with molten paraffin<sup>4</sup> heated to 60°, and place on top of a preheated warming platform that is designed for histological work. Using forceps, remove the Graftskin specimens from the cassette, and place the specimens in individual molds. Orient the specimens in the molds to enable cutting of a cross-or longitudinal section. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain the specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15 to 30 minutes, and clamp the paraffin block into the block holder of the microtome. Fill a histological tissue-flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,<sup>5</sup> and heat to a temperature 5° lower than the melting point of the paraffin. Properly mount the paraffin block into a microtome, adjusting as necessary. Set the microtome to make 5- $\mu$ m thick cuts with a blade angle of  $5 \pm 2^\circ$ . Insert into the knife holder a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife, and cut a ribbon that contains 6 to 10 sections of Graftskin. Pick up the ribbon with forceps, and stretch it across the tissue-flotation water bath. Separate 2 to 3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 hour. The microscope slide with affixed tissue is sequentially immersed in 3 changes of a suitable histological, aliphatic xylene substitute,<sup>6</sup> 5 minutes per step, followed by two changes of dehydrated alcohol, 3 minutes per step. Sequentially immerse the slide in alcohol (for 3 minutes), running water rinse (3 minutes), *Hematoxylin staining solution* (6 minutes), running water rinse (7 minutes), 0.3% *Acid alcohol* (6 seconds), running water rinse (5 minutes), *Bluing agent* (1 second), running water rinse (5 minutes), *Eosin solution* (2 minutes), 2 changes of alcohol (3 minutes each step), 4 changes of dehydrated alcohol (3 minutes each step), and 4 changes of a suitable histological xylene substitute (3 minutes each step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue, using a suitable coverslip mountant.

**MICROSCOPIC SPECIFICATIONS**—A light microscope with 4 $\times$ , 10 $\times$ , 20 $\times$ , and 40 $\times$  objectives installed in a revolving nose-piece; a 10 $\times$  widefield ocular with 10 to 19 mm per 100 microdisk reticle installed; and a 10 $\times$  widefield ocular with grid reticle installed.

**MICROSCOPIC AND MORPHOLOGICAL CHARACTERISTICS**—Score the 3 Graftskin sections for epidermal and dermal aspects, using the light microscope. Evaluate the slides from each of the sections taken. Average the aspect values for each section ( $n = 3$ ) to determine the overall aspect score for the Graftskin unit. When examined microscopically, Graftskin shows a bilayered construct resembling the epidermal and dermal layers of human skin. Using *USP Graftskin Reference Photomicrographs* of passing and failing articles for comparison, Graftskin meets the requirements for epidermal aspects, in-

cluding epidermal coverage, epidermal development, and keratinocyte aspect, and meets the requirements for dermal aspects, including dermal matrix thickness, fibroblast density, and matrix aspect, as described below.

**Epidermal aspects** (see *USP Graftskin Reference Photomicrograph 1* for an example of a passing unit)—

**Epidermal coverage**—Ninety-five percent or more of the dermal matrix present on the slide is covered with epidermal keratinocytes.

**Epidermal development**—Seventy percent or more of the Graftskin epithelium is composed of 3 distinct cell layers (see *USP Graftskin Reference Photomicrograph 2* for an example of a failing unit). The basal cell layer of the epithelium is at least 1 cell thick, consisting of keratinocytes with a cuboidal-columnar shape (see *USP Graftskin Reference Photomicrograph 3* for an example of a failing unit). The suprabasal layer is composed of stratified cells and is at least 5 cells thick. Suprabasal cells closest to the basal layer are cuboidal in shape; cells become progressively stratified the closer they are to the uppermost, squamous cell layer. The squamous cell layer on the apical surface is cornified and at least 1 cell thick (see *USP Graftskin Reference Photomicrograph 4* for an example of a failing unit). The uppermost cell layer of the epithelium is analogous to the stratum corneum of human skin and is composed of one or more rows of flat, scaly cells that are nonliving and keratinized (see *USP Graftskin Reference Photomicrograph 5* for an example of a failing unit).

**Keratinocyte aspect**—Ninety-five percent or more of the basal keratinocytes have basophilic cytoplasm that neither has distinct vacuoles nor is necrotic (see *USP Graftskin Reference Photomicrograph 6* for an example of a failing unit). Eighty percent or more of suprabasal cells (excluding those in the upper 20% of the cell layer closest to the squamous layer) have basophilic cytoplasm. Furthermore, these basophilic suprabasal cells do not have distinct vacuoles and are neither necrotic nor keratinized (see *USP Graftskin Reference Photomicrographs 7 and 8* for examples of failing units).

**Dermal aspects** (see *USP Graftskin Reference Photomicrograph 1* for an example of a passing unit)—Five randomly selected fields per slide will be evaluated for dermal matrix thickness and fibroblast density. The 5 fields will be averaged to obtain the final value for each section.

**Dermal matrix thickness**—The Graftskin dermal layer is not less than 40  $\mu$ m thick and is composed of several rows of flat dermal cells.

**Fibroblast density**—The dermal matrix contains an average of at least 4 nonpyknotic nuclei present per microscopic field (field = 20 grid squares of reticle when using the 10 $\times$  widefield ocular and 40 $\times$  objective).

**Matrix aspect**—At least 95% of the dermal matrix collagen stains uniformly with no large holes or inclusions present (see *USP Graftskin Reference Photomicrographs 9 and 10* for examples of failing units).

**Gene expression profile**—

**RNA extraction solution**—Use an aqueous phenol and guanidine isothiocyanate solution suitable for RNA extraction.<sup>7</sup>

**DEPC-treated water**—Add 0.2 mL of diethylpyrocarbonate (DEPC) to 100 mL of sterile Purified Water, shake vigorously, and allow to stand for at least 12 hours. Autoclave the resulting solution for 15 minutes, using the liquid cycle, to inactivate residual DEPC. Prepare fresh as needed.

**5X Reaction buffer**—Prepare a solution of potassium chloride, magnesium chloride, and tris(hydroxymethyl)amino-methane hydrochloride having concentrations of 375 mM, 15 mM, and 250 mM, respectively. Adjust to a pH of 8.3.

**10X Reaction buffer**—Prepare a solution of potassium chloride and tris(hydroxymethyl)aminomethane hydrochloride

<sup>4</sup> A suitable paraffin for use is Paraplast® X-Tra Tissue Embedding Medium ASTM, melting point 50° to 54°, which can be obtained from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>5</sup> A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific R & D Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.

<sup>6</sup> A suitable histological xylene substitute can be obtained from Shandon, Inc., 171 Industry Drive, Pittsburgh, PA 15275.

<sup>7</sup> A suitable RNA extraction solution is Trizol® reagent, which can be obtained from Invitrogen Corp., 1600 Faraday Ave., P.O. Box 6482, Carlsbad, CA 92008.

ride having concentrations of 500 mM and 100 mM, respectively. Adjust to a pH of 8.3.

**Oligo-deoxythymidine solution**—Prepare a 20-mM oligo-deoxythymidine (primer length: 18) solution, using a suitable buffer.<sup>8</sup>

**dNTP solution I**—Using a suitable buffer,<sup>8</sup> prepare a solution of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate in which the concentration of each component is 10 mM.

**dNTP solution II**—Prepare a solution, in water, of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate, in which the concentration of each component is 10 mM.

**Ribonuclease inhibitor solution**—Prepare a solution containing 40 units of ribonuclease inhibitor per mL of a suitable buffer.<sup>8</sup>

**Reverse transcriptase solution**—Prepare a solution containing 200 units of reverse transcriptase per  $\mu\text{L}$  of a solution of sodium chloride, edetate disodium, dithiothreitol, nonylphenol polyoxyethylene ether, glycerin, and tris(hydroxymethyl)aminomethane hydrochloride having concentrations of 0.1 M, 0.1 M, 1.0 M, 0.01%, 50%, and 200 mM, respectively. Adjust to a pH of 7.5.

**DNA primer pairs**—Prepare individual 20- $\mu\text{M}$  solutions of the following DNA primer pairs,<sup>9</sup> using deoxyribonuclease- and ribonuclease-free water.

**Transforming growth factor  $\beta$ —**

TGF $\beta$ 1-3'	agg ctc caa atg tag ggg cag g
TGF $\beta$ 1-5'	gcc ctg gac acc aac tat tgc t

**Interleukin-1 $\alpha$ —**

IL1 $\alpha$ -3'	tag tgc cgt gag ttt ccc aga aga aga
	gga gg
IL1 $\alpha$ -5'	caa gga gag cat ggt ggt agt agc
	aac caa cg

**Interleukin-4—**

IL4-3'	acg tac tct ggt tgg ctt cct tca cag
	gac ag
IL4-5'	cgg caa ctt tga cca cgg aca caa gtg
	cga ta

**Platelet-derived growth factor A—**

PDGF-A-3'	ctg ctt cac cga gtg cta caa tac ttg
	ct
PDGF-A-5'	aga agt cca ggt gag gtt aga gga
	gcat

**Glyceraldehyde-3-phosphate dehydrogenase—**

G3PDH-3'	cat gtg ggc cat gag gtc cac cac
G3PDH-5'	tga agg tcg gag tca acg gat ttg gt

**DNA polymerase solution**—Prepare a solution containing 5 units of deoxyribonucleic acid polymerase per mL of a solution of potassium chloride, edetate disodium, dithiothreitol, polyoxyethylene (20) sorbitan monolaurate, nonylphenol polyoxyethylene ether, glycerin, and tris(hydroxymethyl)aminomethane hydrochloride, having concentrations of 100 mM, 0.1 mM, 1 mM, 0.5%, 0.5%, 50%, and 20 mM, respectively. Adjust to a pH of 8.0.

**RNA extraction procedure**—Remove three 2-cm diameter circular sections from every 30 cm<sup>2</sup> of Graftskin (not less than 30% of the total unit area), using the appropriate size biopsy punch. Transfer each piece of tissue to individual polypropylene microcentrifuge tubes. Add 1.0 mL of *RNA extraction solution* to each tube, homogenize by repetitive pipetting, and incubate the samples for 5 minutes at room temperature. To each tube add 0.2 mL of chloroform, mix on a vortex mixer, and centrifuge at 12,000 *g* for 15 minutes at 2° to 8°. Transfer the upper, aqueous phase to a second tube, add 0.5 mL of isopropanol, and incubate for 30 minutes to overnight at -20°. Centrifuge at 12,000 *g* for 15 minutes, discard the supernatants by aspiration, and add 75% alcohol to each pellet. Mix the sample on a vortex mixer, centrifuge at 12,000 *g* for 2 minutes, and discard the supernatants by aspiration without disturbing the RNA pellets. Recentrifuge at 12,000 *g* for 2 minutes, and remove the remaining supernatants with a small-volume (20  $\mu\text{L}$  or smaller capacity) micropipet. Air-dry the pellets for 5 minutes at room temperature by keeping the microcentrifuge cap off, and resuspend each pellet in 50  $\mu\text{L}$  of *DEPC-treated water*. Bring absorbance into linear range by diluting 5  $\mu\text{L}$  of each suspension with 195  $\mu\text{L}$  of *DEPC-treated water*. Transfer the samples to suitable quartz microplates or cuvettes and determine the absorbance of the RNA solution at wavelengths of 260 and 280 nm, using a spectrophotometer and *DEPC-treated water* as the blank. The ratio of the absorbance at 260 versus 280 nm should be greater than or equal to 1.65. If this ratio is less than 1.65, mix the resuspended pellet by repetitive pipetting, and repeat the dilution step and absorbance measurement. If this fails to raise the absorbance ratio, repeat the RNA extraction for that sample by adding 1 mL of *RNA extraction solution*, and proceed as above, beginning with "incubate the sample for 5 minutes at room temperature". Determine the concentration of RNA, in  $\mu\text{g}$  per mL, using the following equation:

$$40AD$$

in which *A* is the absorbance at 260 nm, and *D* is the dilution factor. Adjust the volume of the RNA solutions with additional *DEPC-treated water* to bring the concentration of RNA to about 80  $\mu\text{g}$  per mL. If the absorbance at 260 nm is less than 0.05, discard the sample, and repeat the RNA extraction on a fresh sample.

**Synthesis of cDNA**—To separate, individual thin-walled polymerase chain reaction (PCR) tubes add 12.5  $\mu\text{L}$  of the RNA solution from samples 1, 2, and 3 (3 reaction tubes total). Add 1  $\mu\text{L}$  of *Oligo-deoxythymidine solution* to each tube, and incubate at 72° for 2 minutes to anneal the oligo-deoxythymidine to the mRNA. Place the tubes in an ice bath, and to each tube add 4  $\mu\text{L}$  of *5X Reaction buffer*, 1  $\mu\text{L}$  of *dNTP solution I*, 0.5  $\mu\text{L}$  of *Ribonuclease inhibitor solution*, and 1  $\mu\text{L}$  of *Reverse transcriptase solution*. Incubate at 42° for 1 hour to synthesize cDNA, and then incubate at 94° for 5 minutes to inactivate the reverse transcriptase. To each tube add 80  $\mu\text{L}$  of *DEPC-treated water*, and mix.

**Polymerase chain reaction amplification of cDNA**—For each of the five *DNA primer pairs*, label five individual centrifuge tubes (five tubes total). Add the following to each centrifuge tube: *DEPC-treated water*, 135.8  $\mu\text{L}$ ; *dNTP solution II*, 10.5  $\mu\text{L}$ ; *10X Reaction buffer*, 21  $\mu\text{L}$ ; the appropriate 5' primer, 3.5  $\mu\text{L}$ ; the appropriate 3' primer, 3.5  $\mu\text{L}$ ; and 25 mM magnesium chloride, 12.6  $\mu\text{L}$ . Close, mix on a vortex mixer, and pulse spin in a microcentrifuge. Add 2.1  $\mu\text{L}$  of *DNA polymerase solution* to each centrifuge tube, and mix by repetitive pipetting. For each primer pair, transfer 27  $\mu\text{L}$  of the resulting solution to five thin-walled PCR tubes. There should be a total of 25 PCR tubes. Add the following to the PCR tubes of each primer set:

<sup>8</sup>A suitable buffer can be obtained from the RT-for-PCR Kit, BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA 94303-4230.

<sup>9</sup>Suitable DNA primer pairs can be obtained from BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA 94303-4230.

PCR tube number	
1	3 $\mu$ L Graftskin sample 1 cDNA
2	3 $\mu$ L Graftskin sample 2 cDNA
3	3 $\mu$ L Graftskin sample 3 cDNA
4	3 $\mu$ L cDNA positive control <sup>10</sup>
5 Negative control	3 $\mu$ L DEPC-treated water

Repeat for the remaining primer pairs. The positive control contains authentic cDNA of *Transforming growth factor  $\beta$* , *Interleukin-1 $\alpha$* , *Interleukin-4*, *Platelet-derived growth factor A*, and *Glyceraldehyde-3-phosphate dehydrogenase*, as appropriate for each primer set. Pulse spin the PCR tubes in a microcentrifuge to mix, and place the tubes in a single PCR thermal cycler. Cycling conditions are as follows.

Melting temperature	94°
Melting time	45 seconds
Anneal temperature	58°
Anneal time	45 seconds
Elongation temperature	72°
Elongation time	2 minutes
Number of cycles	30
Final elongation temperature	72°
Final elongation time	2 minutes

Terminate the PCR amplification by heating each tube to 72° for 7 minutes.

#### ELECTROPHORESIS IDENTIFICATION—

**Tris-boric acid buffer**—Prepare a solution containing 89 mM of tris(hydroxymethyl) aminomethane, 89 mM of boric acid, and 2 mM of edetate disodium per L.

**6X Loading buffer**—Prepare a solution containing 15% of a branched polymeric sucrose (400 kDa), 0.25% bromophenol blue, and 0.25% xylene cyanole FF.

**Ethidium bromide solution**—Prepare a solution of ethidium bromide in *Tris-boric acid buffer* having a concentration of 10 mg per mL.

**Agarose gel**—Prepare a horizontal 2% agarose<sup>11</sup> gel in *Tris-boric acid buffer*. Once the gel is set, remove the comb, and place the gel into the electrophoresis chamber with the comb end of the gel situated closest to the cathode terminal. Fill the electrophoresis chamber with *Tris-boric acid buffer* until the buffer reaches 3 to 5 mm over the surface of the gel.

**100-bp DNA ladder markers**—Prepare a solution containing 10 DNA fragments covering the range of 100 to 1000 base pairs (bp) in 100-bp increments, with a total DNA content of approximately 100 ng per  $\mu$ L (15–20 ng of DNA per band) in an appropriate buffer.<sup>12</sup>

**Procedure**—Dilute the 25 PCR samples prepared in the *Polymerase chain reaction amplification of cDNA with 6X Loading buffer* so that the final concentration of the buffer is one-sixth of its original concentration. Load 5  $\mu$ L of the *100-bp DNA ladder markers* in the first lane of the agarose gel. Load 10  $\mu$ L of each PCR sample into each gel well, and attach the cathode to the terminal close to the loaded wells. Attach the anode to the terminal farthest from the loaded wells, and apply 120 V to the gel. Run the gel until the bromophenol blue is about two-thirds the length of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Ethidium bromide solution* to cover the gel. Slowly agitate the gel on a shaker table for 30 minutes. Completely remove the *Ethidium bromide solution* from the tray, add an equal amount of *Tris-*

*boric acid buffer*, and slowly agitate the gel on a shaker table for 60 minutes. Place the gel on a 312-nm UV light source, photograph the gel, and inspect the image for bands that have migrated from each individual well. If a band appears, it is verified for size in base pairs by comparing it to the lane for the 100-bp DNA ladder marker. If a band appears and it is of the appropriate size, it is considered positive. The analysis is considered valid if the positive controls show the appropriately sized cDNA–PCR products, no PCR product bands appear in the negative controls, and all bands are observed to be visually discrete. The lanes of the agarose gel that correspond to Graftskin show cDNA bands for *Interleukin-1 $\alpha$*  (expected PCR product band size of 491 base pairs, limit of detection not less than  $9.6 \times 10^{-21}$  moles); *Platelet-derived growth factor* (expected PCR product band size of 304 base pairs, limit of detection not less than  $1.5 \times 10^{-20}$  moles); *Transforming growth factor- $\beta$ 1* (expected PCR product band size of 161 base pairs, limit of detection not less than  $1.5 \times 10^{-20}$  moles); and *Glyceraldehyde-3-phosphate dehydrogenase* (expected PCR product band size of 983 base pairs); but not *Interleukin-4* (expected PCR product band size of 344 base pairs, limit of detection not less than  $1.5 \times 10^{-22}$  moles). If one of the replicates tested yields results discordant with the other two replicates, repeat the assay, and accept only if all 3 replicates are concordant.

#### Barrier integrity assessment—

**Ham's F-12 tissue culture medium**—Prepare a solution that contains the following:

Component	mg per mL
L-Alanine	8.91
L-Arginine hydrochloride	210.7
L-Asparagine monohydrate	15.01
L-Aspartic acid	13.30
L-Cysteine hydrochloride monohydrate	35.12
L-Glutamic acid	14.70
L-Glutamine	146.2
Aminoacetic acid	7.51
L-Histidine hydrochloride monohydrate	20.96
L-Isoleucine	3.94
L-Leucine	13.12
L-Lysine hydrochloride	36.54
L-Methionine	4.48
L-Phenylalanine	4.96
L-Proline	34.53
L-Serine	10.51
L-Threonine	11.91
L-Tryptophan	2.04
L-Tyrosine disodium	6.71
L-Valine	11.71
Calcium chloride	44.00
Cupric sulfate, pentahydrate	0.0025
Ferric sulfate, heptahydrate	0.834
Potassium chloride	223.7
Magnesium chloride	57.22
Sodium chloride	7599.0
Sodium phosphate, dibasic	142.0
Zinc sulfate, heptahydrate	0.863
D-Biotin	0.0073
D-Calcium pantothenate	0.238
Choline chloride	13.96
Folic acid	1.30
Hypoxanthine	4.04
Inositol	18.02
Niacinamide	0.0366
Pyridoxine hydrochloride	0.0617

<sup>10</sup>A suitable cDNA positive control can be obtained from BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA 94303-4230.

<sup>11</sup>An agarose suitable for electrophoresis analysis of Graftskin cytokine PCR product is SeaKem® GTG agarose and can be obtained from BioWhittaker Molecular Applications, Inc., 191 Thomaston St., Rockland, ME 04841.

<sup>12</sup>A suitable buffered solution of *100-bp DNA ladder markers* can be obtained from BioWhittaker Molecular Applications, Inc., 191 Thomaston St., Rockland, ME 04841.



Component	mg per mL
Riboflavin	0.0376
Thiamine hydrochloride	0.337
Thymidine	0.727
Cyanocobalamin	1.36
$\alpha$ -Lipoic acid	0.206
Linoleic acid	0.0841
Dextrose	1801.6
Phenol red, sodium	1.30
Sodium pyruvate	110.0
Putrescine dihydrochloride	0.161
Sodium bicarbonate	1176.0

*Tritiated water*: 2.0  $\mu$ Ci/mL (see *Radioactivity* <821>).

*Percutaneous absorption apparatus*—Prepare the apparatus as described below.<sup>13</sup>

**Six-well cell culture plate**—The dimensions are inner diameter, about 35 mm; depth, about 18 mm.

**Cell culture well insert**—Each well is a plastic cylinder with inner length, about 15 mm; inner diameter, about 24 mm; outer diameter, about 27 mm, with a flanged end extending about 4 mm from the outer diameter. The inner diameter opposite the flanged end is covered by a taut polycarbonate membrane having a porosity of 3  $\mu$ m. The flange should allow the *Cell culture well insert* to be suspended in the well of a *Six-well cell culture plate*, leaving a 3-mm space between the bottom of the *Cell culture well insert* and the inner bottom surface of the *Six-well cell culture plate*.

*Percutaneous absorption insert*—Use a polytetrafluoroethylene cylinder having the following dimensions: length, about 20 mm; inner diameter, about 20 mm; outer diameter, about 23 mm with a flanged end extending about 3 mm from the outer diameter. Ten mm from the flanged end of the cylinder, the inner diameter begins to funnel so that the inner diameter at about 10 mm from the flanged end is about 20 mm, and the inner diameter at about 15 mm from the flanged end is about 8 mm. From about 15 mm to about 20 mm from the flanged end, the inner diameter remains at 8 mm. The outer diameter of the cylinder remains constant at about 23 mm. The flanged end is considered to be the top of the component.

*Silicon grease*—Use high-vacuum silicon grease suitable for glass.<sup>14</sup>

**Procedure**—Fill each well of the *Six-well cell culture plate* with 1.5 mL of *Ham's F-12 tissue culture medium*. Remove two 2-cm circular sections from every 30 cm<sup>2</sup> of *Graftskin* (not less than 20% of the total unit area), using the appropriate size biopsy punch. Transfer each excised section to a separate *Cell culture well insert*, dermal side down on the polycarbonate membrane. Using forceps, gently smooth out the section to remove any wrinkles. Apply a narrow ring of *Silicon grease* to the underside of the *Percutaneous absorption insert*, and place the insert into the *Cell culture well insert*, grease side down, onto the epidermal surface of the *Graftskin* biopsy, with slight pressure to form a tight seal. Do not allow any grease to enter the 8-mm diameter exposed area of the *Graftskin* surface. Place the *Cell culture well insert* containing the *Percutaneous absorption insert* into one of the wells of the *Six-well cell culture plate* containing 1.5 mL of *Ham's F-12 tissue culture medium*. Apply 1.0 mL of *Tritiated water* to the exposed surface of the *Graftskin* unit in the *Percutaneous absorption insert*, and incubate at ambient temperature for 6 hours. At the end of each hour, transfer the *Cell culture well insert* containing the *Percutaneous absorption*

*insert* to a new well within the *Six-well cell culture plate* containing 1.5 mL of fresh *Ham's F-12 tissue culture medium*. After the 6-hour incubation, remove the *Cell culture well insert*. Remove a 0.5-mL aliquot of *Ham's F-12 tissue culture medium* from each well of the *Six-well cell culture plate*, and transfer into individual scintillation vials. Dispense 0.5 mL of *Tritiated water* to a separate scintillation vial as a control; to each scintillation vial add 4.5 mL of a suitable scintillation cocktail,<sup>15</sup> and gently mix. Place the scintillation vials into a liquid scintillation counter, and count the emissions in the tritium spectrum for 60 seconds. Average the counts for each of the six time points (punch average) and duplicate sections (unit average). Determine the percent penetration per hour by the formula:

$$150(C_S/C_C)$$

in which  $C_S$  are the counts per minute of the 0.5-mL aliquot of the *Ham's F-12 tissue culture medium* taken at the end of the incubation period; and  $C_C$  are the counts per minute in the 0.5-mL aliquot of *Tritiated water*. Not more than 1.97% penetration is found.

#### Metabolic activity assessment—

*Dulbecco's modified Eagle's tissue culture medium*—Prepare a solution that contains the following components.

Component	mg per L
Calcium chloride	264.9
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

**MTT solution**—Dissolve 0.33 g of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Dulbecco's modified Eagle's tissue culture medium*, with constant stirring. Pass the solution through a suitable size filter having a 0.2- $\mu$ m porosity.

<sup>13</sup>A suitable *Percutaneous absorption apparatus*, not including the *Percutaneous absorption insert*, is a Costar® 6-well culture cluster, flat bottom with lid, and a Costar® Transwell®, 24 mm in a 6-well cluster plate with lid and can be obtained from Corning Life Sciences, 45 Nagog Park, Acton, MA 01720.

<sup>14</sup>A suitable *Silicon grease* is High Vacuum Silicon Lubricant for Glass and can be obtained from Dow Corning Corporation, P.O. Box 0994, Midland, MI 48686-0994.

<sup>15</sup>A suitable scintillation cocktail is Optiphase®, Supermix®, Perkin-Elmer Life Sciences, Inc., 549 Albany St., Boston, MA 02118.

**0.04 N Acidified isopropyl alcohol**—Add 3.45 mL of hydrochloric acid to 1 L of isopropyl alcohol, and mix thoroughly. Store at room temperature no longer than 6 months.

**Procedure**—Immerse the Graftskin in separate 40.0-mL portions of *MTT solution*, making sure that about 20 mL of *MTT solution* is under the test article, and 20 mL of *MTT solution* is on the surface. Take care not to produce any bubbles. Incubate for 3 hours at 37°, in an environment of air enriched with 10% carbon dioxide. After incubation, remove from the 37°, 10% carbon dioxide-enriched air environment. Transfer the Graftskin to a suitable cutting surface, and, using an appropriate biopsy punch, remove three 8-mm diameter circular sections from every 30 cm<sup>2</sup> of Graftskin (5% of unit area). Transfer each punch to individual snap-top test tubes. Add 0.9 mL of *0.04 N Acidified isopropyl alcohol* to each tube, making sure that the tissue is completely submerged. If not submerged, use forceps to place the sample into the *0.04 N Acidified isopropyl alcohol*. Cap each tube tightly, place on an orbital shaker, and shake for 1 hour at a moderate setting. After 1 hour, remove the tubes from the orbital shaker, and mix each tube on a vortex mixer. Inspect the tubes to make sure that the tissue samples continue to be submerged. If not, use forceps or another device to resubmerge the tissues. Return the tubes to the orbital shaker, and continue to shake for an additional 1 hour. Remove the tubes from the orbital shaker, mix the tubes on a vortex mixer, and transfer a 0.2-mL aliquot to a suitable 96-well flat-bottom plate. Read the absorbance of each sample at 570 nm, using 0.2 mL of *0.04 N Acidified isopropyl alcohol* as the blank. The average absorbance value is  $\geq 0.237$ .

## Gramicidin

Gramicidin.

Gramicidin [1405-97-6].

» Gramicidin is an antibacterial substance produced by the growth of *Bacillus brevis* Dubos (Fam. Bacillaceae). It may be obtained from tyrothricin. It has a potency of not less than 900 µg of gramicidin per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Gramicidin RS

**Identification, Ultraviolet Absorption** (197U)—

*Solution:* 50 µg per mL.

*Medium:* alcohol.

**Melting temperature, Class Ia** (741): not lower than 229°, determined after drying.

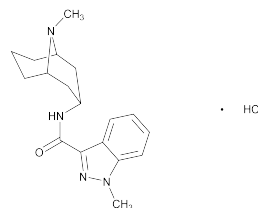
**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

**Residue on ignition** (281): not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay**—Proceed with Gramicidin as directed under *Antibiotics—Microbial Assays* (81).

## Granisetron Hydrochloride



$C_{18}H_{24}N_4O \cdot HCl$  348.87

1*H*-Indazole-3-carboxamide, 1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-, monohydrochloride, *endo*-. 1-Methyl-*N*-(9-methyl-*endo*-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide monohydrochloride [107007-99-8].

» Granisetron Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of  $C_{18}H_{24}N_4O \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at room temperature.

**USP Reference standards** (11)—

USP Granisetron Hydrochloride RS

USP Granisetron Related Compound A RS

(2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide).

USP Granisetron Related Compound B RS

(*N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide).

USP Granisetron Related Compound E RS

((1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]nonan-3-amine, acetate salt).

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** It meets the requirements of the test for *Chloride* (191).

**pH** (791): between 4.0 and 6.5, in a solution in carbon dioxide-free water, containing 10 mg per mL.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): not more than 20 ppm.

**Limit of granisetron related compound E**—

*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture.

*Diluent:* a mixture of acetonitrile and water (80:20).

*Test solution*—Transfer 250 mg of Granisetron Hydrochloride to a 5-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.

*Standard solution*—Dissolve an accurately weighed quantity of USP Granisetron Related Compound E RS in *Diluent* to obtain a solution containing 0.25 mg per mL. [NOTE—USP Granisetron Related Compound E RS is the acetate salt of (1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]

*Application volume:* 2 µL.

*Developing solvent system*—A mixture of ethyl acetate, isopropyl alcohol, and ammonium hydroxide (50:30:6.5).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621), and develop the chromatogram until the solvent front has moved about half of the length of the plate. Dry the plate in air, and expose it to

iodine vapor for 30 minutes. Any spot corresponding to granisetron related compound E obtained from the *Test solution* is not more intense than the corresponding spot obtained from the *Standard solution* (0.5%).

#### Related compounds—

[NOTE—Protect all solutions containing granisetron hydrochloride from light.]

*Mobile phase* and *System suitability solution*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.005 mg per mL.

*Identification solution*—Prepare a solution in *Mobile phase* containing 0.01 mg per mL of USP Granisetron Related Compound A RS and 0.005 mg per mL of USP Granisetron Related Compound B RS.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Chromatographic system* (see *Chromatography* <621>)—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between granisetron related compound C and granisetron is not less than 3.5; and the tailing factor for the granisetron peak is not more than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution*, the *Identification solution*, and the *Test solution* into the chromatograph, record the chromatograms for about two times the retention time of granisetron, identify the peaks on the basis of their relative retention times as given in *Table 1*, and measure the peak responses. Calculate the percentage of each impurity in the portion of Granisetron Hydrochloride taken by the formula:

$$100(1 / F)(C_S / C_T)(r_U / r_S)$$

in which  $F$  is the relative response factor given in *Table 1*;  $C_S$  and  $C_T$  are the concentrations, in mg per mL, of granisetron hydrochloride in the *Standard solution* and the *Test solution*, respectively;  $r_U$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the peak response for granisetron obtained from the *Standard solution*: in addition to not exceeding the limits in *Table 1*, not more than 1.0% of total impurities is found. Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.

#### Assay—

*Mobile phase*—Dilute 1.6 mL of phosphoric acid with water to 800 mL, add 200 mL of acetonitrile, and mix. Add 1.0 mL of hexylamine, and mix. Adjust with triethylamine to

a pH of  $7.5 \pm 0.05$  (about 4 mL is needed). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.

*System suitability solution*—Transfer 2 mL of a solution in *Mobile phase* containing about 1.0 mg of Granisetron Hydrochloride per mL to a colorless glass vial, stopper it, and either expose the solution to sunlight for 4 hours or place it under a UV lamp for 16 hours (granisetron undergoes partial degradation to granisetron related compound C). A degradation of at least about 0.3% of granisetron to granisetron related compound C must be obtained, as shown by the appearance of a corresponding peak in the chromatogram. If it is not obtained, again expose the solution to sunlight or place it under a UV lamp.

*Assay preparation*—Transfer about 50 mg of Granisetron Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 305-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between granisetron related compound C and granisetron is not less than 3.5; and the tailing factor for the granisetron peak is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_{18}H_{24}N_4O \cdot HCl$  in the portion of Granisetron Hydrochloride taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of granisetron hydrochloride in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Table 1

Name	Relative Retention Time	Relative Response Factor ( $F$ )	Limit (%)
Granisetron related compound D <sup>1</sup>	0.4	1.0	0.1
Granisetron related compound B <sup>2</sup>	0.5	0.59	0.5
Granisetron related compound A <sup>3</sup>	0.7	1.0	1.0
Granisetron related compound C <sup>4</sup>	0.8	1.0	0.2
Granisetron	1.0	n/a	n/a
Any other individual impurity	n/a	1.0	0.1

<sup>1</sup>1-Methyl-1H-indazole-3-carboxylic acid.

<sup>2</sup>N-[(1R,3R,5S)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1H-indazole-3-carboxamide.

<sup>3</sup>2-Methyl-N-[(1R,3R,5S)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2H-indazole-3-carboxamide.

<sup>4</sup>N-[(1R,3R,5S)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1H-indazole-3-carboxamide.

## Granisetron Hydrochloride Injection

» Granisetron Hydrochloride Injection is a sterile solution of Granisetron Hydrochloride in Water for Injection. It contains the equivalent of not less than 93.0 percent and not more than 107.0 percent of the labeled amount of granisetron ( $C_{18}H_{24}N_4O$ ). It may contain suitable preservatives.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, protected from light, and store at controlled room temperature.

**Labeling**—It meets the requirements for *Labeling* under *Injections* (1). Label it to indicate the name and the quantity of any added preservative.

### USP Reference standards (11)—

USP Endotoxin RS

USP Granisetron Hydrochloride RS

USP Granisetron Related Compound B RS

(*N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide).

USP Granisetron Related Compound C RS

*N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

USP Granisetron Related Compound D RS

1-Methyl-1*H*-indazole-3-carboxylic acid.

### Identification—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

*Developing solvent*—Prepare a mixture of methylene chloride, alcohol, water, and ammonium hydroxide (60:40:5:2).

*Standard solution*—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in water or alcohol to obtain a solution having a concentration of granisetron that matches the concentration of granisetron in the *Test solution*. To calculate the concentration of granisetron in the *Standard solution*, use the molecular weights of granisetron (312.41) and granisetron hydrochloride (348.87).

*Test solution*—Use the undiluted Injection.

*Procedure*—Separately apply equal volumes of the *Standard solution* and the *Test solution*, equivalent to about 4 to 5 µg of granisetron, to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, dry the spots under a current of warm air for about 5 minutes, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent* prior to use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, dry the plate under a current of warm air, and examine the plate under short-wavelength UV light: the principal spot from the *Test solution* corresponds in appearance and  $R_f$  value to that of the *Standard solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 25 USP Endotoxin Units per mg of granisetron.

**Sterility** (71): meets the requirements.

**pH** (791): between 4.0 and 6.0.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Related compounds**—[NOTE—Perform the determination under subdued light, and use amber autosampler vials and low-actinic glassware.]

*Buffer solution pH 2.0*, *Mobile phase*, *System suitability preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation* prepared as directed in the *Assay*.

*Test solution*—Use the undiluted Injection.

*Procedure*—Separately inject equal volumes (about 15/L µL), where *L* is the labeled amount, in mg, of granisetron per mL of Injection of the *Standard solution* and the *Test solution*, into the chromatograph. Record the chromatograms for at least three times the retention time of the granisetron peak, identify the impurities listed in *Table 1*, and measure the peak responses. Calculate the percentage of each impurity relative to the labeled content of granisetron in the portion of Injection taken by the formula:

$$100(1/F)(312.41/348.87)(C_S/C_T)(r_i/r_S)$$

in which *F* is the relative response factor as listed in *Table 1*; 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively;  $C_S$  is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of granisetron in the *Test solution*, based on the label claim;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_S$  is the peak response of the granisetron peak, obtained from the *Standard solution*. Disregard the peak due to granisetron related compound A that elutes at the relative retention time of about 0.5–0.6 as this impurity is controlled in the drug substance monograph. Not more than 0.7% of granisetron related compound C is found, not more than 1.3% of total specified impurities is found, and not more than 0.5% of any unspecified impurity is found. The reporting level for impurities is 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor ( <i>F</i> )
Granisetron related compound A <sup>1</sup>	0.5–0.6	—
Granisetron related compound B <sup>2</sup>	0.7	0.8
Granisetron	1.0	—
Granisetron related compound C <sup>3</sup>	1.2	1.0
Granisetron related compound D <sup>4</sup>	2.1–2.3	1.5

<sup>1</sup> 2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.

<sup>2</sup> *N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.

<sup>3</sup> *N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

<sup>4</sup> 1-Methyl-1*H*-indazole-3-carboxylic acid.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—[NOTE—Perform the determination under subdued light, and use amber autosampler vials and low-actinic glassware.]

*Buffer solution pH 2.0*—Dissolve 15.6 g of monobasic sodium phosphate dihydrate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute with water to 1000 mL.

*Mobile phase*—Prepare a mixture of *Buffer solution pH 2.0*, methanol, and tetrahydrofuran (75:24:1.1), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability preparation*—Dissolve suitable quantities of USP Granisetron Hydrochloride RS, USP Granisetron Related Compound B RS, USP Granisetron Related Compound C RS, and USP Granisetron Related Compound D RS in a mixture of water and methanol (75:25), to obtain a solution containing about 0.1 mg of each component per mL. Dilute with water to obtain a solution containing about *L* µg

of each component per mL, where *L* is the labeled amount, in mg, of granisetron per mL of Injection.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in water to obtain a solution having a known concentration of about  $(0.11 \times L)$  mg of granisetron hydrochloride per mL, where *L* is the labeled amount, in mg, of granisetron per mL of Injection.

**Assay preparation**—Use the Injection diluted 1:10 with water.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm  $\times$  15-cm column that contains 4- $\mu$ m polar endcapped packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *System suitability preparation*, and identify the components based on the information listed in *Table 1*. Record the peak responses as directed for *Procedure*: the resolution, *R*, between the granisetron and granisetron related compound C peaks is not less than 2. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing for the granisetron peak is not more than 3; and the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15/*L*  $\mu$ L), where *L* is the labeled amount, in mg, of granisetron per mL of Injection, of the *Standard preparation* and the *Assay preparation* into the chromatograph. Record the chromatograms for at least three times the retention time of the granisetron peak, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of granisetron ( $C_{18}H_{24}N_4O$ ) in each mL of the Injection by the formula:

$$100(312.41 / 348.87)(C/L)(r_U / r_S)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; *C* is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard preparation*; *L* is as defined above; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Granisetron Hydrochloride Oral Suspension

### DEFINITION

Granisetron Hydrochloride Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of granisetron hydrochloride ( $C_{18}H_{24}N_4O \cdot HCl$ ). Prepare Granisetron Hydrochloride Oral Suspension (0.056 mg/mL) equivalent to 0.05 mg of Granisetron per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Granisetron (as Hydrochloride)	5 mg (5.6 mg)
Vehicle: A mixture of Vehicle for Oral Solution, NF, and Vehicle for Oral Suspension, NF, (1:1), a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder with a pestle, or add *Granisetron Hydrochloride* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a *Granisetron Hydrochloride* suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile and 50 mM monobasic sodium dihydrogen phosphate (3:17). Adjust with phosphoric acid to a pH of 7.0. Make adjustments if necessary (see *Chromatography* <621>, *System Suitability*.)

**Standard stock solution:** 1.0 mg/mL USP Granisetron Hydrochloride RS

**Standard solution:** Transfer 2.5 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 25  $\mu$ g/mL of granisetron hydrochloride.

**Sample solution:** Shake the Oral Suspension thoroughly by hand. Pipet 5.0 mL into a 10-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a nominal concentration of 25  $\mu$ g of granisetron hydrochloride/mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L10

**Flow rate:** 1.0 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of the granisetron peak is 7.0 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 2.0% for the replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of  $C_{18}H_{24}N_4O \cdot HCl$  in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of granisetron hydrochloride in the *Standard solution* ( $\mu$ g/mL)

*C<sub>U</sub>* = nominal concentration of granisetron hydrochloride in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

• **pH** <791>: 4.0–4.5

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or controlled cold temperature.
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or when stored at controlled cold temperature
- **USP REFERENCE STANDARDS** <11>  
USP Granisetron Hydrochloride RS

## Granisetron Hydrochloride Tablets

» Granisetron Hydrochloride Tablets contain an amount of Granisetron Hydrochloride equivalent to not less than 92.0 percent and not more than 108.0 percent of the labeled amount of granisetron ( $C_{18}H_{24}N_4O$ ).

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at controlled room temperature.

**USP Reference standards** (11)—

USP Granisetron Hydrochloride RS

USP Granisetron Related Compound B RS

(N-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide).

USP Granisetron Related Compound C RS

N-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

USP Granisetron Related Compound D RS

1-Methyl-1*H*-indazole-3-carboxylic acid.

**Identification**—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

*Developing solvent*—Prepare a mixture of methylene chloride, alcohol, water, and ammonium hydroxide (60:40:5:2).

*Standard solution*—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in 0.1 N hydrochloric acid to obtain a solution containing 0.44 mg of granisetron hydrochloride per mL.

*Test solution*—Transfer a number of Tablets, equivalent to about 2 mg of granisetron, to a suitable container, add 5.0 mL of 0.1 N hydrochloric acid, and sonicate for about 3 minutes. Pass through a 0.45-μm filter.

*Procedure*—Separately apply 20 μL each of the *Standard solution* and the *Test solution* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, dry the spots under a current of warm air for about 5 minutes, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent*. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, dry the plate under a cold air stream for about 10 minutes, and examine the plate under short-wavelength UV light: the principal spot from the *Test solution* corresponds in appearance and *R<sub>f</sub>* value to that of the *Standard solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* pH 6.5 phosphate buffer, prepared by dissolving 6.8 g of monobasic potassium phosphate in 800 mL of water, adjusting to pH 6.5 with 1 N sodium hydroxide, and diluting to 1 L with water; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Buffer solution pH 2.0, Mobile phase, Diluent, System suitability preparation, and Standard preparation*—Prepare as directed in the *Assay*.

*Standard solution*—Transfer 5.0 mL of the *Standard preparation* to a 250-mL volumetric flask, and dilute with *Diluent* to volume.

*Test solution*—Pass a portion of the solution under test through a 0.45-μm filter. If necessary, further dilute 5 mL of this solution with *Diluent* to (5 × *L*) mL, where *L* is the Tablet label claim, in mg.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not less than 0.8 and not more than 1.5; and the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 100 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and meas-

ure the peak responses. Calculate the amount of C<sub>18</sub>H<sub>24</sub>N<sub>4</sub>O dissolved by the formula:

$$(312.41 / 348.87)(r_U \times C_S \times 500 \times D \times 100) / (r_S \times L)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses for the *Test solution* and the *Standard solution*, respectively; *C<sub>S</sub>* is the concentration, in mg per mL, of the *Standard solution*; 500 is the volume, in mL, of the *Medium*; *D* is the dilution factor of the *Test solution*; 100 is the conversion factor to percentage; and *L* is the Tablet label claim, in mg.

*Tolerances*—Not less than 75% (Q) of the labeled amount of C<sub>18</sub>H<sub>24</sub>N<sub>4</sub>O is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—[NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.]

*Buffer solution pH 2.0, Mobile phase, Diluent, System suitability preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the *Assay*.

*Test solution*—Use the *Assay preparation*, prepared as directed in the *Assay*.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least three times the retention time of the granisetron peak, identify the impurities listed in *Table 1*, and measure the peak responses. Calculate the percentage of each impurity relative to the labeled content of granisetron in the portion of Tablets taken by the formula:

$$100(1 / F)(312.41 / 348.87)(C_S / C_U)(r_i / r_S)$$

in which *F* is the relative response factor as listed in *Table 1*; 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; *C<sub>S</sub>* is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard solution*; *C<sub>U</sub>* is the concentration, in mg per mL, of granisetron in the *Test solution*, based on the label claim; *r<sub>i</sub>* is the peak response of each impurity obtained from the *Test solution*; and *r<sub>S</sub>* is the peak response of the granisetron peak, obtained from the *Standard solution*. Disregard the peak due to granisetron related compound A that elutes at the relative retention time of about 0.5–0.6, as this impurity is controlled in the drug substance monograph. Not more than 0.7% of granisetron related compound C is found, not more than 1.3% of total specified impurities is found, and not more than 0.5% of any unspecified impurity is found. The reporting level for impurities is 0.1%.

**Table 1**

Name	Relative Retention Time	Relative Response Factor ( <i>F</i> )
Granisetron related compound A <sup>1</sup>	0.5–0.6	—
Granisetron related compound B <sup>2</sup>	0.7	0.8
Granisetron	1.0	—

<sup>1</sup> 2-Methyl-N-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.

<sup>2</sup> N-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.

<sup>3</sup> N-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

<sup>4</sup> 1-Methyl-1*H*-indazole-3-carboxylic acid.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor (F)
Granisetron related compound C <sup>3</sup>	1.2	1.0
Granisetron related compound D <sup>4</sup>	2.1–2.3	1.5

<sup>1</sup> 2-Methyl-N-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.

<sup>2</sup> N-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.

<sup>3</sup> N-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

<sup>4</sup> 1-Methyl-1*H*-indazole-3-carboxylic acid.

**Assay**—[NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.]

**Buffer solution pH 2.0**—Dissolve 15.6 g of monobasic sodium phosphate dihydrate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute with water to 1000 mL.

**Mobile phase**—Prepare a mixture of **Buffer solution pH 2.0**, methanol, and tetrahydrofuran (75:24:1.1), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Use **Buffer solution pH 2.0**.

**System suitability preparation**—Dissolve suitable amounts of USP Granisetron Hydrochloride RS, USP Granisetron Related Compound B RS, USP Granisetron Related Compound C RS, and USP Granisetron Related Compound D RS in **Diluent**, to obtain a solution having about 0.1 mg of granisetron hydrochloride per mL and about 0.01 mg of each of granisetron related compounds B, C, and D.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in **Diluent** to obtain a solution having a known concentration of about 0.11 mg of granisetron hydrochloride per mL.

**Assay preparation**—Prepare a solution containing about 0.1 mg of granisetron (base) per mL, based on the label claim, using the following procedure: fill a suitable volumetric flask with **Diluent**, add 5 Tablets and sonicate for approximately 20 minutes until the Tablets disintegrate completely. Pass a portion of this solution through a 0.45-μm membrane filter, discarding the first few mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 300-nm detector, and 4.6-mm × 15-cm column that contains 4-μm polar endcapped packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *System suitability preparation*, and identify the components based on the information listed in *Table 1*. Record the peak responses as directed for *Procedure*: the column efficiency, determined from the granisetron peak, is not less than 1200 theoretical plates; the tailing factor for the granisetron peak is not less than 0.8 and not more than 1.5; the resolution, *R*<sub>s</sub>, between the granisetron and granisetron related compound C peaks is not less than 2. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of granisetron (C<sub>18</sub>H<sub>24</sub>N<sub>4</sub>O) in each Tablet taken by the formula:

$$100(312.41 / 348.87)(CV / NL)(r_U / r_S)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; C is

the concentration, in mg per mL, of granisetron hydrochloride in the *Standard preparation*; V is the volume, in mL, of the *Assay preparation*; N is the number of Tablets taken to prepare the *Assay preparation*; L is the Tablet label claim, in mg; and *r*<sub>U</sub> and *r*<sub>S</sub> are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Green Soap

» Green Soap is a potassium soap made by the saponification of suitable vegetable oils, excluding coconut oil and palm kernel oil, without the removal of glycerin.

Green Soap may be prepared as follows:

The Vegetable Oil . . . . .	380 g
Oleic Acid . . . . .	20 g
Potassium Hydroxide (total alkali 85 percent) . . . . .	91.7 g
Glycerin . . . . .	50 mL
Purified Water, a sufficient quantity to make about . . . . .	1000 g

Mix the oil and the Oleic Acid, and heat the mixture to about 80°. Dissolve the Potassium Hydroxide in a mixture of the Glycerin and 100 mL of Purified Water, and add the solution, while it is still hot, to the hot oil. Stir the mixture vigorously until emulsified, then heat while continuing the stirring, until the mixture is homogeneous and a test portion will dissolve to give a clear solution in hot water. Add hot purified water to make the product weigh 1000 g, continuing the stirring until the Soap is homogeneous.

**Packaging and storage**—Preserve in well-closed containers.

**Water**—Place about 5 g of Soap, quickly weighed to the nearest centigram, in the distilling flask of the apparatus for *Moisture Method by Toluene Distillation* (921). (The Soap is most conveniently weighed in a boat of metal foil, of a size that will just pass through the neck of the flask.) Place 250 mL of toluene and 10 g of anhydrous barium chloride in the flask, connect the flask through a ground-glass joint to the distilling apparatus, fill the receiving tube with toluene, and determine the water as directed, beginning with "Heat the flask gently." The volume of water found corresponds to not more than 52.0% by weight of the Soap taken.

**Alcohol-insoluble substances**—Dissolve about 5 g of Soap, rapidly and accurately weighed, in 100 mL of hot neutralized alcohol, collect the residue, if any, on a tared filter, thoroughly wash it with hot neutralized alcohol, and dry at 105° for 1 hour: the weight of the residue so obtained is not more than 3.0% of the weight of the Soap taken. Retain the solution for the test for *Free alkali hydroxides*, and retain the residue for the test for *Alkali carbonates*.

**Free alkali hydroxides**—To the combined filtrate and washings obtained in the test for *Alcohol-insoluble substances* add 0.5 mL of phenolphthalein TS. If a pink color is produced, titrate the solution with 0.1 N sulfuric acid VS until the pink color is just discharged. Each mL of 0.1 N sulfuric acid is equivalent to 5.611 mg of KOH. The volume of 0.1 N sulfuric acid VS consumed corresponds to not more than 0.25% of KOH.

**Alkali carbonates**—Wash the filter containing the *Alcohol-insoluble substances* with 50 mL of boiling water, cool, add methyl orange TS, and titrate the filtrate with 0.1 N sulfuric acid VS. Not more than 0.5 mL of 0.10 N sulfuric acid per g of Soap originally taken is required (0.35% as  $K_2CO_3$ ).

**Unsaponified matter**—A solution of Soap in hot water (1 in 20) is nearly clear.

**Characteristics of the liberated fatty acids**—Dissolve about 30 g of Soap in 300 mL of hot water in a beaker, add gradually 60 mL of 2 N sulfuric acid, and heat on a steam bath until the liberated acids form a transparent layer. Decant the fatty acids into a separator, and wash them with 50-mL portions of hot water until the last washing, when cool, is neutral to methyl orange TS. Transfer the fatty acids to a dry beaker, and allow them to stand in a warm oven until any water that may be present has separated. Then filter the acids through a dry filter in the warm oven. Determine the *Acid Value* (see *Fats and Fixed Oils* (401)) of about 1 g, accurately weighed, of the fatty acids: it is not more than 205. Determine the *Iodine Value* (see *Fats and Fixed oils* (401)) of 150 to 200 mg, accurately weighed, of the fatty acids: it is not less than 85.

## Green Soap Tincture

» Prepare Green Soap Tincture as follows:

Green Soap .....	650 g
Suitable essential oil(s) Alcohol .....	316 mL
Purified Water, a sufficient quantity to make .....	1000 mL

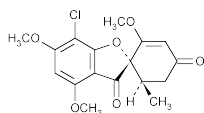
Mix the oil(s) and Alcohol, dissolve in this the Green Soap by stirring or by agitation, set the solution aside for 24 hours, filter through paper, and add water to make 1000 mL.

**Packaging and storage**—Preserve in tight containers.

**Identification**—Transfer 10 mL to a flask containing 10 mL of water, and carefully add 1 mL of sulfuric acid: fatty material separates.

**Alcohol content, Method II** (611): between 28.0% and 32.0% of  $C_2H_5OH$ .

## Griseofulvin



$C_{17}H_{17}ClO_6$  352.77

Spiro[benzofuran-[2](3*H*),1'-2cyclohexene]-3,4'-dione, 7-chloro-2',4,6-trimethoxy-6'-methyl-, (1'*S*-trans)-, 7-Chloro-2',4,6-trimethoxy-6' $\beta$ -methylspiro[benzofuran-2(3*H*), 1'-[2]cyclohexene]-3,4'-dione [126-07-8].

» Griseofulvin has a potency of not less than 900  $\mu$ g of  $C_{17}H_{17}ClO_6$  per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Griseofulvin RS

USP Griseofulvin Permeability Diameter RS

**Identification**—

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 217° and 224°.

**Specific rotation** (781S): between +348° and +364°.

*Test solution:* 10 mg per mL, in dimethylformamide.

**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Permeability diameter**—Determine the apparent particle size in  $\mu$ m by the air-permeation method, using a suitable subsieve sizer. Weigh  $1.819 \pm 0.001$  g of Griseofulvin, and transfer to the compression tube of the apparatus. Compact with moderate pressure so that a uniform porosity is achieved. Pass dry compressed air through the tube, and measure the air pressure with a water manometer. Read the porosity, and calculate the apparent particle size from the instrument equation. Repeat the porosity readings at successively higher degrees of compaction until the apparent particle size reaches a minimum value. Calculate the observed permeability diameter, in square meters per g, taken by the formula:

$$6 / (1.455MF)$$

in which  $M$  is the minimum apparent particle size; and  $F$  is a factor, obtained from the accompanying table, interpolation being used if necessary, to correct the apparent particle size to the true particle size at a given porosity reading.

Porosity Reading	$F$	Porosity Reading	$F$
0.80	1.3771	0.56	1.7353
0.76	1.4142	0.52	1.8528
0.72	1.4573	0.48	2.0076
0.68	1.5082	0.44	2.2203
0.64	1.5690	0.40	2.5298
0.60	1.6432		

Concomitantly determine the observed permeability diameter of a similar preparation of USP Griseofulvin Permeability Diameter RS. Calculate the permeability diameter of the Griseofulvin taken by the formula:

$$O_U (A_5 / O_5)$$

in which  $O_U$  is the observed permeability diameter of the specimen,  $A_5$  is the assigned permeability diameter of USP Griseofulvin Permeability Diameter RS, and  $O_5$  is the observed permeability diameter of USP Griseofulvin Permeability Diameter RS: it is between 1.3 and 1.7 square meters per g.

**Heavy metals, Method II** (231): 0.0025%.

**Assay**—

*Mobile phase*—Prepare a suitable filtered mixture of water, acetonitrile, and tetrahydrofuran (60:35:5). Degas for 5 minutes before use, and stir continuously during use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).



**Standard preparation**—Dissolve an accurately weighed quantity of USP Griseofulvin RS in methanol to obtain a solution having a known concentration of about 1.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.125 mg of USP Griseofulvin RS in each mL.

**Assay preparation**—Transfer about 62 mg of Griseofulvin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. The relative standard deviation for replicate injections of *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the peak responses for the major peaks. Calculate the quantity, in µg of C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub>, in each mg of the Griseofulvin taken by the formula:

$$500(CP / W_U)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Griseofulvin RS in the *Standard preparation*; P is the content, in µg of C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub> per mg, of USP Griseofulvin RS; W<sub>U</sub> is the quantity, in mg, of Griseofulvin taken; and r<sub>U</sub> and r<sub>S</sub> are the griseofulvin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Griseofulvin Capsules

» Griseofulvin Capsules contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of griseofulvin (C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub>).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the griseofulvin contained is known as griseofulvin (microsize).

**USP Reference standards** <11>—  
USP Griseofulvin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—

*Medium*: water containing 5.4 mg of sodium lauryl sulfate per mL; 1000 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub> dissolved from UV absorbances at the wavelength of maximum absorbance at about 291 nm of filtered portions of the solution under test, suitably diluted with a solution of methanol and water (4:1), if necessary, in comparison with a *Standard solution* having a known concentration of USP Griseofulvin RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub> is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—Transfer the contents of 1 Capsule to a suitable container, add an accurately measured volume of methanol sufficient to yield a concentration of griseofulvin not greater than 1 mg per mL, shake by me-

chanical means for 1 hour, or longer if necessary to disperse the specimen completely, and sonicate for 1 minute. Centrifuge a portion of this solution, and quantitatively dilute an accurately measured volume of the clear supernatant to obtain a test solution containing about 10 µg of griseofulvin per mL. Concomitantly determine the absorbances of the test solution and a *Standard solution* of USP Griseofulvin RS in methanol having a known concentration of about 10 µg per mL at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of griseofulvin (C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub>) in the Capsule taken by the formula:

$$(CL / D)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Griseofulvin RS in the *Standard solution*; L is the labeled quantity, in mg, of griseofulvin in the Capsule; D is the concentration, in µg per mL, of griseofulvin in the test solution, based on the labeled quantity per Capsule and the extent of dilution; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the test solution and the *Standard solution*, respectively.

**Loss on drying** <731>—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 1.0% of its weight.

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Griseofulvin*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 125 mg of griseofulvin, to a 100-mL volumetric flask. Add about 70 mL of methanol, shake by mechanical means for 30 minutes, dilute with methanol to volume, and mix. Filter a portion of this solution, discarding the first 5 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Griseofulvin*. Calculate the quantity, in mg, of griseofulvin (C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub>) in the portion of Capsules taken by the formula:

$$PC(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Griseofulvin RS in the *Standard preparation*, and the other terms are as defined therein.

## Griseofulvin Oral Suspension

» Griseofulvin Oral Suspension contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of griseofulvin (C<sub>17</sub>H<sub>17</sub>ClO<sub>6</sub>). It contains one or more suitable colors, diluents, flavors, preservatives, and wetting agents.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the griseofulvin contained is known as griseofulvin (microsize).

**USP Reference standards** <11>—  
USP Griseofulvin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.5 and 7.5.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Griseofulvin*.

*Sodium chloride solution*—Dissolve a suitable quantity of sodium chloride in water to obtain a solution containing about 0.1 g per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 125 mg of griseofulvin, to a glass-stoppered, 50-mL centrifuge tube. Add 20 mL of *Sodium chloride solution* and 20 mL of methylene chloride. Insert the stopper into the tube, and mix by rotating the tube for 10 minutes. Separate the phases by centrifugation, carefully remove the lower methylene chloride layer with a needle and syringe, and filter through methylene chloride-pretreated anhydrous sodium sulfate into a 100-mL volumetric flask. Repeat the extraction with two additional 20-mL portions of methylene chloride, combining the extracts in the volumetric flask. Dilute with methylene chloride to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, and evaporate on a steam bath under a stream of nitrogen to dryness. Transfer 4.0 mL of *Mobile phase* to the flask, swirl to dissolve the residue, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Griseofulvin*. Calculate the quantity, in mg, of griseofulvin ( $C_{17}H_{17}ClO_6$ ) in each mL of the Oral Suspension taken by the formula:

$$(PC / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Griseofulvin RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and the other terms are as defined therein.

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**Griseofulvin Tablets**


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» Griseofulvin Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of griseofulvin ( $C_{17}H_{17}ClO_6$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the griseofulvin contained is known as griseofulvin (microsize).

**USP Reference standards** (11)—

USP Griseofulvin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution** (711)—

*Medium*: water containing 40.0 mg of sodium lauryl sulfate per mL; 1000 mL.

*Apparatus 2*: 75 rpm.

*Time*: 90 minutes.

*Procedure*—Determine the amount of  $C_{17}H_{17}ClO_6$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 291 nm of filtered portions of the solution under test, suitably diluted with a solution of methanol and water (4:1), if necessary, in comparison with a Standard

solution having a known concentration of USP Griseofulvin RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{17}ClO_6$  is dissolved in 90 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Transfer 1 Tablet to a suitable container, add an accurately measured volume of methanol sufficient to yield a concentration of griseofulvin not greater than 1 mg per mL, shake by mechanical means for 1 hour, or longer if necessary to disperse the specimen completely, and sonicate for 1 minute. Centrifuge a portion of this solution, and quantitatively dilute an accurately measured volume of the clear supernatant to obtain a test solution containing about 10 µg of griseofulvin per mL. Concomitantly determine the absorbances of the test solution and a Standard solution of USP Griseofulvin RS in methanol having a known concentration of about 10 µg per mL at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of griseofulvin ( $C_{17}H_{17}ClO_6$ ) in the Tablet taken by the formula:

$$(CL / D)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Griseofulvin RS in the Standard solution; L is the labeled quantity, in mg, of griseofulvin in the Tablet; D is the concentration, in µg per mL, of griseofulvin in the test solution, based on the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the test solution and the Standard solution, respectively.

**Loss on drying** (731)—Dry about 100 mg of finely ground Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Griseofulvin*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets, and proceed as directed for *Assay preparation* in the Assay under *Griseofulvin Capsules*, beginning with "transfer an accurately weighed portion."

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Griseofulvin*. Calculate the quantity, in mg, of griseofulvin ( $C_{17}H_{17}ClO_6$ ) in the portion of Tablets taken by the formula:

$$PC(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Griseofulvin RS in the *Standard preparation*, and the other terms are as defined therein.

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**Ultramicronsize Griseofulvin Tablets**


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» Ultramicronsize Griseofulvin Tablets are composed of ultramicronsize crystals of Griseofulvin dispersed in Polyethylene Glycol 6000 or dispersed by other suitable means. They contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of griseofulvin ( $C_{17}H_{17}ClO_6$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Griseofulvin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—

*Medium*: water containing 5.4 mg of sodium lauryl sulfate per mL; 1000 mL.

*Apparatus 2*: 75 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{17}H_{17}ClO_6$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 291 nm of filtered portions of the solution under test, suitably diluted with a solution of methanol and water (4:1), if necessary, in comparison with a Standard solution having a known concentration of USP Griseofulvin RS in the same medium.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{17}ClO_6$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

*Procedure for content uniformity*—Proceed as directed for *Procedure for content uniformity* in the test for *Uniformity of dosage units* under *Griseofulvin Tablets*.

**Loss on drying** <731>—Dry about 100 mg of finely ground Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Griseofulvin*.

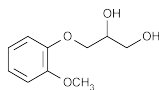
*Assay preparation*—Weigh and finely powder not less than 20 Tablets and proceed as directed for *Assay preparation* in the *Assay* under *Griseofulvin Capsules*, beginning with “transfer an accurately weighed portion.”

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Griseofulvin*. Calculate the quantity, in mg, of griseofulvin ( $C_{17}H_{17}ClO_6$ ) in the portion of Tablets taken by the formula:

$$PC(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Griseofulvin RS in the *Standard preparation*, and the other terms are as defined therein.

## Guaifenesin



$C_{10}H_{14}O_4$  198.22

1,2-Propanediol, 3-(2-methoxyphenoxy)-, (±)-.  
(±)-3-(o-Methoxyphenoxy)-1,2-propanediol [93-14-1].

» Guaifenesin contains not less than 98.0 percent and not more than 102.0 percent of  $C_{10}H_{14}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Guaifenesin RS

USP Guaiacol RS

**Identification**—

**A: Infrared Absorption** <197K>—

**B: Ultraviolet Absorption** <197U>—

*Solution*: 40 µg per mL.

*Medium*: methanol.

**C**: Mix about 5 mg with 1 drop of formaldehyde and a few drops of sulfuric acid: a deep cherry red to purple color is produced.

**Melting range** <741>: between 78° and 82°, but the range between beginning and end of melting does not exceed 3°.

**Loss on drying** <731>—Dry it in vacuum, but at a pressure not below 10 mm of mercury, at 60° to constant weight: it loses not more than 0.5% of its weight.

**Heavy metals, Method I** <231>—The limit is 0.0025%.

**Chromatographic purity**—

*Solution A, Solution B, and Mobile phase*—Proceed as directed in the *Assay*.

*Chromatographic system*—Proceed as directed in the *Assay*. To evaluate the system suitability requirements, use the *Resolution solution* and the *Standard preparation* prepared as directed in the *Assay*.

*Test solution*—Dissolve about 20 mg of Guaifenesin in 10 mL of *Solution B*.

*Diluted test solution*—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Solution B* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Test solution* and the *Diluted test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. All of the peaks are baseline resolved. Calculate the percentage of each impurity in the portion of Guaifenesin taken by the formula:

$$F(r_i / r_S)$$

in which *F* is a response factor equal to 0.63 for the guaiaicol peak, having a relative retention time of 1.4, and 1.0 for all other impurities; *r<sub>i</sub>* is the area of each peak, other than that of the main guaifenesin peak, obtained from the *Test solution*; and *r<sub>S</sub>* is the area of the main peak obtained from the *Diluted test solution*: not more than 1.5% of 2-(2-methoxyphenoxy)-1,3-propanediol (guaifenesin β-isomer), the peak for which occurs at a relative retention time of about 0.9, is found; not more than 0.03% of guaiaicol is found; not more than 0.5% of any other individual impurity is found; and not more than 1.0% of total impurities, excluding guaifenesin β-isomer and guaiaicol, is found.

**Assay**—

*Solution A*—Prepare a mixture of water and glacial acetic acid (990:10).

*Solution B*—Use acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Resolution solution*—Prepare a solution in *Solution B* containing about 0.5 mg of USP Guaifenesin RS and 0.02 mg of USP Guaiaicol RS in each mL.

*Standard preparation*—Prepare a solution of USP Guaifenesin RS in *Solution B* having a known concentration of about 0.5 mg per mL.

*Assay preparation*—Transfer about 25 mg of Guaifenesin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Solution B* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–32	80→50	20→50	linear gradient
32–35	50→80	50→20	linear gradient

Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for guaifenesin  $\beta$ -isomer, 1.0 for guaifenesin, and 1.3 for guaiaicol; and the resolution,  $R$ , between guaifenesin and guaiaicol is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity of  $C_{10}H_{14}O_4$  in the portion of Guaifenesin taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Guaifenesin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of  $C_{10}H_{14}O_4$  in the portion of Guaifenesin taken. To this value, add the percentage of guaifenesin  $\beta$ -isomer found in the test for *Chromatographic purity*.

## Guaifenesin Capsules

» Guaifenesin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ( $C_{10}H_{14}O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Guaifenesin RS

**Identification**—

**A:** Triturate a portion of the contents of Capsules, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and evaporate 1 mL of the filtrate on a watch glass. Mix the residue with 1 drop of formaldehyde and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

**B:** The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that of the guaifenesin peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* <711>—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of  $C_{10}H_{14}O_4$  dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_{10}H_{14}O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

*Mobile phase*, *Benzoic acid solution*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Guaifenesin Tablets*.

*Assay preparation*—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared

container, and determine the average content weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion, equivalent to about 200 mg of guaifenesin, to a 100-mL volumetric flask. Proceed as directed for *Assay preparation* in the *Assay* under *Guaifenesin Tablets*, beginning with “add about 60 mL of water.”

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Guaifenesin Tablets*. Calculate the quantity, in mg, of guaifenesin ( $C_{10}H_{14}O_4$ ) in the portion of Capsule contents taken by the formula:

$$5C(r_U / r_S)$$

in which the terms are as defined therein.

## Guaifenesin for Injection

» Guaifenesin for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ( $C_{10}H_{14}O_4$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, and store at controlled room temperature.

**Labeling**—It meets the requirements for *Labeling* under *Injections* <1>. Label it to indicate that it is for veterinary use only. The label states that it is intended for injection only by the intravenous route in horses.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Guaifenesin RS

**Identification**, *Infrared Absorption* <197K>.

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* <1>.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Bacterial endotoxins** <85>—It contains not more than 0.05 Endotoxin Unit per mg of guaifenesin.

**Assay**—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Guaifenesin RS in chloroform to obtain a solution having a known concentration of about 40  $\mu$ g per mL.

*Assay preparation*—Constitute a container of Guaifenesin for Injection with a volume of warm (30° to 35°) water, accurately measured, corresponding to the volume of solvent specified in the labeling. Transfer an accurately measured volume of the constituted solution, equivalent to about 100 mg of guaifenesin, to a 250-mL separator containing 10 mL of a saturated solution of sodium bicarbonate. Extract with four 25-mL portions of chloroform, combining the chloroform extracts in a second 250-mL separator. Wash the combined chloroform extracts with 5 mL of 1 N hydrochloric acid. Filter the washed chloroform extracts through chloroform-moistened filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 276 nm, using chloroform to zero the instrument. Calculate the quantity, in mg, of guaifenesin ( $C_{10}H_{14}O_4$ ) in each mL of the

constituted solution of Guaifenesin for Injection taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Guaifenesin RS in the *Standard preparation*,  $V$  is the volume, in mL, of constituted solution taken to prepare the *Assay preparation*, and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Guaifenesin Oral Solution

» Guaifenesin Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ( $\text{C}_{10}\text{H}_{14}\text{O}_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Guaifenesin RS

**Identification**—The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 2.3 and 3.0.

**Alcohol content**, *Method I* (611) (if present): between 90.0% and 115.0% of the labeled amount of  $\text{C}_2\text{H}_5\text{OH}$ .

**Assay**—

*Mobile phase*, *Benzoic acid solution*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Guaifenesin Tablets*.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 200 mg of guaifenesin, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix.

*Procedure*—Proceed as directed in the *Assay* under *Guaifenesin Tablets*. Calculate the quantity, in mg, of guaifenesin ( $\text{C}_{10}\text{H}_{14}\text{O}_4$ ) in each mL of the Oral Solution taken by the formula:

$$5C/V(r_U/r_S)$$

in which  $V$  is the volume, in mL, of Oral Solution taken; and the other terms are as defined therein.

## Guaifenesin Tablets

» Guaifenesin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ( $\text{C}_{10}\text{H}_{14}\text{O}_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Guaifenesin RS

**Identification**—

**A:** Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and evaporate 1 mL of the filtrate on a watch glass. Mix the residue with 1 drop of formaldehyde and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

**B:** The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that of the guaifenesin peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $\text{C}_{10}\text{H}_{14}\text{O}_4$  dissolved in filtered portions of the solution under test from UV absorbances at the wavelength of maximum absorbance at about 274 nm in comparison with a *Standard solution* having a known concentration of USP Guaifenesin RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $\text{C}_{10}\text{H}_{14}\text{O}_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase*—Prepare a suitable filtered and degassed mixture of water, methanol, and glacial acetic acid (60:40:1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Benzoic acid solution*—Dissolve a suitable quantity of benzoic acid in methanol to obtain a solution containing about 2 mg per mL.

*Resolution solution*—Dissolve a suitable quantity of guaifenesin in water, with shaking, to obtain a solution containing about 2 mg per mL. Transfer 2.0 mL of this solution and 5.0 mL of *Benzoic acid solution* to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix to obtain a solution containing about 40  $\mu\text{g}$  of guaifenesin and 100  $\mu\text{g}$  of benzoic acid per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Guaifenesin RS quantitatively in water, with shaking, to obtain a solution having a known concentration of about 2 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 40  $\mu\text{g}$  per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of guaifenesin, to a 100-mL volumetric flask, add about 60 mL of water, and shake for about 15 minutes. Dilute with water to volume, filter if necessary to obtain a clear solution, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the guaifenesin and benzoic acid peaks is not less than 3.0 (the relative retention times are about 0.7 for guaifenesin and 1.0 for benzoic acid). Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.5%.

*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ( $C_{10}H_{14}O_4$ ) in the portion of Tablets taken by the formula:

$$5C(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Guaifenesin RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Guaifenesin and Codeine Phosphate Oral Solution

» Guaifenesin and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ( $C_{10}H_{14}O_4$ ) and codeine phosphate ( $C_{18}H_{21}NO_4 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, at controlled room temperature.

**USP Reference standards** (11)—

USP Codeine Phosphate RS

USP Guaifenesin RS

**Identification**—

**A:** The retention time of the codeine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for codeine phosphate*.

**B:** The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for guaifenesin*.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 2.3 and 3.0 if it contains alcohol, 5.0 to 5.5 if it does not contain alcohol.

**Alcohol content** (if present)—

*Standard stock solution*—Dilute 10.0 mL of dehydrated alcohol with water to 100.0 mL.

*Internal standard solution*—Dilute 10 mL of acetone with water to 100 mL.

*Standard preparation*—Transfer 10.0 mL of *Standard stock solution* and 8.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution contains 0.02% (v/v) of alcohol.

*Test preparation*—Transfer 25/ $k$  mL of Oral Solution ( $k$  being the labeled percentage [v/v] of  $C_2H_5OH$  in the Oral Solution), accurately measured, to a 25-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm  $\times$  1.8-m column that contains 5% liquid phase G16 on 100- to 120-mesh support S1A, and is programmed to maintain the column temperature at 50° for 5 minutes after injection, to increase to 100° at the rate of

20° per minute, to maintain the temperature at 100° for 2 minutes, and then to return to 50° before the next injection. The injection port is maintained at about 200°, and the detector block is maintained at about 250°. The carrier gas is dry helium flowing at a rate of about 30 mL per minute. Chromatograph the *Standard preparation*, and record the chromatogram as directed for *Procedure*: the relative retention times are about 0.3 for acetone and 1.0 for alcohol ( $C_2H_5OH$ ); the resolution,  $R$ , between the acetone and alcohol peaks is not less than 2; the tailing factors for the acetone and alcohol peaks are not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 2  $\mu\text{L}$ ) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of alcohol ( $C_2H_5OH$ ) in the portion of Oral Solution taken by the formula:

$$(1250P/V)(R_U / R_S)$$

in which  $P$  is the percentage (v/v) of alcohol ( $C_2H_5OH$ ) in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken to prepare the *Test preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of alcohol to that of acetone obtained from the *Test preparation* and the *Standard preparation*, respectively: between 90.0% and 115.0% of the labeled amount of alcohol ( $C_2H_5OH$ ) is found.

**Assay for codeine phosphate**—

*Internal standard solution*—Place about 45 mg of hydrocodone bitartrate in a flask containing about 5 mL of water, add 1 mL of 0.5 N sodium hydroxide and 50 mL of chloroform, insert the stopper into the flask, and shake by mechanical means for about 20 minutes. Allow the layers to separate, and filter the chloroform layer.

*Standard preparation*—Prepare a Standard stock solution of USP Codeine Phosphate RS in 0.1 N hydrochloric acid having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this Standard stock solution to a suitable flask. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution*, and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of codeine phosphate, to a 200-mL flask containing about 5 mL of water, and swirl. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution*, and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm  $\times$  0.6-m column that contains 3% liquid phase G3 on 100- to 120-mesh support S1A, and is stabilized for isothermal operation. The column temperature is maintained at about 210°, the injection port temperature at about 250°, and the detector block temperature at about 300°. The carrier gas is dry helium flowing at a rate of about 25 mL per minute. Chromatograph the *Standard preparation*, and record the chromatogram as directed for *Procedure*: the relative retention times are about 0.75 for codeine and 1.0 for hydrocodone; the resolution,  $R$ , between the codeine and hydrocodone peaks is not less than 1; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 2  $\mu\text{L}$ ) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot$

$\text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.37)(5C/V)(R_U/R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the Standard stock solution used to prepare the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the codeine peak to the hydrocodone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for guaifenesin—

**Internal standard solution**—Prepare a solution of dipropyl phthalate in chloroform containing about 12.5 mg per mL.

**Standard preparation**—Prepare a Standard stock solution of USP Guaifenesin RS in 0.1 N hydrochloric acid having a known concentration of about 4 mg per mL. Transfer 5.0 mL of this Standard stock solution to a suitable glass-stoppered flask, add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution*, and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer. Extract the aqueous layer with two 20-mL portions of chloroform, and combine the three chloroform extracts. Transfer 5.0 mL of the combined chloroform extracts to a suitable glass-stoppered flask, add 1 mL of trifluoroacetic anhydride, and allow to stand for not less than 1 hour.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of guaifenesin, to a 200-mL flask containing about 5 mL of water, and swirl. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution*, and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer. Extract the aqueous layer with two 20-mL portions of chloroform, and combine the three chloroform extracts. Transfer 5.0 mL of the combined chloroform extracts to a suitable glass-stoppered flask, add 1 mL of trifluoroacetic anhydride, and allow to stand for not less than 1 hour.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm  $\times$  1.2-m column that contains 3% liquid phase G6 on 100- to 120-mesh support S1A. The column temperature is maintained at about 170°, the injection port temperature is maintained at about 250°, and the detector block temperature is maintained at about 300°. The carrier gas is dry helium flowing at a rate of about 45 mL per minute. Chromatograph the *Standard preparation*, and record the chromatogram as directed for *Procedure*: the relative retention times are about 0.6 for the trifluoroacetyl derivative of guaifenesin and 1.0 for the trifluoroacetyl derivative of dipropyl phthalate; the resolution,  $R$ , between the guaifenesin and dipropyl phthalate peaks is not less than 1; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 1  $\mu\text{L}$ ) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ( $\text{C}_{10}\text{H}_{14}\text{O}_4$ ) in the portion of Oral Solution taken by the formula:

$$(W/V)(R_U/R_S)$$

in which  $W$  is the quantity, in mg, of USP Guaifenesin RS taken to prepare the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the guaifenesin peak to the dipropyl phthalate peak obtained

from the *Assay preparation* and the *Standard preparation*, respectively.

## Guaifenesin and Pseudoephedrine Hydrochloride Capsules

» Guaifenesin and Pseudoephedrine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ( $\text{C}_{10}\text{H}_{14}\text{O}_4$ ) and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Guaifenesin RS

USP Pseudoephedrine Hydrochloride RS

#### Identification—

**A:** The retention time of the guaifenesin peak relative to that of the benzoic acid peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for guaifenesin*.

**B:** The retention time of the pseudoephedrine peak in the chromatogram of the *Assay preparation* relative to that of the dextromethorphan peak corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for pseudoephedrine hydrochloride*.

#### Uniformity of dosage units (905)—

**Procedure for content uniformity of pseudoephedrine hydrochloride**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*, preparing the *Assay preparation* as follows. Transfer 1 Capsule to a 100-mL volumetric flask, add about 50 mL of water, heat on a steam bath for about 5 minutes, and allow to cool. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

#### Assay for guaifenesin—

**Mobile phase**—Prepare a mixture of water, methanol, and glacial acetic acid (60:40:1.5). Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of benzoic acid in methanol containing about 2 mg per mL.

**Standard stock solution**—Prepare a solution in water having known concentrations of about 20 mg of USP Guaifenesin RS and 20J mg of USP Pseudoephedrine Hydrochloride RS per mL,  $J$  being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of guaifenesin per Capsule.

**Standard preparation**—Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a second 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix. Each mL of this solution contains about 0.1 mg of USP Guaifenesin RS, 0.1J mg of USP Pseudoephedrine Hydrochloride RS, and 0.1 mg of benzoic acid.

**Assay preparation**—Transfer an accurately counted number of Capsules, equivalent to about 2000 mg of guaifenesin, to a 100-mL volumetric flask, add about 50 mL of water, and heat on a steam bath for about 15 minutes. Allow to cool, dilute with water to volume, and mix. Transfer 10.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a third 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 276-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.65 for guaifenesin and 1.0 for benzoic acid; the resolution,  $R$ , between the guaifenesin peak and the benzoic acid peak is not less than 3.0; the tailing factors for the guaifenesin peak and the benzoic acid peak are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.5%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ( $C_{10}H_{14}O_4$ ) in each Capsule taken by the formula:

$$20,000(C / N)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Guaifenesin RS in the *Standard preparation*;  $N$  is the number of Capsules taken; and  $R_U$  and  $R_S$  are the ratios of the guaifenesin peak response to the benzoic acid peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for pseudoephedrine hydrochloride—

**Mobile phase**—To 3.5 g of docusate sodium add 500 mL of methanol, 350 mL of water, 145 mL of tetrahydrofuran, and 5 mL of glacial acetic acid, mix, and pass through a filter having a porosity of 0.5  $\mu$ m or less. Make any necessary adjustments (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Prepare a solution of dextromethorphan hydrobromide in methanol containing about 1.2 mg per mL.

**Standard stock solution**—Prepare as directed in the *Assay for guaifenesin*.

**Standard preparation**—Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains about 2 mg of USP Guaifenesin RS, 2/ mg of USP Pseudoephedrine Hydrochloride RS, and 0.06 mg of dextromethorphan hydrobromide.

**Assay preparation**—Transfer 10.0 mL of the stock solution used to prepare the *Assay preparation* in the *Assay for guaifenesin* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 263-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.55 for pseudoephedrine and 1.0 for dextromethorphan; the resolution,  $R$ , between the pseudoephedrine peak and the dextromethorphan peak is not less than 1.5; the tailing factors for the pseudoephedrine peak and the dextromethorphan peak are not more than 1.5 and 2.5, respectively; and the relative standard deviation for replicate injections determined for the pseudoephedrine peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the

quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in each Capsule taken by the formula:

$$1000(C / N)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*;  $N$  is the number of Capsules taken; and  $R_U$  and  $R_S$  are the ratios of the pseudoephedrine peak area response to the dextromethorphan peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Guaifenesin, Pseudoephedrine Hydrochloride, and Dextromethorphan Hydrobromide Capsules

» Guaifenesin, Pseudoephedrine Hydrochloride, and Dextromethorphan Hydrobromide Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ( $C_{10}H_{14}O_4$ ), pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Dextromethorphan Hydrobromide RS

USP Guaifenesin RS

USP Pseudoephedrine Hydrochloride RS

#### Identification—

**A:** The retention time of the guaifenesin peak relative to that of the benzoic acid peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for guaifenesin*.

**B:** The retention times of the pseudoephedrine and dextromethorphan peaks in the chromatogram of the *Assay preparation* relative to that of the brompheniramine peak correspond to those in the chromatogram of the *Standard preparation* as obtained in the *Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide*.

#### Uniformity of dosage units <905>—

**Procedure for content uniformity of pseudoephedrine hydrochloride and dextromethorphan hydrobromide**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide*, preparing the *Assay preparation* as follows. Transfer 1 Capsule to a 100-mL volumetric flask, add about 50 mL of water, heat on a steam bath for about 15 minutes, and allow to cool. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

#### Assay for guaifenesin—

**Mobile phase, Internal standard solution, Standard stock solution, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for guaifenesin* under *Guaifenesin and Pseudoephedrine Hydrochloride Capsules*.

**Assay preparation**—Transfer an accurately counted number of Capsules, equivalent to about 2000 mg of guaifenesin, to a 100-mL volumetric flask, add about 50 mL of water, and heat on a steam bath for about 15 minutes. Allow to cool, dilute with water to volume, and mix (stock solution). Transfer 10.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a third 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix.



**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ( $C_{10}H_{14}O_4$ ) in each Capsule taken by the formula:

$$20,000(C / N)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Guaifenesin RS in the *Standard preparation*, *N* is the number of Capsules taken, and  $R_U$  and  $R_S$  are the ratios of the guaifenesin peak area response to the benzoic acid peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide—

**Mobile phase**—To 3.5 g of docusate sodium add 500 mL of methanol, 350 mL of water, 145 mL of tetrahydrofuran, and 5 mL of glacial acetic acid, mix, and pass through a filter having a porosity of 0.5  $\mu$ m or less. Make any necessary adjustments (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Prepare a solution of brompheniramine maleate in methanol containing about 0.3 mg per mL.

**Standard stock solution**—Prepare a solution in water having known concentrations of about 20 mg of USP Guaifenesin RS, 20/ mg of USP Pseudoephedrine Hydrochloride RS per mL, and 20/ mg of USP Dextromethorphan Hydrobromide RS per mL, *I* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of guaifenesin per Capsule, and *I'* being the ratio of the labeled amount, in mg, of dextromethorphan hydrobromide to the labeled amount, in mg, of guaifenesin per Capsule.

**Standard preparation**—Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation**—Transfer 10.0 mL of the stock solution used to prepare the *Assay preparation* in the *Assay for guaifenesin* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 263-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.4 for pseudoephedrine, 0.75 for dextromethorphan, and 1.0 for brompheniramine; the resolution, *R*, between the peaks is not less than 1.5; the tailing factors for the pseudoephedrine peak, the dextromethorphan peak, and the brompheniramine peak are not more than 1.5, 2.5, and 3.0, respectively; and the relative standard deviation for replicate injections determined for the pseudoephedrine peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in each Capsule taken by the formula:

$$1000(C / N)(R_U / R_S)$$

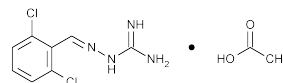
in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *N* is the number of Capsules taken; and  $R_U$  and  $R_S$  are the ratios of the pseudoephedrine peak area response to the brompheniramine peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each Capsule taken by the formula:

$$1000(370.33 / 352.31)(C / N)(R_U / R_S)$$

in which 370.33 and 352.31 are the molecular weights of dextromethorphan hydrobromide and anhydrous dextromethorphan hydrobromide, respectively; *C* is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; *N* is the number of Capsules taken; and  $R_U$  and  $R_S$  are the ratios of the dextromethorphan peak area response to the brompheniramine peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Guanabenz Acetate



$C_8H_8Cl_2N_4 \cdot C_2H_4O_2$  291.13

Hydrazinecarboximidamide, 2-[(2,6-dichlorophenyl)methylene]-, monoacetate.

[(2,6-Dichlorobenzylidene)amino]guanidine monoacetate [23256-50-0].

» Guanabenz Acetate contains not less than 98.0 percent and not more than 101.5 percent of the labeled amount of  $C_{10}H_{12}N_4O_2Cl_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Guanabenz Acetate RS

**Identification, Infrared Absorption** <197K>.

**pH** <791>: between 5.5 and 7.0, in a solution (7 in 1000).

**Loss on drying** <731>—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.2%.

**Limit of 2,6-dichlorobenzaldehyde**—

**Internal standard solution 1**—Dissolve 100 mg of *p*-chlorobenzaldehyde in 100 mL of chloroform, and mix.

**Internal standard solution 2**—Dilute 1.0 mL of *Internal standard solution 1* to 10.0 mL with chloroform, and mix.

**Standard solution**—Prepare a solution of 2,6-dichlorobenzaldehyde in chloroform containing 1.0 mg per mL.

**Standard preparation**—Transfer 4.0 mL of *Standard solution* and 1.0 mL of *Internal standard solution 1* to a 10-mL volumetric flask, dilute with chloroform to volume, and mix.

**Test preparation**—Transfer 200 mg of Guanabenz Acetate to a 30-mL glass-stoppered centrifuge tube. Add 10 mL of 0.1 N hydrochloric acid, shake to dissolve, add 1.0 mL of *Internal standard solution 2*, and shake. Centrifuge, and transfer a portion of the lower layer to a stoppered container. [NOTE—The lower layer must be removed within 10 minutes of adding the acid to the centrifuge tube.]

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 1.8-m  $\times$  3-mm column packed with 20% phase G1 on 80- to 100-mesh support S1A. The column is maintained at a temperature of about 190°, the injection port at about 225°, and the detector at about 250°. Nitrogen is used as the carrier gas at a flow rate of about 30 mL per minute.

**Procedure**—Separately inject 2- $\mu$ L portions of the *Standard preparation* and the *Test preparation*, successively, into the gas chromatograph. The resolution between 2,6-dichlorobenzaldehyde and *p*-chlorobenzaldehyde is not less than 3.0, and the relative retention time for *p*-chlorobenzaldehyde is 0.5 and for 2,6-dichlorobenzaldehyde is 1.0. The relative peak response ratio obtained from the *Test preparation* does not exceed that obtained from the *Standard preparation* (0.2%).

#### Chromatographic purity—

**Methanolic formic acid**—Prepare a mixture of formic acid and methanol (1 in 2000).

**Aminoguanidine bicarbonate solution**—Transfer 100 mg of aminoguanidine bicarbonate to a test tube, add 0.05 mL of formic acid, and warm gently to effect solution. Quantitatively transfer the contents of the test tube to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

**Standard solution A**—Transfer 10 mg of USP Guanabenz Acetate RS to a 100-mL volumetric flask, and dissolve in 50 mL of *Methanolic formic acid*. Add 1.0 mL of the *Aminoguanidine bicarbonate solution*, dilute with *Methanolic formic acid* to volume, and mix.

**Standard solution B**—Transfer 5.0 mL of *Standard solution A* to a 10-mL volumetric flask, dilute with *Methanolic formic acid* to volume, and mix.

**Standard solution C**—Transfer 2.0 mL of *Standard solution A* to a 10-mL volumetric flask, dilute with *Methanolic formic acid* to volume, and mix.

**Test solution**—Prepare a solution of guanabenz acetate containing 10 mg per mL in *Methanolic formic acid*.

**Procedure**—Prepare a chromatographic chamber containing a mixture of chloroform, methanol, and ammonium hydroxide (60:40:1) as the developing solvent, and allow it to equilibrate for at least 30 minutes before use. Prewash a plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* (621)) by placing it in the chromatographic chamber, allowing the solvent front to rise to the top of the plate, drying it in air and activating it by heating at 105° for 20 minutes. Within 30 minutes after preparation, separately apply 10- $\mu$ L portions of *Standard solutions A, B, and C, the Test solution, and Methanolic formic acid*. Allow the spots to dry, and place the plate in the chromatographic chamber. When the solvent has moved about three-fourths of the length of the plate, remove the plate and allow it to air-dry for about 30 minutes. Examine the plate under short-wavelength UV light. Estimate the amount of any secondary spots (other than any secondary spot with the same  $R_f$  as the *Methanolic formic acid*) observed in the chromatogram of the *Test solution* by comparison with the *Standard solutions*. Place the plate in a chamber saturated with iodine vapors for about 10 minutes. Remove and examine the plate. Estimate the amount of any spot in the chromatogram of the *Test solution* that has an  $R_f$  corresponding to the  $R_f$  of the spot produced by the aminoguanidine bicarbonate by comparison with the *Standard solutions*. No individual secondary spot is greater in size or intensity than the spot produced by *Standard solution B* (0.5%), and the total of any such spots observed is not more than 1%.

**Assay**—Dissolve about 200 mg of Guanabenz Acetate, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.12 mg of  $C_{10}H_{12}N_4O_2Cl_2$ .

## Guanabenz Acetate Tablets

» Guanabenz Acetate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guanabenz ( $C_8H_8N_4Cl_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Guanabenz Acetate RS

**Identification**—Transfer an amount of powdered Tablets, equivalent to about 8 mg of guanabenz, to a 60-mL separator. Add 10 mL of 0.1 N hydrochloric acid, and shake to disperse the powder. Shake the mixture with three 10-mL portions of chloroform, discarding the chloroform phase each time. Add 5 mL of 1 N sodium hydroxide, and extract with two 25-mL portions of ether, filtering the ether extracts. Evaporate the combined extracts with the aid of a current of air to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as that of a similar preparation of USP Guanabenz Acetate RS.

#### Dissolution (711)—

**Medium:** water; 1000 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_8H_8N_4Cl_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 272 nm of filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a *Standard solution* having a known concentration of USP Guanabenz Acetate RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_8H_8N_4Cl_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Chromatographic purity—

*Extracting solvent, Mobile phase, Standard preparation I, System suitability solution, Chromatographic system, and Assay preparation*—Proceed as directed in the Assay.

**Standard preparation II**—Pipet 2 mL of *Standard preparation I* into a 100-mL volumetric flask, dilute with *Extracting solvent* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay, except to substitute *Standard preparation II* for *Standard preparation I*. Calculate the quantity of any impurity observed having a relative retention time corresponding to the component eluting before guanabenz obtained from the *System suitability solution*. The amount of any such impurity observed is not more than 2%.

#### Assay—

**Extracting solvent**—Dissolve 8.2 g of sodium acetate in 20 mL of water, add 5.7 mL of glacial acetic acid, dilute to 1 L with methanol, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and phosphoric acid (57:43:0.3), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation I**—Transfer about 25 mg of USP Guanabenz Acetate RS, accurately weighed, to a 250-mL volumetric flask. Add 25 mL of water, shake to dissolve the solids, dilute with *Extracting solvent* to volume, and mix.

**Assay preparation**—Transfer 10 Tablets to a 500-mL volumetric flask. Add 50 mL of water, stir by mechanical means until the solids are well dispersed, add 400 mL of *Extracting solvent*, and stir for 45 minutes. Dilute with *Extracting solvent* to volume, mix, and centrifuge a portion of the mixture

until a clear supernatant is obtained. If necessary, dilute a portion of the supernatant quantitatively with a mixture of *Extracting solvent* and water (9:1) to obtain a solution containing about 0.08 mg of guanabenz per mL.

**System suitability solution**—Transfer about 30 mg of guanabenz acetate to a 100-mL stoppered flask. Add about 50 mL of 0.1 N hydrochloric acid, heat on a steam bath for 60 minutes, and allow the solution to cool.

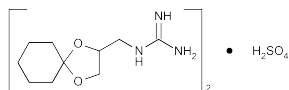
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 245-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of *Standard preparation I* and record the peak responses as directed for *Procedure*; the relative standard deviation is not more than 2.0%. Inject a volume (about 20 µL) of the *System suitability solution* into the chromatograph and record the chromatogram: the resolution between guanabenz and the peak eluting before it is not less than 1.6.

**Procedure**—Separately inject equal volumes (about 20 µL) of *Standard preparation I* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_8H_8N_4Cl_2$  in the portion of Tablets taken by the formula:

$$(231.08 / 291.13)(CD)(r_U / r_S)$$

in which 231.08 and 291.13 are the molecular weights of guanabenz and guanabenz acetate, respectively; C is the concentration, in mg per mL, of USP Guanabenz Acetate RS in *Standard preparation I*; D is the *Assay preparation* dilution factor, in mL per Tablet; and  $r_U$  and  $r_S$  are the peak responses of the *Assay preparation* and *Standard preparation I*, respectively.

## Guanadrel Sulfate



$(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$  524.63  
Guanidine (1,4-dioxaspiro[4.5]dec-2-ylmethyl)-, sulfate (2:1).  
(1,4-Dioxaspiro[4.5]dec-2-ylmethyl)guanidine sulfate (2:1)  
[22195-34-2].

» Guanadrel Sulfate contains not less than 97.0 percent and not more than 103.0 percent of  $(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Guanadrel Sulfate RS

**Identification**—The IR absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Guanadrel Sulfate RS.

**Loss on drying** <731>—Dry it at room temperature at a pressure not exceeding 5 mm of mercury for 16 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** <31>: 0.002%.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of 530 mL of water and 470 mL of methanol containing about

6.35 g of *dl*-10-camphorsulfonic acid sodium salt and 0.8 g of ammonium nitrate. Adjust with glacial acetic acid, if necessary, to a pH of between 5.0 and 5.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Guanadrel Sulfate RS in *Mobile phase* to obtain a solution having a known concentration of about 10 mg per mL.

**Assay preparation**—Transfer about 100 mg of Guanadrel Sulfate, accurately weighed, to a container, add 10.0 mL of *Mobile phase*, and mix.

**Resolution solution**—Dissolve suitable quantities of USP Guanadrel Sulfate RS and ethylparaben in *Mobile phase* to obtain a solution having known concentrations of about 10 mg and 12 mg, respectively, in each mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a refractive index detector and a 4- to 4.6-mm × 25- to 30-cm stainless steel column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between guanadrel and ethylparaben is not less than 1.6, and the relative retention times are about 0.8 for guanadrel and 1.0 for ethylparaben. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.5%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$  in the portion of Guanadrel Sulfate taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Guanadrel Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Guanadrel Sulfate Tablets

» Guanadrel Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guanadrel sulfate  $[(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4]$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—  
USP Guanadrel Sulfate RS

**Identification**—To a portion of 1 finely powdered Tablet, equivalent to about 4 mg of guanadrel sulfate, add 2 mL of a 1% aqueous alkaline solution of 1-naphthol (containing 6 g of sodium hydroxide and 16 g of sodium carbonate per 100 mL of water) and 1 mL of 2,3-butanedione solution (1 in 2000), and mix. Allow to stand at room temperature: an intense, pinkish-red color develops.

**Dissolution** <711>—

**Medium**: pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2**: 50 rpm.

**Time**: 20 minutes.

**Standard solution**—Dissolve an accurately weighed quantity of USP Guanadrel Sulfate RS in *Medium* to obtain a solu-

tion having a known concentration of about 0.5 mg per mL. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Medium* to volume, and mix.

**Working color reagent preparation**—Transfer 50.0 mL of the *Color reagent preparation*, prepared as directed for *Procedure for content uniformity* in *Uniformity of dosage units*, to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Color reagent blank**—[NOTE—Prepare this solution concurrently with the preparation of the *Standard solution* and the solution under test.] Transfer 40.0 mL of *Medium* to a container, add 8.0 mL of *Working color reagent preparation*, and mix.

**Procedure**—After 20 minutes, withdraw a portion of the solution under test, and filter immediately. For Tablets containing less than 25 mg per Tablet, transfer 20.0 mL of the filtered solution to a container. For Tablets containing 25 mg or more, transfer 10.0 mL of the filtered solution and 10.0 mL of *Medium* to a container. Transfer 20.0 mL of the *Standard solution* to another, similar container. Separately add 4.0 mL of *Working color reagent preparation* to the solution of the test specimen and the *Standard solution*, and mix. Using a suitable spectrophotometer, determine the absorbances of the solutions obtained from the test specimen and the *Standard solution*, in 5-cm cells, at the wavelength of maximum absorbance at about 494 nm, using the *Color reagent blank* in the reference cell. [NOTE—Once the *Working color reagent preparation* has been added to the filtered dissolution specimens and mixed, determine the absorbances so that none of the solutions stands for less than 20 minutes or more than 80 minutes.] Calculate the amount of  $(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$  dissolved by comparison of the absorbances obtained with the solutions obtained from the *Standard solution* and the solution under test.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$  is dissolved in 20 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—

**Color reagent preparation**—Prepare separately sodium nitroferricyanide solution (1 in 10), potassium ferricyanide solution (1 in 10), and sodium hydroxide solution (1 in 10), and store in separate amber-colored bottles. Mix an equal and sufficient volume of each of these solutions, in the order listed, and allow to stand for about 15 minutes. The solution changes from a deep red-black to a yellow-green color. Prepare a 1 in 10 dilution of the solution in water to obtain the *Color reagent preparation*. [NOTE—Prepare this *Color reagent preparation* on the day of use. The 10% aqueous solutions are stable for about 2 months.]

**Standard preparation**—Dissolve an accurately weighed quantity of USP Guanadrel Sulfate RS in water to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 10.0 mL of this solution to a container, and proceed as directed under *Procedure*.

**Test preparation**—Transfer 1 Tablet to a 100-mL volumetric flask, dilute with water to volume, and shake the flask vigorously for about 4 minutes. Transfer not less than 25 mL of the solution to a vial, and centrifuge for 10 minutes. Transfer an accurately measured volume of this solution, equivalent to 1 mg of guanadrel sulfate, to another container, and dilute, if necessary, with an accurately measured volume of water to a volume of 10.0 mL. Mix, and proceed as directed under *Procedure*.

**Procedure**—Separately add 4.0 mL of the *Color reagent preparation* to the *Standard preparation*, the *Test preparation*, and 10.0 mL of water to provide the blank. Mix the solutions, and allow to stand for 10 minutes. Within 5 minutes, determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 494 nm, with a suitable spectrophotometer, against the reagent

blank. Calculate the quantity, in mg, of  $(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of guanadrel sulfate in the Tablet; *C* is the concentration, in mg per mL, of USP Guanadrel Sulfate RS in the *Standard preparation*; *D* is the concentration, in mg per mL, of guanadrel sulfate in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the solution under test and the *Standard preparation*, respectively.

#### Assay—

**Mobile phase, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the Assay under *Guanadrel Sulfate*.

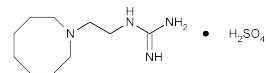
**Assay preparation**—Transfer a number of Tablets, equivalent to about 100 mg of guanadrel sulfate, to a container. Add an accurately measured volume of *Mobile phase* to obtain a final concentration of about 10 mg of guanadrel sulfate per mL of *Mobile phase*, shake by mechanical means for 20 minutes, and centrifuge if necessary.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Guanadrel Sulfate*. Calculate the quantity, in mg, of guanadrel sulfate  $[(C_{10}H_{19}N_3O_2)_2 \cdot H_2SO_4]$  in the Tablets taken by the formula:

$$VC(r_U / r_S)$$

in which *V* is the volume, in mL, of *Mobile phase* added to the Tablets, and the other terms are as defined in the Assay under *Guanadrel Sulfate*.

## Guanethidine Monosulfate



$C_{10}H_{22}N_4 \cdot H_2SO_4$  296.39

Guanidine, [2-(hexahydro-1(2*H*)-azocinyl)ethyl]-, sulfate (1:1).

[2-(Hexahydro-1(2*H*)-azocinyl)ethyl]guanidine sulfate (1:1) [645-43-2].

» Guanethidine Monosulfate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{10}H_{22}N_4 \cdot H_2SO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Guanethidine Monosulfate RS

**Identification**—

**A: Infrared Absorption** (197M).

**B:** Dissolve 2.5 mg in 10 mL of water. Add 2 mL of a solution prepared by dissolving 500 mg of 1-naphthol, 3 g of sodium hydroxide, and 8 g of sodium carbonate in water to make 50 mL, and 1 mL of a solution of 2,3-butanedione (1 in 2000). Allow to stand at room temperature: an intense, pinkish-red color develops.

**pH** (791): between 4.7 and 5.7, in a solution containing 20 mg per mL.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.001%.

**Assay—**

*Sodium nitroferricyanide–potassium ferricyanide solution—*Dissolve 1 g of sodium nitroferricyanide and 1 g of potassium ferricyanide in water to make 100 mL, and mix.

*Standard preparation—*Dissolve a suitable quantity of USP Guanethidine Monosulfate RS, accurately weighed, in 1 N sulfuric acid to obtain a solution having a known concentration of about 1 mg per mL.

*Assay preparation—*Transfer about 50 mg of Guanethidine Monosulfate, accurately weighed, to a 50-mL volumetric flask, dissolve in 1 N sulfuric acid, add 1 N sulfuric acid to volume, and mix.

*Procedure—*Pipet 2 mL each of the *Assay preparation* and the *Standard preparation*, and 2 mL of 1 N sulfuric acid to provide the blank, into separate glass-stoppered, 40-mL centrifuge tubes. Add 10.0 mL of water to each tube, and mix. To each tube add 10.0 mL of *Sodium nitroferricyanide–potassium ferricyanide solution*, mix, add 4.0 mL of 1 N sodium hydroxide, mix, and allow to stand for 20 minutes, accurately timed. Concomitantly determine the absorbances of both solutions in 1-cm cells against the blank at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $C_{10}H_{22}N_4 \cdot H_2SO_4$  in the Guanethidine Monosulfate taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Guanethidine Monosulfate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Guanethidine Monosulfate Tablets

» Guanethidine Monosulfate Tablets contain an amount of guanethidine monosulfate ( $C_{10}H_{22}N_4 \cdot H_2SO_4$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guanethidine sulfate [ $(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$ ].

**Packaging and storage—**Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Guanethidine Monosulfate RS

**Identification, Infrared Absorption** (197M)—Obtain the test specimen as follows. Transfer a quantity of powdered Tablets, equivalent to about 120 mg of guanethidine monosulfate, to a glass-stoppered flask, add 20 mL of water, shake by mechanical means for 30 minutes, and filter, discarding the first few mL of the filtrate. Transfer 10 mL of the filtrate to a separator, add 2 mL of 0.1 N sodium hydroxide and 2 mL of a saturated solution of picric acid in 0.1 N sodium hydroxide, and mix. Extract with 20 mL of chloroform, filter the chloroform extract through cotton, and collect in a beaker. Evaporate the chloroform extract with the aid of a stream of nitrogen to dryness.

**Dissolution** (711)—

*Medium:* water; 500 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure—*Determine the amount of  $(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$  dissolved, employing the procedure set forth in the *Assay* and making any necessary modifications.

*Tolerances—*Not less than 75% (Q) of the labeled amount of  $(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Borate solution—*Transfer 12.4 g of boric acid to a 1000-mL volumetric flask, dissolve in 100 mL of 1.0 N sodium hydroxide, dilute with water to volume, and mix.

*Borate buffer—*Mix 400 mL of *Borate solution* with 600 mL of 0.1 N sodium hydroxide, and adjust with 1.0 N sodium hydroxide or 1.0 N hydrochloric acid to a pH of  $12.3 \pm 0.1$ .

*Picrate reagent—*Transfer 15 g of picric acid to a 1000-mL volumetric flask, dissolve in 750 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix.

*Standard preparation—*Prepare a solution in 0.1 N sulfuric acid having a known concentration of about 0.3 mg of USP Guanethidine Monosulfate RS per mL.

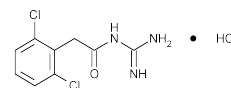
*Assay preparation—*Finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of guanethidine sulfate, to a 200-mL volumetric flask, add 150 mL of 0.1 N sulfuric acid, shake by mechanical means for 30 minutes, dilute with 0.1 N sulfuric acid to volume, and mix. Filter, and discard the first 25 mL of the filtrate.

*Procedure—*Pipet 5 mL each of the *Standard preparation* and the *Assay preparation* into separate 125-mL separators. To each separator add 20 mL of *Borate buffer* and 20 mL of *Picrate reagent*. Extract with three 20-mL portions of chloroform, filtering the extracts through chloroform-pretreated cotton, and collect the extracts in low-actinic 100-mL volumetric flasks. Rinse the cotton with 20 mL of chloroform, adding the rinsings to the volumetric flasks, dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 412 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of guanethidine sulfate [ $(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$ ] in the portion of Tablets taken by the formula:

$$(247.35 / 296.39)(200)(C)(A_U / A_S)$$

in which 247.35 is one-half the molecular weight of guanethidine sulfate; 296.39 is the molecular weight of guanethidine monosulfate; C is the concentration, in mg per mL, of USP Guanethidine Monosulfate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Guanfacine Hydrochloride



$C_9H_9Cl_2N_3O \cdot HCl$  282.55

Benzeneacetamide, *N*-(aminoiminomethyl)-2,6-dichloro-, monohydrochloride.

*N*-Amidino-2-(2,6-dichlorophenyl)acetamide monohydrochloride [29110-48-3].

» Guanfacine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_9H_9Cl_2N_3O \cdot HCl$ , calculated on the dried basis. [Caution—Guanfacine Hydrochloride is a potent antihypertensive drug. Minimize flying dust, and avoid all bodily and respiratory contact with this substance.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Guanfacine Hydrochloride RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals, Method II** (231): 0.002%.

NOTE—Prepare all solutions in the tests for *Related compounds* and *Chromatographic purity* immediately prior to use, and apply to plates as quickly as possible.

**Related compounds**—

*Spray reagent*—[Caution—Avoid contact with *o*-tolidine. Prepare and use this *Spray reagent* in a well-ventilated hood.] Dissolve 50 mg of *o*-tolidine in 100 mL of alcohol, and mix.

*Chlorine chamber*—Transfer 1.5 g of potassium permanganate to a 100-mL beaker, dissolve in and dilute with water to volume, and mix. Transfer 25 mL of this solution to a beaker, and place the beaker inside a chromatographic chamber. Pipet 10 mL of hydrochloric acid into the beaker, and cover the chamber.

*Developing solvent system*—Prepare a fresh mixture of ethyl acetate, glacial acetic acid, and acetonitrile (70:25:3).

*Standard solutions*—Dissolve accurately weighed quantities of USP Guanfacine Hydrochloride RS and guanidine hydrochloride in methanol to obtain a solution having a known concentration of 0.4 mg each of USP Guanfacine Hydrochloride RS and guanidine hydrochloride per mL. Quantitatively dilute this solution with methanol to obtain *Standard solutions* having the following compositions:

Standard Solution	Dilution	Concentration (µg RS and Guanidine Hydrochloride per mL)	Percentage (% for Comparison with Test Specimen)
1	(undiluted)	400	2.0
2	(1 in 2)	200	1.0
3	(1 in 4)	100	0.5
4	(1 in 8)	50	0.25

*Test solution*—Dissolve an accurately weighed quantity of Guanfacine Hydrochloride in methanol to obtain a solution having a concentration of about 20 mg per mL.

*Procedure*—Use a thin-layer chromatographic plate (see *Chromatography* (621) coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with *Developing solvent system*. Remove the plates from the chamber, and allow to dry. Separately apply 10 µL each of the *Standard solutions* and the *Test solution* to the chromatographic plate. Allow the spots to dry, and develop the chromatogram in *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 1 hour. Examine the plate under short-wavelength UV light. Place the dried plate in the *Chlorine chamber* for 15 minutes, remove, and allow the excess chlorine to evaporate by air drying for 5 minutes. Spray the plate with *Spray reagent*, and examine: any spot due to guanidine hydrochloride observed in the chromatogram of the *Test solution* is not greater in size or intensity than the guanidine hydrochloride spot obtained from *Standard solution* 3 (0.5%); no other individual impurity spot observed in the

chromatogram of the *Test solution* is greater in size or intensity than the guanfacine hydrochloride spot obtained from *Standard solution* 4 (0.25%); and the sum of all impurities found, including guanidine hydrochloride, is not more than 1.0%.

**Chromatographic purity**—

*Spray reagent 1*—Prepare a mixture of tertiary butyl alcohol and water (9:1).

*Spray reagent 2*—Dissolve 5 g of 4,4'-tetramethyldiaminodiphenylmethane in 20 mL of glacial acetic acid, add 10 mL of water, and mix (*Solution 1*). Dissolve 6 g of potassium iodide in 120 mL of water, and mix (*Solution 2*). Dissolve 0.3 g of ninhydrin in 10 mL of glacial acetic acid, dilute with water to 100 mL, and mix (*Solution 3*). Mix *Solution 1* and *Solution 2*, and add 9 mL of *Solution 3*.

*Developing solvent system*—Prepare a fresh mixture of hexanes, diisopropyl ether, toluene, and glacial acetic acid (60:30:5:3).

*Reference solutions*—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in a mixture of methanol and water (9:1) to obtain a solution having a concentration of 1 mg per mL (*Reference solution 1*). Quantitatively dilute this solution with a mixture of methanol and water (9:1) to obtain *Reference solution 2* and *Reference solution 3* having known concentrations of 0.5 and 0.25 mg per mL of 2,6-dichlorophenylacetic acid, respectively.

*Test solution*—Prepare a solution of Guanfacine Hydrochloride in a mixture of methanol and water (9:1), containing 100 mg per mL.

*Procedure*—Use a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with *Developing solvent system*. Remove the plates from the chamber, and allow to dry. Separately apply 25 µL of each of the *Reference solutions* and the *Test solution* to the chromatographic plate. Allow the spots to dry, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for 30 minutes. Examine the plate under short-wavelength UV light. Spray the plate with *Spray reagent 1*, wait for 1 minute, and then spray with *Spray reagent 2*. Place the wet plate under short-wavelength UV light for 10 minutes, remove, and observe under white light: no spot observed in the chromatogram of the *Test solution*, other than that due to guanfacine hydrochloride, is greater in size or intensity than the principal spot obtained from *Reference solution 2* (0.5%); and the sum of all impurities found is not more than 1.0%.

**Assay**—

*Dilute phosphoric acid*—Prepare a mixture of water and phosphoric acid (4:1).

*Buffer solution*—Dissolve 68 g of monobasic potassium phosphate in water, dilute with water to 1000 mL, and mix. Dilute 100 mL of this solution with water to 1000 mL, add 5 mL of triethylamine, mix, and adjust with *Dilute phosphoric acid* to a pH of 3.0.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (79:21). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in a mixture of acetonitrile and water (3:1) to obtain a solution having a known concentration of about 1 mg of USP Guanfacine Hydrochloride RS per mL. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Transfer an accurately weighed quantity of about 50 mg of Guanfacine Hydrochloride to a 50-mL volumetric flask, dissolve in and dilute with a mixture

of acetonitrile and water (3:1) to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the capacity factor,  $k'$ , is between 2 and 5; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_9H_9Cl_2N_3O \cdot HCl$  in the portion taken by the formula:

$$1.25C(r_U/r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the guanfacine hydrochloride peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Guanfacine Tablets

» Guanfacine Tablets contain an amount of Guanfacine Hydrochloride ( $C_9H_9Cl_2N_3O \cdot HCl$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guanfacine ( $C_9H_9Cl_2N_3O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Guanfacine Hydrochloride RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**B:** It responds to the *Thin-Layer Chromatographic Identification Test* (201), the test solution and the *Standard solution* being prepared at a concentration of 2 mg per mL in methanol, and a solvent system consisting of a mixture of ethyl acetate, glacial acetic acid, and water (5:2:2) being used.

**Dissolution** <711>—

*Medium:* water; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of  $C_9H_9Cl_2N_3O$  dissolved, employing the procedure set forth in the *Assay* and making any necessary modifications.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amount of  $C_9H_9Cl_2N_3O$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**pH 2.5 Diethylamine phosphate solution**—Add 10.3 mL of diethylamine to about 70 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to 100 mL, and mix.

**Reagent solution**—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in *Mobile phase*, and

dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 18 µg per mL.

**Mobile phase**—Dissolve 600 mg of monobasic potassium phosphate and 3 mL of pH 2.5 Diethylamine phosphate solution in 480 mL of water, and mix. Adjust with 0.2 N sodium hydroxide to a pH of 4.0. While swirling, add 520 mL of acetonitrile. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Prepare a solution of butylparaben in *Mobile phase* containing 0.5 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.23 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, and add 5.0 mL each of the *Reagent solution* and the *Internal standard solution*. Dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of guanfacine, to a 100-mL volumetric flask. Add 50 mL of *Mobile phase*, and heat on a steam bath for 5 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for guanfacine, 0.6 for 2,6-dichlorophenylacetic acid, and 1.0 for butylparaben; the resolution,  $R$ , between guanfacine and 2,6-dichlorophenylacetic acid is not less than 1.5, and the resolution,  $R$ , between 2,6-dichlorophenylacetic acid and butylparaben is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guanfacine ( $C_9H_9Cl_2N_3O$ ) in the portion of Tablets taken by the formula:

$$(246.09/282.55)(0.25C)(R_U/R_S)$$

in which 246.09 and 282.55 are the molecular weights of guanfacine and guanfacine hydrochloride, respectively;  $C$  is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of guanfacine to butylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

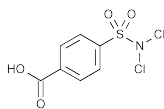
## Gutta Percha

» Gutta Percha is the coagulated, dried, purified latex of the trees of the genera *Palaquium* and *Payena* and most commonly *Palaquium gutta* (Hooker) Baillon (Fam. Sapotaceae).

**Packaging and storage**—Preserve under water in well-closed containers, protected from light.

**Residue on ignition** (281): not more than 1.7%.

## Halazone



$C_7H_5Cl_2NO_4S$  270.09  
Benzoic acid, 4-[(dichloroamino)sulfonyl]-;  
*p*-(Dichlorosulfamoyl)benzoic acid [80-13-7].

### DEFINITION

Halazone contains NLT 91.5% and NMT 100.5% of  $C_7H_5Cl_2NO_4S$ , calculated on the dried basis.

### IDENTIFICATION

#### • PROCEDURE

**Sample:** 100 mg

**Analysis:** Add the *Sample* to 5 mL of a sodium bromide solution (1 in 10).

**Acceptance criteria:** Bromine is liberated from the mixture.

### ASSAY

#### • PROCEDURE

**Sample:** 150 mg

**Analysis:** Add the *Sample* to 10 mL of 2.5 N sodium hydroxide in a 250-mL iodine flask, and stir well to dissolve. Add 75 mL of water, then promptly add 15 mL of potassium iodide solution (1 in 10), and mix. Acidify with 10 mL of 6 N acetic acid, and titrate immediately with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 6.752 mg of  $C_7H_5Cl_2NO_4S$ .

**Acceptance criteria:** 91.5%–100.5% on the dried basis

### IMPURITIES

#### Organic Impurities

#### • PROCEDURE: READILY CARBONIZABLE SUBSTANCES TEST (271)

**Sample:** 100 mg

**Analysis:** Dissolve the *Sample* in 0.5 mL of sulfuric acid.

**Acceptance criteria:** No blackening occurs, although some effervescence may take place.

### SPECIFIC TESTS

• **LOSS ON DRYING (731):** Dry a sample over phosphorus pentoxide for 4 h: it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

## Halazone Tablets for Solution

» Halazone Tablets for Solution contain not less than 90.0 percent and not more than 135.0 percent of the labeled amount of  $C_7H_5Cl_2NO_4S$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label the Halazone Tablets for Solution to indicate that they are not intended to be swallowed.

**Identification**—Finely powder a number of Halazone Tablets for Solution, equivalent to about 150 mg of halazone: a portion of the powder, equivalent to about 100 mg of halazone, responds to the *Identification* test under *Halazone*.

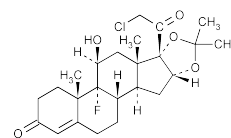
**Disintegration (701):** 10 minutes.

**Uniformity of dosage units (905):** meet the requirements, except that if the average value of the dosage units tested is between 100.0 percent and 135.0 percent, *Criterion (B) (3)* applies.

**pH (791):** not less than 7.0, in a solution of 1 Halazone Tablet for Solution, containing 4 mg of halazone, in 200 mL of water.

**Assay**—Transfer a counted number of Halazone Tablets for Solution, equivalent to about 160 mg of halazone, to a suitable container, and proceed as directed in the *Assay* under *Halazone*. Each mL of 0.1 N sodium thiosulfate is equivalent to 6.752 mg of  $C_7H_5Cl_2NO_4S$ .

## Halcinonide



$C_{24}H_{32}ClFO_5$  454.96

Pregn-4-ene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11 $\beta$ ,16 $\alpha$ )-.

21-Chloro-9-fluoro-11 $\beta$ ,16 $\alpha$ ,17-trihydroxypregn-4-ene-3,20-dione cyclic 16,17-acetal with acetone [3093-35-4].

» Halcinonide contains not less than 97.0 percent and not more than 102.0 percent of  $C_{24}H_{32}ClFO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards (11)**—

USP Halcinonide RS

**Identification, Infrared Absorption (197K).**

**Specific rotation (781S):** between +150° and +160°.

*Test solution:* 20 mg per mL, in chloroform.

**Loss on drying (731)**—Dry it in vacuum at 100° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition (281):** not more than 0.2%.

**Chromatographic purity**—Prepare the test solution by dissolving 50 mg of Halcinonide in 5.0 mL of a mixture of chloroform and methanol (1:1). Divide the area of a suitable thin-layer chromatographic plate (see *Chromatography (621)*) coated with a 0.25-mm layer of chromatographic silica gel mixture, into three equal sections, the first two sections to be used for the test solution and the third section for the blank. Apply 100  $\mu$ L of the test solution to appropriate sections of the plate, drying each solution as it is applied with a current of warm air. Using a continuous elution chromatographic chamber, develop the chromatogram in a solvent system consisting of a mixture of chloroform and ethyl acetate (5:1) for about 2 hours. Remove the plate from the developing chamber, dry in an oven at 90° for 15 minutes, and locate the bands by viewing under short-wavelength UV light. Mark the principal band and any secondary bands. Quantitatively remove the silica gel containing these bands, including a corresponding blank segment, and transfer to separate glass-stoppered, 50-mL centrifuge tubes, combining the impurities if more than one impurity is present. Add 30.0 mL of dehydrated alcohol to the tubes containing the principal band and the corresponding blank, and add 10.0 mL of dehydrated alcohol to the tubes containing the combined impurities and the corresponding blank. Insert stoppers in the tubes, and shake gently on a reciprocating shaker for about 60 minutes. Centrifuge, dilute the principal band eluate and its corresponding blank eluate with an



equal volume of dehydrated alcohol, and mix. Determine the absorbances of the clear supernatant eluates in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using dehydrated alcohol as the blank. Calculate the percentage of chromatographic impurities by the formula:

$$100A_i / (A_i + 6A_u)$$

in which  $A_i$  is the absorbance of the combined impurity bands eluate, corrected for the corresponding blank; and  $A_u$  is the absorbance of the principal band eluate, corrected for the corresponding blank. Not more than 3.0% is found.

#### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Halcinonide RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 15 µg per mL.

**Assay preparation**—Weigh accurately about 30 mg of Halcinonide, transfer to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of  $C_{24}H_{32}ClFO_5$  in the portion of Halcinonide taken by the formula:

$$2C(A_u / A_s)$$

in which  $C$  is the concentration, in µg per mL, of USP Halcinonide RS in the *Standard preparation*; and  $A_u$  and  $A_s$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Halcinonide Cream

» Halcinonide Cream is Halcinonide in a suitable cream base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{32}ClFO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Halcinonide RS

**Identification**—Transfer a quantity of Cream, equivalent to about 2 mg of halcinonide, to a glass-stoppered, 50-mL centrifuge tube, add 15 mL of warm water, and shake for 2 minutes to disperse. Add 20 mL of chloroform, and shake for 5 minutes. Cool in an ice bath, then centrifuge. Transfer the chloroform layer to a conical flask. Repeat the extraction with an additional 15 mL of chloroform, and combine the chloroform extracts. Evaporate the chloroform extracts on a steam bath under a current of air nearly to dryness, and dissolve the residue in 10.0 mL of chloroform. Apply 20 µL of this solution and 20 µL of a solution of USP Halcinonide RS in chloroform having a concentration of 0.2 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and ethyl acetate (5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, and view the chromatogram under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test

solution corresponds to that obtained from the Standard solution.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill (755):** meets the requirements.

#### Assay—

**Solvent A**—On the day of use, prepare a mixture of acetonitrile and water (2:1).

**Mobile phase**—Mix approximately equal volumes of acetonitrile and water, adjusting the composition as necessary to achieve acceptable chromatography.

**Internal standard solution**—Transfer 15 mg of Progesterone to a 100-mL volumetric flask. Dissolve in hexanes-saturated *Solvent A*, dilute with hexanes-saturated *Solvent A* to volume, and mix.

**Standard preparation**—Transfer about 20 mg of USP Halcinonide RS, accurately weighed, to a 100-mL volumetric flask, dissolve in *Solvent A*, dilute with *Solvent A* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask. Add 4.0 mL of *Internal standard solution*, dilute with hexanes-saturated *Solvent A* to volume, and mix.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 0.5 mg of halcinonide, to a glass-stoppered, 50-mL centrifuge tube, add 12 mL of hexanes-saturated *Solvent A* and 20 mL of hexanes, and shake for 1 minute. Place in a heated ultrasonic bath at  $58 \pm 2^\circ$  for 20 minutes, initially shaking for 1 to 2 minutes to ensure dispersion, and at about 5-minute intervals thereafter, on a vibratory mixer. Cool, centrifuge, and transfer the lower layer to a 25-mL volumetric flask. Add 5 mL of hexanes-saturated *Solvent A* to the tube, mix for 1 minute, then centrifuge. Transfer the lower layer to the volumetric flask, and repeat the extraction with an additional 5 mL of hexanes-saturated *Solvent A*, combining the extracts in the flask. Add 2.0 mL of *Internal standard solution* to the flask, dilute with hexanes-saturated *Solvent A* to volume, and mix. If necessary, clarify a portion of the solution by centrifugation.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 1.5, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.2 for progesterone and 1.0 for halcinonide. Calculate the quantity, in mg, of  $C_{24}H_{32}ClFO_5$  in the portion of Cream taken by the formula:

$$25C(R_u / R_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Halcinonide RS in the *Standard preparation*; and  $R_u$  and  $R_s$  are the ratios of the peak responses of halcinonide to internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Halcinonide Ointment

» Halcinonide Ointment is Halcinonide in a suitable ointment base. It contains not less than

90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{32}ClFO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Halcinonide RS

**Identification**—It responds to the *Identification* test under *Halcinonide Cream*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

**Mobile phase**—Mix approximately equal volumes of acetonitrile and water, adjusting the ratio of solvents as necessary to achieve acceptable chromatography.

**Internal standard solution**—Dissolve Butylparaben in acetonitrile to obtain a solution having a concentration of 6  $\mu$ g per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Halcinonide RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.04 mg per mL. Mix 5.0 mL of this solution with 5.0 mL of the *Mobile phase*. Each mL of the *Standard preparation* has a known concentration of about 0.02 mg of USP Halcinonide RS.

**Assay preparation**—Transfer an accurately weighed quantity of Ointment, equivalent to about 1 mg of halcinonide, to a glass-stoppered, 50-mL centrifuge tube, and add 25.0 mL of *Internal standard solution* and 5.0 mL of hexane. Place in a water bath at  $58 \pm 2^\circ$  for 3 minutes, then mix in a vortex mixer for about 1 minute until the specimen is well dispersed. Repeat the above-specified heating and mixing step one more time. Cool in an ice-methanol bath for 15 minutes or until the two phases separate, centrifuging if necessary. Transfer 5.0 mL of the lower layer into a 15-mL centrifuge tube, add 5.0 mL of *Mobile phase*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2.0, and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for butylparaben and 1.0 for halcinonide. Calculate the quantity, in mg, of  $C_{24}H_{32}ClFO_5$  in the portion of Ointment taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Halcinonide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of halcinonide to internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Halcinonide Topical Solution

» Halcinonide Topical Solution is Halcinonide in a suitable aqueous vehicle. It contains not less than

90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{32}ClFO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Halcinonide RS

**Identification**—It responds to the *Identification* test under *Halcinonide Cream*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Assay**—

**Mobile phase**—Mix approximately equal volumes of acetonitrile and water, adjusting the ratio of solvents as necessary to achieve acceptable chromatography.

**Internal standard solution**—Transfer 15 mg of Progesterone to a 50-mL volumetric flask. Dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Transfer about 20 mg of USP Halcinonide RS, accurately weighed, to a 100-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

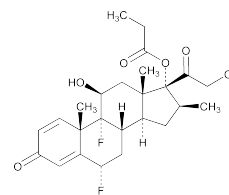
**Assay preparation**—Transfer an accurately measured quantity of Topical Solution, equivalent to about 1 mg of halcinonide, to a 50-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system and Procedure**—Proceed as directed in the *Assay* under *Halcinonide Cream*. Calculate the quantity, in mg, of  $C_{24}H_{32}ClFO_5$  in the portion of Topical Solution taken by the formula:

$$50C(R_U / R_S)$$

in which the terms are as defined therein.

## Halobetasol Propionate



$C_{25}H_{31}ClF_2O_5$  484.96  
Pregna-1,4-diene-3,20-dione, 21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (6 $\alpha$ ,11 $\beta$ ,16 $\beta$ )-; 21-Chloro-6 $\alpha$ ,9-difluoro-11 $\beta$ ,17-dihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17-propionate [66852-54-8].

### DEFINITION

Halobetasol Propionate contains NLT 98.0% and NMT 102.0% of  $C_{25}H_{31}ClF_2O_5$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** Acetonitrile and water (9:11)

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	64.5	35.5
22	64.5	35.5
23	100	0
30	100	0

**Standard solution:** 0.2 mg/mL of USP Halobetasol Propionate RS in acetonitrile

**Sample solution:** 0.2 mg/mL of Halobetasol Propionate in acetonitrile

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.8 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>25</sub>H<sub>31</sub>ClF<sub>2</sub>O<sub>5</sub> in the portion of Halobetasol Propionate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

• **RESIDUE ON IGNITION** <281>: NMT 0.2%

• **HEAVY METALS**, *Method II* <231>: NMT 20 ppm

**Organic Impurities**• **PROCEDURE**

**Mobile phase, Standard solution, and Sample solution:** Proceed as directed in the Assay.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.8 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 22,000 theoretical plates

**Tailing factor:** NLT 0.9 and NMT 1.1 for halobetasol propionate

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Halobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of any individual impurity from the *Sample solution*

$r_T$  = sum of responses for all the peaks from the *Sample solution*

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
21-Chloro diflorasone <sup>a</sup>	0.75	0.15
21-Acetate 17-propionate diflorasone <sup>b</sup>	0.88	0.15
11-Propionate 21-chloro diflorasone <sup>c</sup>	0.95	0.15
Halobetasol propionate	1.0	—
9-Chloro halobetasol propionate <sup>d</sup>	1.12	0.15
6-Chloro halobetasol propionate <sup>e</sup>	1.24	0.15
Any individual, unspecified degradation product	—	0.10

<sup>a</sup> 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione.

<sup>b</sup> 6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-acetate 17-propionate.

<sup>c</sup> 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 11-propionate.

<sup>d</sup> 9,21-Dichloro-6α,9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

<sup>e</sup> 6α,21-Dichloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

**SPECIFIC TESTS**

• **LOSS ON DRYING** <731>: Dry a sample in a vacuum at 70° for 3 h: it loses NMT 1.0% of its weight.

• **OPTICAL ROTATION**, *Specific Rotation* <781S>

**Sample solution:** 10 mg/mL in dioxane

**Acceptance criteria** Between +87° and +99°

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed, light resistant containers. Store between 2° and 8°.

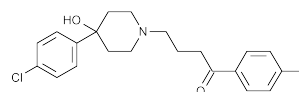
• **USP REFERENCE STANDARDS** <11>

USP Halobetasol Propionate RS

Pregna-1,4-diene-3,20-dione, 21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (6α,11β,16β)-.

21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

C<sub>25</sub>H<sub>31</sub>ClF<sub>2</sub>O<sub>5</sub> 484.96

**Haloperidol**

C<sub>21</sub>H<sub>23</sub>ClFNO<sub>2</sub> 375.86

1-Butanone, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-.

4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone [52-86-8].

» Haloperidol contains not less than 98.0 percent and not more than 102.0 percent of  $C_{21}H_{23}ClFNO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Haloperidol RS

USP Haloperidol Related Compound A RS

4,4'-Bis[4-*p*-chlorophenyl]-4-hydroxypiperidino]butyrophenone.

$C_{32}H_{36}Cl_2N_2O_3$  567.56

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 20 µg per mL.

*Medium:* dilute hydrochloric acid (1 in 100) in isopropyl alcohol (1 in 9).

Absorptivities at 245 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 149° and 155°, determined after drying in vacuum at 60° for 3 hours.

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Limit of haloperidol related compound A**—

*Test solution*—Dissolve about 80 mg of Haloperidol, accurately weighed, in 80 mL of isopropyl alcohol in a 100-mL volumetric flask. Add 10 mL of dilute hydrochloric acid (1 in 100), dilute with isopropyl alcohol to volume, and mix.

*Standard solution*—Prepare a solution containing 800 µg per mL of USP Haloperidol RS and 8 µg per mL of USP Haloperidol Related Compound A RS in isopropyl alcohol containing 10 mL of dilute hydrochloric acid (1 in 100) in each 100 mL of solution.

*Procedure*—Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 335 nm, with a suitable spectrophotometer, using isopropyl alcohol containing 10 mL of dilute hydrochloric acid (1 in 100) in each 100 mL of solution as the blank. The absorbance of the *Test solution* is not greater than that of the *Standard solution*, corresponding to not more than 1.0%.

**Assay**—Dissolve about 125 mg of Haloperidol, accurately weighed, in 25 mL of glacial acetic acid, add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.05 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 18.79 mg of  $C_{21}H_{23}ClFNO_2$ .

## Haloperidol Injection

### DEFINITION

Haloperidol Injection is a sterile solution of Haloperidol in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ). It may contain a suitable preservative.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer solution:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

**Mobile phase:** Methanol and *Buffer solution* (55:45)

**Standard stock solution:** 1 mg/mL of USP Haloperidol RS in methanol. Sonicate to aid in dissolution.

**Standard solution:** 0.2 mg/mL of USP Haloperidol RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** Nominally, 0.2 mg/mL of haloperidol in *Mobile phase* from a volume of Injection

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 247 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 0.8 mL/min

**Injection size:** 10 µL

**Run time:** 2.5 times the retention time of haloperidol

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 71.4 USP Endotoxin Units/mg of haloperidol.
- PH (791):** 3.0–3.8
- OTHER REQUIREMENTS:** It meets the requirements under *Injections* (1).

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS  
USP Haloperidol RS

## Haloperidol Oral Solution

### DEFINITION

Haloperidol Oral Solution is a solution of Haloperidol in Water, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer solution:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

**Mobile phase:** Methanol and *Buffer solution* (55:45)

**Standard stock solution:** 1 mg/mL of USP Haloperidol RS in methanol. Sonicate to aid in dissolution.

**Standard solution:** 0.2 mg/mL of USP Haloperidol RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** Nominally, 0.2 mg/mL of haloperidol in *Mobile phase* from a volume of Haloperidol Oral Solution. Filter a portion to use in the analysis.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 247 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 0.8 mL/min

**Injection size:** 10 μL

**Run time:** 2.5 times the retention time of haloperidol

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- DELIVERABLE VOLUME** <698>: Meets the requirements for oral solution packaged in multiple-unit containers
- UNIFORMITY OF DOSAGE UNITS** <905>: Meets the requirements for oral solution packaged in single-unit containers

**SPECIFIC TESTS**

- PH** <791>: 2.75–3.75

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS** <11>  
USP Haloperidol RS

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**Haloperidol Tablets**

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**DEFINITION**

Haloperidol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol and 0.05 M monobasic potassium phosphate buffer (60:40). Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.0.

**Standard solution:** 0.1 mg/mL of USP Haloperidol RS in *Mobile phase*

**Sample solution:** Nominally 0.1 mg/mL of Haloperidol prepared as follows. Transfer an equivalent of about 10 mg of haloperidol from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 60 mL of *Mobile phase*, sonicate for 10 min, and shake by mechanical means for about 1 h. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 20 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 15 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION** <711>

**Medium:** Simulated gastric fluid TS without enzyme; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 60 min

**Mobile phase:** Prepare as directed in the *Assay*.

**Standard solution:** A known concentration of USP Haloperidol RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 50 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Tolerances:** NLT 80% (Q) of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ) is dissolved.

**• UNIFORMITY OF DOSAGE UNITS** <905>**Procedure for content uniformity**

**Standard solution:** 20 μg/mL of USP Haloperidol RS in warm methanol

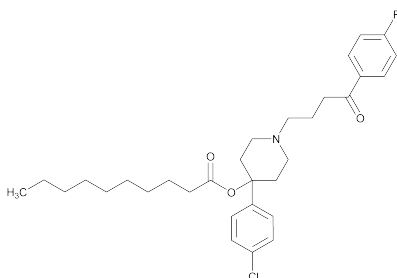
**Sample solution:** 20 μg/mL of haloperidol from 1 finely powdered Tablet in warm methanol. Shake for 15 min and filter, discarding the first 20 mL of the filtrate.

**Instrumental conditions**(See *Spectrophotometry and Light-Scattering* <851>.)**Mode:** UV**Analytical wavelength:** 245 nm**Cell:** 1 cm**Blank:** Methanol**Analysis:** Concomitantly determine the absorbances of the *Standard solution* and *Sample solution* at the wavelength of maximum absorbance.Calculate the percentage of the labeled amount of haloperidol ( $C_{21}H_{23}ClFNO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Haloperidol RS in the *Standard solution* ( $\mu\text{g/mL}$ ) $C_U$  = nominal concentration of haloperidol in the *Sample solution* ( $\mu\text{g/mL}$ )**Acceptance criteria:** Meet the requirements**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** <11>  
USP Haloperidol RS

**Haloperidol Decanoate**

$C_{31}H_{41}ClFNO_3$  530.11  
Decanoic acid, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl ester;  
Decanoic acid, ester with 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone [74050-97-8].

**DEFINITION**Haloperidol Decanoate contains NLT 97.0% and NMT 103.0% of  $C_{31}H_{41}ClFNO_3$ , calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION** <197M>
- **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** <191>  
**Sample solution:** Mix 0.1 g of the sample with 0.5 g of anhydrous sodium carbonate in a porcelain crucible. Heat over open flame for 10 min. Allow to cool. Dissolve the residue in 5 mL of dilute nitric acid, and filter. Dilute 1 mL of the filtrate with 1 mL of water.  
**Acceptance criteria:** Meets the requirements of the silver nitrate precipitate test

**ASSAY**• **PROCEDURE****Solution A:** 27 g/L of tetrabutylammonium hydrogen sulfate in water**Solution B:** Acetonitrile**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	40	60
35	40	60
40	80	20
45	80	20

**Standard solution:** 0.2 mg/mL of USP Haloperidol Decanoate RS in methanol**Sample solution:** 0.2 mg/mL of Haloperidol Decanoate in methanol**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4-mm  $\times$  10-cm; 3- $\mu\text{m}$  packing L1**Flow rate:** 1.5 mL/min**Injection size:** 10  $\mu\text{L}$ **System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of Haloperidol Decanoate ( $C_{31}H_{41}ClFNO_3$ ) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Haloperidol Decanoate RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Haloperidol Decanoate in the *Sample solution* (mg/mL)**Acceptance criteria:** 97.0%–103.0% on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%
- **HEAVY METALS, Method II** <231>: NMT 20 ppm
- **ORGANIC IMPURITIES**

**Solution A, Solution B, and Mobile phase:** Proceed as directed in the Assay.**System suitability solution:** 0.05 mg/mL each of USP Haloperidol Decanoate RS and USP Bromperidol Decanoate RS in methanol**Standard stock solution:** 0.2 mg/mL of USP Haloperidol Decanoate RS in methanol**Standard solution:** 0.05 mg/mL of USP Haloperidol Decanoate RS in methanol, from *Standard stock solution***Sample solution:** 10 mg/mL of Haloperidol Decanoate in methanol**Chromatographic system:** Proceed as directed in the Assay.**System suitability****Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 1.5 between haloperidol decanoate and bromperidol decanoate**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Haloperidol Decanoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution*

- $r_s$  = peak response of haloperidol decanoate from the *Standard solution*  
 $C_s$  = concentration of USP Haloperidol Decanoate RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Haloperidol Decanoate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Haloperidol <sup>a</sup>	0.09	0.50
Haloperidol octanoate <sup>b</sup>	0.6	0.50
Haloperidol nonanoate <sup>c</sup>	0.79	0.50
Haloperidol decanoate-dechloro analog <sup>d</sup>	0.89	0.50
2-Fluorohaloperidol decanoate <sup>e</sup>	0.97	0.50
Haloperidol decanoate	1.0	—
Bromperidol decanoate <sup>f</sup>	1.05	—
Haloperidol undecanoate <sup>g</sup>	1.10	0.50
Haloperidol decanoate-3-ethyl analog <sup>h</sup>	1.13	0.50
Haloperidol decanoate-4-piperidinol analog <sup>i</sup>	1.17	0.50
Haloperidol dodecanoate <sup>j</sup>	1.21	0.50
Haloperidol decanoate-3-chlorobiphenyl analog <sup>k</sup>	1.22	0.50
Haloperidol decanoate-4-chlorobiphenyl analog <sup>l</sup>	1.24	0.50
Any other individual, unspecified impurity	—	0.10
Total impurities	—	1.0

<sup>a</sup> 4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4-fluorobutyrophenone.

<sup>b</sup> 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl octanoate.

<sup>c</sup> 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl nonanoate.

<sup>d</sup> 1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-phenylpiperidin-4-yl decanoate.

<sup>e</sup> 4-(4-Chlorophenyl)-1-(4-(2-fluorophenyl)-4-oxobutyl)piperidin-4-yl decanoate.

<sup>f</sup> Used only for system suitability.

<sup>g</sup> 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl undecanoate.

<sup>h</sup> 4-(4-Chlorophenyl)-1-[4-(3-ethyl-4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

<sup>i</sup> 4-(4-Chlorophenyl)-1-(4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl)-4-oxobutylpiperidin-4-yl decanoate.

<sup>j</sup> 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl dodecanoate.

<sup>k</sup> 4-(4'-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

<sup>l</sup> 4-(3'-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample in a vacuum at 30° under phosphorous pentoxide desiccant: it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Bromperidol Decanoate RS
  - Decanoic acid, 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl ester.
  - C<sub>31</sub>H<sub>41</sub>BrFNO<sub>3</sub> 574.56
  - USP Haloperidol Decanoate RS

## Halothane



C<sub>2</sub>HBrClF<sub>3</sub> 197.38  
 Ethane, 2-bromo-2-chloro-1,1,1-trifluoro-, (±)-;  
 (±)-2-Bromo-2-chloro-1,1,1-trifluoroethane [151-67-7].

### DEFINITION

Halothane contains NLT 0.008% and NMT 0.012% of thymol, by weight, as a stabilizer.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197S)

**Sample solution:** 1 in 25

**Medium:** Carbon disulfide

**Acceptance criteria:** Meets the requirements

### OTHER COMPONENTS

#### • THYMOL CONTENT

**Buffer:** Use pH 8.0 Alkaline Borate Buffer (see *Reagents, Indicators, and Solutions—Solutions*).

**Chlorimide solution:** 4 mg/mL of 2,6-dibromoquinonechlorimide in dehydrated alcohol. [NOTE—Prepare a fresh solution for each assay.]

**Standard thymol solution:** 0.1 mg/mL of thymol in 0.25 N sodium hydroxide

**Standard solution A:** Pipet 1 mL of *Standard thymol solution* into a 100-mL volumetric flask, and add 0.25 N sodium hydroxide to make the final volume 5 mL. Add 10 mL of *Buffer*, mix by gentle swirling, and add 1 mL of *Chlorimide solution*. Allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and dilute with water to volume.

**Standard solution B:** Pipet 3 mL of *Standard thymol solution* into a 100-mL volumetric flask. Proceed as directed for *Standard solution A*, beginning with "add 0.25 N sodium hydroxide..."

**Standard solution C:** Pipet 5 mL of *Standard thymol solution* into a 100-mL volumetric flask. Proceed as directed for *Standard solution A*, beginning with "Add 10 mL of *Buffer*..."

**Sample solution:** Place 2 mL of Halothane in a 100-mL volumetric flask containing 5 mL of 0.25 N sodium hydroxide, and mix by gentle swirling. Evaporate the halothane under a stream of nitrogen, and add 10 mL of *Buffer* and 1 mL of *Chlorimide solution*. Swirl gently, and allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and add water to volume.

**Blank solution:** Pipet 5.0 mL of 0.25 N sodium hydroxide into a 100-mL volumetric flask. Add 10 mL of *Buffer*, mix by gentle swirling, and add 1 mL of *Chlorimide solution*. Allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and dilute with water to volume.

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Vis

**Analytical wavelength:** 590 nm

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Sample solution*, and *Blank solution*  
 Measure the absorbances of *Standard solution A*, *Standard solution B*, and *Standard solution C* relative to the *Blank solution*. Plot the readings, and draw the curve of best fit. Read the absorbance of the *Sample solution*, and by reference to the *Standard thymol curve*, calculate the percentage of thymol in the weight of Halothane taken.

Acceptance criteria: 0.008%–0.012%, by weight

## IMPURITIES

### • LIMIT OF NONVOLATILE RESIDUE

**Analysis:** Evaporate 50 mL in a tared dish on a steam bath to dryness, dry the residue at 105° for 2 h, and weigh.

**Acceptance criteria:** NMT 1 mg of the residue remains.

### • CHLORIDE AND BROMIDE

**Analysis:** Shake 25 mL with 25 mL of water for 5 min, and allow the liquids to separate completely. Draw off the water layer, and to 10 mL add 1 drop of nitric acid and 5 drops of silver nitrate TS.

**Acceptance criteria:** No opalescence is produced.

### • ORGANIC IMPURITIES

**Standard solution:** Add 1.0 µL of 1,1,2-trichloro-1,2,2-trifluoroethane to 20.0 mL of Halothane.

**Sample solution:** Halothane

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 3-m × 2-mm stainless steel; 20% G24 on support S1AB

**Temperatures**

**Column:** 60°

**Injection port:** 200°

**Detector:** 200°

**Carrier gas:** Nitrogen

**Flow rate:** 15 mL/min

**Injection volume:** 2 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention times for 1,1,2-trichloro-1,2,2-trifluoroethane and halothane are 5 and 13 min, respectively.]

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** 0.005%; the total area of all peaks (except that of halothane) recorded from the *Sample solution* does not exceed that due to the added 1,1,2-trichloro-1,2,2-trifluoroethane in the *Standard solution*.

## SPECIFIC TESTS

### • ACIDITY OR ALKALINITY

**Analysis:** Shake 20 mL with 20 mL of carbon dioxide-free water for 3 min, and allow the layers to separate.

**Acceptance criteria:** The aqueous layer requires NMT 0.1 mL of 0.010 N sodium hydroxide or NMT 0.6 mL of 0.010 N hydrochloric acid for neutralization, using bromocresol purple TS as the indicator.

### • DISTILLING RANGE, *Method II* <721>: NLT 95% distills within a 1° range between 49° and 51°, and NLT 100% distills between 49° and 51°, a correction factor of 0.040°/mm being applied as necessary.

### • REFRACTIVE INDEX <831>: 1.369–1.371 at 20°

### • SPECIFIC GRAVITY <841>: 1.872–1.877 at 20°

### • WATER DETERMINATION, *Method I* <921>: NMT 0.03%

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers, preferably of Type NP glass, and avoid exposure to excessive heat. Dispense it only in the original container.

### • USP REFERENCE STANDARDS <11>

USP Halothane RS

» Helium contains not less than 99.0 percent, by volume, of He.

**Packaging and storage**—Preserve in cylinders.

**NOTE**—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.

**Identification**—The flame of a burning splinter of wood is extinguished when inserted into an inverted test tube filled with Helium. [NOTE—Use caution.] A small balloon filled with Helium shows decided buoyancy.

**Odor**—Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.

**Carbon monoxide**—Pass 1000 ± 50 mL through a carbon monoxide detector tube (see under *Reagents* in the section *Reagents, Indicators, and Solutions*) at the rate specified for the tube: the indicator change corresponds to not more than 0.001%.

**Air**—Not more than 1.0% of air is present, determined as directed in the *Assay*.

**Assay**—Introduce a specimen of Helium into a gas chromatograph by means of a gas sampling valve. Select the operating conditions of the gas chromatograph such that the standard peak signal resulting from the following procedure corresponds to not less than 70% of the full-scale reading. Preferably, use an apparatus corresponding to the general type in which the column is 6 m in length and 4 mm in inside diameter and is packed with porous polymer beads, which permits complete separation of nitrogen and oxygen from Helium, although the nitrogen and oxygen may not be separated from each other. Use industrial grade helium (99.99%) as the carrier gas, with a thermal-conductivity detector, and control the column temperature: the peak response produced by the assay specimen exhibits a retention time corresponding to that produced by an air-helium certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and indicates not more than 1.0% of air when compared to the peak response of the air-helium certified standard, and not less than 99.0%, by volume, of He.

## Heparin Lock Flush Solution

### DEFINITION

Heparin Lock Flush Solution is a sterile preparation of Heparin Sodium Injection with sufficient Sodium Chloride to make it isotonic with blood. Its potency is NLT 90.0% and NMT 120.0% of the potency stated on the label in terms of USP Heparin Units. It contains NMT 1.00% of sodium chloride (NaCl). It may contain a suitable preservative.

### ASSAY

#### • ANTI-FACTOR IIa POTENCY

**pH 8.4 buffer:** Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

**Antithrombin solution:** Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Anti-thrombin IU/mL. Dilute this solution with pH 8.4 buffer to obtain a solution having a concentration of 0.125 Anti-thrombin IU/mL.

## Helium

He 4.00

Helium.

Helium [7440-59-7].



**Thrombin human solution:** Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

**Stopping solution:** 20% (v/v) solution of acetic acid

**Standard solutions:** Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Lock Flush Solution similar to those obtained for the *Standard solutions*.

#### Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200  $\mu$ L) of *Anti-thrombin solution* to each tube containing one volume (50–100  $\mu$ L) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50  $\mu$ L of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100  $\mu$ L of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100  $\mu$ L of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* <851>). Calculate the change in absorbance/min ( $\Delta$ OD/min). The blanks for kinetic measurement are also expressed as  $\Delta$ OD/min and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

**Calculations:** The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

**Parallel-line assay:** For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Lock Flush Solution in USP Units/mL using statistical methods for parallel-line assays.

ar in Lock Flush Solution in USP Units/mL using statistical methods for parallel-line assays.

**Slope ratio assay:** For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Lock Flush Solution in USP Units/mL using statistical methods for slope ratio assays.

**Acceptance criteria:** 90.0%–120.0%

#### • SODIUM CHLORIDE

**Sample solution:** Pipet 10 mL of Solution into a suitable container, dilute with water to about 150 mL, and add 1.5 mL of potassium chromate TS.

**Analysis:** Titrate with 0.1 N silver nitrate. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** <85>: NMT 0.5 USP Endotoxin Unit/mL
- **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements for small-volume injections
- **pH** <791>: 5.0–7.5
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections* <1>.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, prefilled syringes or containers, or in multiple-dose containers, preferably of Type I glass.
- **LABELING:** Label it to indicate the volume of the total contents and to indicate the potency in terms of USP Heparin Units only per mL, except that single unit-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states clearly that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate the organ and species from which the heparin sodium is derived. The label states also that the Solution is intended for maintenance of patency of intravenous injection devices only, and that it is not to be used for anticoagulant therapy. The label states also that in the case of the Solution having a concentration of 10 USP Heparin Units/mL, it may alter, and that in the case of higher concentrations, it will alter, the results of blood coagulation tests.
- **USP REFERENCE STANDARDS** <11>  
USP Endotoxin RS  
USP Heparin Sodium for Assays RS

## Heparin Sodium

### DEFINITION

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process should be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (1050) for general guidance on viral safety evaluation. It is composed of polymers of alternating derivatives of  $\alpha$ -D-glucosamido (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and *O*-sulfated uronic acid ( $\alpha$ -L-iduronic acid or  $\beta$ -D-glucuronic acid). The component activities of the mixture are in ratios corresponding to

those shown by USP Heparin Sodium for Assays RS. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin and heparin cofactor II to potentiate the inactivation of thrombin (factor IIa). Other coagulation proteases in the clotting sequence, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity to anti-factor IIa potency is between 0.9 and 1.1. The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

## IDENTIFICATION

### A. <sup>1</sup>H NMR SPECTRUM

(See *Nuclear Magnetic Resonance* <761>.)

**Standard solution:** NLT 20 mg/mL of USP Heparin Sodium Identification RS in deuterium oxide with 0.02% (w/v) deuterated trimethylsilylpropionic (TSP) acid sodium salt

**System suitability solution:** Prepare 1% (w/w) USP Oversulfated Chondroitin Sulfate RS in *Standard solution*.

**Sample solution:** NLT 20 mg/mL of Heparin Sodium in deuterium oxide with 0.02% (w/v) deuterated TSP

#### Spectrometric conditions

(See *Nuclear Magnetic Resonance* <761>.)

**Mode:** NMR, pulsed (Fourier transform)

**Frequency:** NLT 500 MHz (for <sup>1</sup>H)

**Temperature:** 25°

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for <sup>1</sup>H, acquire a free induction decay (FID) using NLT 16 scans using a 90° pulse and 20-s delay. Record the <sup>1</sup>H NMR spectra of the *Standard solution* and *System suitability solution* at 25°. Collect the <sup>1</sup>H NMR spectrum with a spectral window of at least 10 to -2 ppm and without spinning. The number of transients should be adjusted until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm. The *Standard solution* shall be run at least daily when *Sample solutions* are being run. For all samples, the TSP methyl signal should be set to 0.00 ppm. The chemical shift for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution* should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the <sup>1</sup>H NMR spectrum of the *Sample solution* at 25°. Draw a baseline from 8.00 ppm to 0.10 ppm. The ppm values for H1 of GlcNAc/GlcNS, 6S (signal 1), H1 of IdoA2S (signal 2), the H2 of GlcNS (signal 3), and the methyl of GlcNAc (signal 4) of heparin are present at 5.42, 5.21, 3.28 (doublet centered at 3.28 ppm), and 2.05 ppm, respectively.<sup>1</sup> The ppm values of these signals do not differ by more than ±0.03 ppm. Measure the signal heights above the baseline of signal 1 and signal 2, and calculate the mean of these signal heights. Other signals of variable heights and ppm values, attributable to heparin and HOD, may be seen between signal 2 and 4.55 ppm. Residual solvent signals may be observed in the 0.10–3.00 range. Heparin Sodium must meet the requirements stated in *Residual Solvents* <467>.

#### Suitability requirements

**Number of transients:** Adjust until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm.

**Chemical shift:** The TSP methyl signal should be set to 0.00 ppm for all samples.

**Chemical shifts** (for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate): Should be ob-

served at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively, *System suitability solution*

#### Analysis

**Sample:** *Sample solution*

**Acceptance criteria:** No unidentified signals greater than 4% of the mean of signal height of 1 and 2 are present in the following ranges: 0.10–2.00, 2.10–3.20, and 5.70–8.00 ppm. No signals greater than 200% signal height of the mean of the signal height of 1 and 2 are present in the 3.35–4.55 ppm for porcine heparin.

### B. CHROMATOGRAPHIC IDENTITY

**Solution A:** Dissolve 0.8 g of monobasic sodium phosphate dihydrate in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a filter membrane with pore sizes of 0.45 µm and degas before use.

**Solution B:** Dissolve 0.8 g of monobasic sodium phosphate dihydrate and 280 g of sodium perchlorate monohydrate in 2 L of water and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a filter membrane with pore sizes of 0.45 µm and degas before use.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Elution
0	80	20	Equilibration
60	10	90	Linear gradient
61	80	20	Linear gradient
75	80	20	Re-equilibration

**Standard solution:** NLT 20 mg/mL of USP Heparin Sodium Identification RS in water

**System suitability solution:** Prepare 1% (w/w) USP Oversulfated Chondroitin Sulfate RS and 1% (w/w) USP Dermatan Sulfate RS in *Standard solution*.

**Sample solution:** NLT 20 mg/mL of Heparin Sodium in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 202 nm

**Column:** 2-mm × 25-cm; packing L61

**Guard column:** 2-mm × 50-mm; packing L61

**Column temperature:** Maintain columns at 40°

**Flow rate:** 0.22 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The retention times for dermatan sulfate, heparin, and oversulfated chondroitin sulfate are about 20, 30, and 50 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between the dermatan sulfate and the heparin peaks, and NLT 1.5 between the heparin and the oversulfated chondroitin sulfate

**Relative standard deviation:** NMT 2% for the heparin peak determined from three replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Record the chromatograms, and measure the retention times for the major peaks.

**Acceptance criteria:** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*.

### C. ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO

#### Anti-factor Xa activity

**pH 8.4 buffer:** Dissolve amounts of tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water containing 0.1% of polyethylene glycol 6000 to obtain a solution having concentrations of 0.050, 0.0075, and 0.175 M, respectively. Adjust, if necessary, with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

<sup>1</sup> GlcNAc, *N*-acetylated glucosamine; GlcNS, *N*-sulfated glucosamine; S, sulfate; IdoA, iduronic acid; GlcN, glucosamine; GalN, galactosamine.

**Antithrombin solution:** Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) as directed by the manufacturer, and further dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 1.0 Antithrombin IU/mL.

**Factor Xa solution:** Reconstitute bovine factor Xa as directed by the manufacturer (see *Factor Xa* in *Reagents, Indicators, and Solutions—Reagent Specifications*), and further dilute in *pH 8.4 buffer* to obtain a solution that gives an absorbance value between 0.65 and 1.25 at 405 nm when assayed as described below but using 30  $\mu$ L of *pH 8.4 buffer* instead of 30  $\mu$ L of the *Standard solutions* or the *Sample solutions*. [NOTE—*Factor Xa solution* contains about 3 nanokatalytic units/mL, but can vary depending upon the manufacturer of factor Xa or the substrate used.]

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) specific for factor Xa in water to obtain a concentration of 1 mM.

**Stopping solution:** 20% (v/v) solution of acetic acid

**Standard solutions:** Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least five dilutions in the concentration range between 0.03 and 0.375 USP Heparin Units/mL.

**Sample solutions:** Dissolve or dilute a measured quantity of Heparin Sodium in *pH 8.4 buffer*, and dilute with the same buffer to obtain solutions having activities approximately equal to those of the *Standard solutions*.

#### Analysis

[NOTE—The procedure can also be performed using alternative platforms. Perform the test with each *Standard solution* and *Sample solution* in duplicate.]

To each of a series of suitable plastic tubes placed in a water bath set at 37°, transfer 120  $\mu$ L of *pH 8.4 buffer*. Then separately transfer 30  $\mu$ L of the different dilutions of the *Standard solutions* or the *Sample solutions* to the tubes. Add 150  $\mu$ L of *Antithrombin solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300  $\mu$ L of *Factor Xa solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for 2 min. Add 300  $\mu$ L of *Chromogenic substrate solution*, prewarmed at 37° for 15 min, to each tube, mix, and incubate for exactly 2 min. Add 150  $\mu$ L of *Stopping solution* to each tube, and mix. Prepare a blank for zeroing the spectrophotometer by adding the reagents in reverse order, starting with the *Stopping solution* and ending with the addition of 150  $\mu$ L of *pH 8.4 buffer*, and excluding the *Standard solutions* or the *Sample solutions*. Record the absorbance at 405 nm against the blank.

**Calculations:** Plot the log of the absorbance values of the *Standard solutions* and the *Sample solutions* versus the heparin concentrations in USP Units. Calculate the activity of Heparin Sodium in USP Units/mg using statistical methods for slope ratio assays. Calculate the anti-factor Xa activity of Heparin Sodium:

$$\text{Result} = A \times (S_T/S_S)$$

A = potency of USP Heparin Sodium for Assays RS

$S_T$  = slope of the line for the *Sample solutions*

$S_S$  = slope of the line for the *Standard solutions*

Express the anti-factor Xa activity of the *Sample solution* as USP Heparin Units/mg, calculated on the dried basis. Calculate the ratio of anti-factor Xa activity against anti-factor IIa potency (see the Assay):

Result = anti-factor Xa activity/anti-factor IIa potency

**Acceptance criteria:** 0.9–1.1

- **D. IDENTIFICATION TESTS—GENERAL, Sodium (191):** It meets the requirements of the flame test for sodium.

#### ASSAY

##### • ANTI-FACTOR IIa POTENCY

**pH 8.4 buffer:** Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

**Antithrombin solution:** Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

**Thrombin human solution:** Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

**Stopping solution:** 20% (v/v) solution of acetic acid

**Standard solutions:** Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

#### Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200  $\mu$ L) of *Antithrombin solution* to each tube containing one volume (50–100  $\mu$ L) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50  $\mu$ L of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100  $\mu$ L of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100  $\mu$ L of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a

suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). Calculate the change in absorbance/min ( $\Delta OD/min$ ). The blanks for kinetic measurement are also expressed as  $\Delta OD/min$  and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

**Calculations:** The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

**Parallel-line assay:** For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

**Slope ratio assay:** For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

**Acceptance criteria:** The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

#### OTHER COMPONENTS

- NITROGEN DETERMINATION, Method I (461):** 1.3%–2.5%, calculated on the dried basis, using the procedure for *Nitrates and Nitrites Absent*

#### IMPURITIES

##### Inorganic Impurities

- RESIDUE ON IGNITION (281):** 28.0%–41.0%
- HEAVY METALS, Method II (231):** NMT 30 ppm

##### Organic Impurities

##### PROCEDURE 1: LIMIT OF GALACTOSAMINE IN TOTAL HEX-

**OSAMINE** (a measure of dermatan sulfate and other galactosamine containing impurities)

**Mobile phase:** 14 mM potassium hydroxide

**Glucosamine standard solution:** 1.6 mg/mL of USP

Glucosamine Hydrochloride RS in 5 N hydrochloric acid

**Galactosamine standard solution:** 16  $\mu$ g/mL of USP Galactosamine Hydrochloride RS in 5 N hydrochloric acid

**Standard solution:** Mix equal volumes of *Glucosamine standard solution* and *Galactosamine standard solution*.

**Hydrolyzed standard solution:** Transfer 5 mL of the *Standard solution* to a 7-mL screw-cap test tube, cap, and heat for 6 h at 100°. Cool to room temperature, quantitatively transfer the solution to a 500-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 12 mg of Heparin Sodium to a 7-mL screw-cap test tube, dissolve in 5 mL of 5 N hydrochloric acid, and cap.

**Hydrolyzed sample solution:** Heat the *Sample solution* for 6 h at 100°. Cool to room temperature, and dilute with water (1 in 100).

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** HPLC

**Detector:** Pulsed amperometric detector, set to the following waveform:

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.1	—
2	0.20	+0.1	Begins
3	0.40	+0.1	Ends

Step	Time (s)	Potential (V)	Integration
4	0.41	−2.0	—
5	0.42	−2.0	—
6	0.43	+0.6	—
7	0.44	−0.1	—
8	0.50	−0.1	—

**Column:** 3-mm  $\times$  30-mm amino acid trap column in series with a 3-  $\times$  30-mm guard column and a 3-mm  $\times$  15-cm column that contains packing L69

**Column temperature:** Maintain columns at 30°.

**Flow rate:** 0.5 mL/min

**Pre-equilibration:** At least 60 min with *Mobile phase*

**Injection size:** 10  $\mu$ L

**Elution:** 10 min with *Mobile phase*

**Column cleaning:** At least 10 min with 100 mM potassium hydroxide

**Equilibration:** At least 10 min with *Mobile phase* before each injection

##### System suitability

**Sample:** *Hydrolyzed standard solution*

##### Suitability requirements

**Resolution:** NLT 2 between the galactosamine and glucosamine peaks

**Column efficiency:** NLT 2000 theoretical plates for glucosamine

**Tailing factor:** Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

##### Analysis

**Samples:** *Hydrolyzed standard solution* and *Hydrolyzed sample solution*

Record the chromatograms, and measure the responses for the peaks at the retention time of galactosamine and glucosamine. Calculate the response ratio of galactosamine to glucosamine ( $GalN_R$ ) in the *Hydrolyzed standard solution*:

$$\text{Result} = (GalN_B / GalN_W) \times (GlcN_W / GlcN_B)$$

$GalN_B$  = galactosamine peak area from the *Hydrolyzed standard solution*

$GalN_W$  = weight of galactosamine for the *Standard solution*

$GlcN_W$  = weight of glucosamine for the *Standard solution*

$GlcN_B$  = glucosamine peak area from the *Hydrolyzed standard solution*

Calculate the percentage of galactosamine in the portion of total hexosamine taken:

$$\text{Result} = \{[(GalN_U / GalN_R)] / [(GalN_U / GalN_R) + GlcN_U]\} \times 100$$

$GalN_U$  = galactosamine peak area from the *Hydrolyzed sample solution*

$GalN_R$  = galactosamine response ratio

$GlcN_U$  = glucosamine peak area from the *Hydrolyzed sample solution*

**Acceptance criteria:** The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

##### PROCEDURE 2: NUCLEOTIDIC IMPURITIES

(See *Biotechnology-Derived Articles—Total Protein Assay* (1057), *Method 1*, with the following modifications.)

**Analysis:** Dissolve 40 mg of Heparin Sodium in 10 mL of water. Measure the absorbance of this solution at 260 nm using the light-scattering correction procedure of *Biotechnology-Derived Articles—Total Protein Assay* (1057), *Method 1*.

**Acceptance criteria:** The absorbance of this solution at 260 nm is NMT 0.20.

• **PROCEDURE 3: ABSENCE OF OVERSULFATED CHONDROITIN SULFATE**

**A:** Proceed as directed in *Identification* test A. No features associated with oversulfated chondroitin sulfate are found between 2.12 and 3.00 ppm.

**B:** Proceed as directed in *Identification* test B. No peaks corresponding to oversulfated chondroitin sulfate should be detected eluting after the heparin peak.

• **PROCEDURE 4: PROTEIN IMPURITIES**

**Standard stock solution:** 0.100 mg/mL of bovine serum albumin in water

**Standard solutions:** Dilute portions of the *Standard stock solution* with water to obtain NLT 5 standard solutions having concentrations between 0.005 and 0.100 mg/mL of bovine serum albumin, the concentrations being evenly spaced.

**Sample solution:** 5 mg/mL of Heparin Sodium in water. Prepare in triplicate.

**Blank:** Water

**Lowry reagent A:** Prepare a solution of 10 g/L of sodium hydroxide in water and a solution of 50 g/L of sodium carbonate in water. Mix equal volumes (2V:2V) of each solution, and dilute with water to 5V.

**Lowry reagent B:** Prepare a solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate in water. Mix equal volumes of both solutions (2V:2V), and dilute with water to 5V.

**Lowry reagent C:** Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*.

**Diluted Folin–Ciocalteu’s phenol reagent:** Dilute Folin–Ciocalteu’s phenol reagent 2–4 times with water. The dilution should be chosen such that the pH of the samples (i.e., *Standard solution* and *Sample solution* after addition of *Lowry reagent C* and the *Diluted Folin–Ciocalteu’s phenol reagent*) is  $10.25 \pm 0.25$ .

**Analysis**

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*

To 1 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 5 mL of *Lowry reagent C*. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin–Ciocalteu’s phenol reagent* to each solution, mix immediately, and allow to stand at room temperature for 30 min. Determine the absorbance as directed in *Biotechnology-Derived Articles—Total Protein Assay* (1057), Method 2.

**Calculations:** See *Biotechnology-Derived Articles—Total Protein Assay* (1057), Method 2.

**Acceptance criteria:** NMT 1.0% (w/w) is found.

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.03 USP Endotoxin Unit/USP Heparin Unit.
- **LOSS ON DRYING (731):** Dry a sample in a vacuum at 60° for 3 h: it loses NMT 5.0% of its weight.
- **pH (791):** 5.0–7.5, in a solution (1 in 100)
- **STERILITY TESTS (71):** Where it is labeled as sterile, it meets the requirements.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 40°, preferably at room temperature.
- **LABELING:** Label it to indicate the tissue and the animal species from which it is derived.

• **USP REFERENCE STANDARDS (11)**

USP Dermatan Sulfate RS  
USP Endotoxin RS  
USP Galactosamine Hydrochloride RS  
USP Glucosamine Hydrochloride RS  
USP Heparin Sodium for Assays RS  
USP Heparin Sodium Identification RS  
USP Oversulfated Chondroitin Sulfate RS

## Heparin Sodium Injection

**DEFINITION**

Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. It exhibits a potency NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units/mL.

**ASSAY**

• **ANTI-FACTOR IIa POTENCY**

**pH 8.4 buffer:** Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

**Antithrombin solution:** Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Anti-thrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

**Thrombin human solution:** Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

**Stopping solution:** 20% (v/v) solution of acetic acid

**Standard solutions:** Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Heparin Sodium similar to those obtained for the *Standard solutions*.

**Analysis**

[NOTE—The procedure can also be performed using alternative platforms.] For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested: for example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the

volume (100–200  $\mu\text{L}$ ) of *Antithrombin solution* to each tube containing one volume (50–100  $\mu\text{L}$ ) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50  $\mu\text{L}$  of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100  $\mu\text{L}$  of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100  $\mu\text{L}$  of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). Calculate the change in absorbance/min ( $\Delta\text{OD}/\text{min}$ ). The blanks for kinetic measurement are also expressed as  $\Delta\text{OD}/\text{min}$  and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

**Calculations:** The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

**Parallel-line assay:** For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for parallel-line assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

**Slope ratio assay:** For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Heparin Sodium in USP Units/mL using statistical methods for slope ratio assays. Express the potency of Heparin Sodium/mg, calculated on the dried basis.

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.03 USP Endotoxin Unit/USP Heparin Unit
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **pH (791):** 5.0–7.5
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections* (1)

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store at a temperature below 40°, preferably at room temperature.
- **LABELING:** Label it to indicate the volume of the total contents and the potency in terms of USP Heparin Units only per mL, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of USP Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS  
USP Heparin Sodium for Assays RS

## Anticoagulant Heparin Solution

### DEFINITION

Anticoagulant Heparin Solution is a sterile solution of Heparin Sodium in Sodium Chloride Injection. Its potency is NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units. It contains NLT 0.85% and NMT 0.95% of sodium chloride (NaCl). It may be buffered. It contains no antimicrobial agents. Prepare Anticoagulant Heparin Solution as follows.

Heparin Sodium	75,000 Units
Sodium Chloride Injection, sufficient quantity to make	1000 mL

Add the Heparin Sodium, in solid form or in solution, to the Sodium Chloride Injection, mix, filter if necessary, and sterilize.

### ASSAY

#### • ANTI-FACTOR IIa POTENCY

**pH 8.4 buffer:** Dissolve 6.10 g of tris(hydroxymethyl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

**Antithrombin solution:** Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Anti-thrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

**Thrombin human solution:** Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

**Stopping solution:** 20% (v/v) solution of acetic acid

**Standard solutions:** Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

**Sample solutions:** Proceed as directed for *Standard solutions* to obtain concentrations of Anticoagulant Heparin Solution similar to those obtained for the *Standard solutions*.

#### Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3,

and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200  $\mu$ L) of *Anti-thrombin solution* to each tube containing one volume (50–100  $\mu$ L) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50  $\mu$ L of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100  $\mu$ L of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100  $\mu$ L of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)). Calculate the change in absorbance/min ( $\Delta$ OD/min). The blanks for kinetic measurement are also expressed as  $\Delta$ OD/min and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

**Calculations:** The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

**Parallel-line assay:** For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for parallel-line assays.

**Slope ratio assay:** For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for slope ratio assays.

**Acceptance criteria:** 90.0%–110.0% of the potency stated on the label in terms of USP Heparin Units.

#### • SODIUM CHLORIDE

**Sample solution:** Solution and potassium chromate TS (5:1)

**Analysis:** Titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

#### SPECIFIC TESTS

- **pH (791):** Between 5.0 and 7.5
- **BACTERIAL ENDOTOXINS (85):** It contains NMT 2.5 USP Endotoxin Units/mL.
- **INJECTIONS (1):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, of colorless, transparent Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* (161)).
- **LABELING:** Label it in terms of USP Heparin Units, and to indicate the number of mL of Solution required per 100 mL of whole blood.

#### • USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Heparin Sodium for Assays RS

### Hepatitis B Immune Globulin

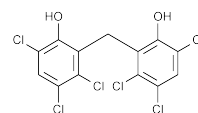
» Hepatitis B Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* (1041)). It is a sterile, nonpyrogenic solution free from turbidity, consisting of globulins derived from the blood plasma of human donors who have high titers of antibodies against hepatitis B surface antigen. It contains not less than 10.0 g and not more than 18.0 g of protein per 100 mL, of which not less than 80 percent is monomeric immunoglobulin G, having no ultracentrifugally detectable fragments, nor aggregates having a sedimentation coefficient greater than 12S. It contains 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative. It has a potency per mL not less than that of the U.S. Reference Hepatitis B Immune Globulin tested by an approved radioimmunoassay for the detection and measurement of antibody to hepatitis B surface antigen. It has a pH between 6.4 and 7.2, measured in a solution diluted to contain 1 percent of protein with 0.15 M sodium chloride. It meets the requirements of the test for heat stability.

**Packaging and storage—**Preserve at a temperature between 2° and 8°.

**Expiration date—**Its minimum expiration date is not later than 1 year after the date of manufacture, such date being that of the first valid potency test of the product.

**Labeling—**Label it to state that it is not for intravenous injection.

### Hexachlorophene



$C_{13}H_6Cl_6O_2$  406.90

Phenol, 2,2'-methylenebis[3,4,6-trichloro-2,2'-Methylenebis[3,4,6-trichlorophenol] [70-30-4].

» Hexachlorophene contains not less than 98.0 percent and not more than 100.5 percent of  $C_{13}H_6Cl_6O_2$ , calculated on the dried basis.

**Packaging and storage—**Preserve in tight, light-resistant containers.

**USP Reference standards (11)—**

USP Hexachlorophene RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** To a solution of about 5 mg in 5 mL of alcohol add 1 drop of ferric chloride TS: a transient purple color is produced immediately.

**Melting range** (741): between 161° and 167°.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Limit of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin**—[*Cautions*—Since 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is an extremely toxic substance, exercise all necessary precautions in the conduct of this procedure.] Dissolve 10.0 g of Hexachlorophene in 50 mL of methanol, transfer to a 1-Liter separator with the aid of 25 mL of methanol, add 25 mL of 2.5 N lithium hydroxide and 225 mL of water, and extract with two 200-mL portions of freshly distilled *n*-hexane. Dry the combined *n*-hexane extracts over anhydrous sodium sulfate, filter, and evaporate to a volume of about 15 mL on a rotary evaporator at a bath temperature not exceeding 40°. Transfer this solution in portions to a 12-mL centrifuge tube, concentrating each time to a volume of 1 mL in a gentle stream of nitrogen in a warm water bath. Rinse the flask with 15 mL of *n*-hexane, and evaporate similarly. Wash down the walls of the tube with 10 mL of *n*-hexane, and again evaporate to a volume of 1.0 mL. Cool, and transfer to a micro-column that has been prepared in the following manner. Place a small plug of glass wool in a 5- × 15-mm pipet, add a small amount of sand and 1.0 g of basic alumina, tap several times to pack down the alumina, and heat in a vacuum oven at 110° for 3 hours. Store under vacuum.

Elute the column with 10 mL of a mixture of *n*-hexane and methylene chloride (9:1), using a portion to rinse the tube. Collect the eluate in a 12-mL graduated centrifuge tube, and concentrate in a gentle stream of nitrogen in a warm water bath to a volume of 1.0 mL.

Inject 2.0 µL of the concentrated eluate into a suitable gas chromatograph connected to a mass spectrograph equipped with a multiple-ion detector (see *Chromatography* (621) and *Mass Spectrometry* (736)). The gas chromatograph is fitted with a 2-mm × 1-m glass column containing liquid phase G1 on support S1. The carrier gas is helium, flowing at the rate of 40 mL per minute. The column temperature is maintained at 250° and the injection port is maintained at 300°. Similarly inject 2.0 µL of a Standard solution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin containing 0.01 µg per mL.\*

The sum of the peak heights at mass values of 320, 322, and 324 obtained from the solution under test is not greater than the sum of the peak heights at the same mass values obtained from the Standard solution. The limit is 1 ppb.

**Assay**—Accurately weigh about 1.5 g of Hexachlorophene, dissolve in 25 mL of alcohol, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 40.69 mg of C<sub>13</sub>H<sub>6</sub>Cl<sub>6</sub>O<sub>2</sub>.

**Packaging and storage**—Preserve in tight, light-resistant, non-metallic containers.

**USP Reference standards** (11)—

USP Hexachlorophene RS

**Identification**—Place a volume of Emulsion, equivalent to about 150 mg of hexachlorophene, in a glass-stoppered, 25-mL graduated cylinder, dilute with a mixture of equal volumes of chloroform and methanol to volume, mix, and allow to stand for about 5 minutes. Apply 10 µL of this solution and 10 µL of a solution of USP Hexachlorophene RS in the same chloroform and methanol mixture containing 6 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of silica gel. Develop the chromatogram in a solvent system consisting of a mixture of toluene and glacial acetic acid (9:1) until the solvent moves to about 10 cm above the point of application. Remove the plate, mark the solvent front and evaporate the solvent in a current of warm air. Spray the plate with dilute nitric acid (1 in 5), and warm on a hot plate until yellow spots appear: the *R<sub>f</sub>* value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**pH** (791)—Place 20 mL of the well-shaken Emulsion and 10 mL of water in a glass-stoppered, 50-mL graduated cylinder, mix, and determine the pH in a suitable pH meter, using a glass electrode and preferably a sleeve-type calomel electrode: the pH is between 5.0 and 6.0.

**Assay—**

**Standard preparation**—Weigh accurately about 50 mg of USP Hexachlorophene RS into a 50-mL volumetric flask, add 10 mL of methanol, shake until dissolved, add methanol to volume, and mix. Pipet 3 mL of this solution into a 100-mL volumetric flask, add 1 mL of dilute hydrochloric acid (1 in 10), add methanol to volume, and mix.

**Assay preparation**—Transfer an accurately weighed portion of Emulsion, equivalent to about 30 mg of hexachlorophene, to a 100-mL volumetric flask, add methanol to volume, and mix. Filter the solution through paper, taking adequate precautions to prevent evaporation. Pipet a 10-mL aliquot of the filtrate into a 100-mL volumetric flask, add 1 mL of dilute hydrochloric acid (1 in 10), and add methanol to volume.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 299 nm, with a suitable spectrophotometer, using a mixture of 99 volumes of methanol and 1 volume of hydrochloric acid as the blank. Calculate the quantity, in mg, of C<sub>13</sub>H<sub>6</sub>Cl<sub>6</sub>O<sub>2</sub> in the portion of Emulsion taken by the formula:

$$C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Hexachlorophene RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Hexachlorophene Cleansing Emulsion

» Hexachlorophene Cleansing Emulsion is Hexachlorophene in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>13</sub>H<sub>6</sub>Cl<sub>6</sub>O<sub>2</sub>. It contains no coloring agents.

\* A solution in anisole is available commercially from KOR Isotopes, Div. of ECO, Inc., 56 Rogers St., Cambridge, Mass. 02142. This solution may be diluted with a mixture of *n*-hexane and methylene chloride (9:1) to the required concentration.

## Hexachlorophene Liquid Soap

» Hexachlorophene Liquid Soap is a solution of Hexachlorophene in a 10.0 to 13.0 percent solution of a potassium soap. It contains, in each 100 g, not less than 225 mg and not more than



260 mg of  $C_{13}H_6Cl_6O_2$ . It may contain suitable water hardness controls.

**NOTE**—The inclusion of nonionic detergents in Hexachlorophene Liquid Soap in amounts greater than 8 percent on a total weight basis may decrease the bacteriostatic activity of the Soap.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Solutions of higher concentrations of hexachlorophene and potassium soap, in which the ratios of these components are consistent with the official limits, may be labeled "For the preparation of Hexachlorophene Liquid Soap, USP," provided that the label indicates also that the soap is a concentrate, and provided that directions are given for dilution to the official strength.

**USP Reference standards** (11)—

USP Hexachlorophene RS

**Identification**—

**A:** Pour about 2 g into a beaker, and add, with stirring, dilute hydrochloric acid (1 in 100) until the mixture is just acid to litmus. To 10 mL of the mixture, in a beaker, add 10 mL of chloroform, and mix. Add 3 or 4 drops of ferric chloride TS, mix, and allow to stand: the chloroform layer becomes purple.

**B:** To 2 mL of the mixture prepared in *Identification* test A, in a test tube, add 2 mL of acetone, and mix. Add 1 mL of titanium trichloride solution (1 in 5), and shake vigorously: a yellow oil separates.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Water**—Place about 5 g, quickly weighed to the nearest centigram, in the distilling flask of the apparatus for *Water Determination—Azeotropic Method* (921). (The Soap is most conveniently weighed in a boat of metal foil, of a size that will just pass through the neck of the flask.) Place 250 mL of toluene and 10 g of anhydrous barium chloride in the flask, connect the flask through a ground-glass joint to the distilling apparatus, fill the receiving tube with toluene, and determine the water as directed, beginning with "Heat the flask gently." The volume of water found corresponds to between 86.5% and 90.0% by weight of the portion of Soap taken.

**Alcohol-insoluble substances**—Dissolve about 5 g, rapidly and accurately weighed, in 100 mL of hot neutralized alcohol, collect the residue, if any, on a tared filter, thoroughly wash it with hot neutralized alcohol, and dry at 105° for 1 hour: the weight of the residue obtained does not exceed 3.0% of the weight of Soap taken. Retain the solution and the residue.

**Free alkali hydroxides**—To the combined filtrate and washings obtained in the test for *Alcohol-insoluble substances*, add 0.5 mL of phenolphthalein TS. If a pink color is produced, titrate the solution with 0.1 N sulfuric acid VS until the pink color is just discharged. Each mL of 0.1 N sulfuric acid is equivalent to 5.61 mg of KOH. The volume of 0.1 N sulfuric acid consumed corresponds to not more than 0.05% of KOH.

**Alkali carbonates**—Wash the filter containing the *Alcohol-insoluble substances* with 50 mL of boiling water, cool, add methyl orange TS, and titrate the filtrate with 0.1 N sulfuric acid VS. Not more than 0.5 mL of 0.1 N sulfuric acid per g of Soap originally taken is required (0.35% calculated as  $K_2CO_3$ ).

**Assay for hexachlorophene**—

*Alkaline buffer*—Dissolve 6.07 g of tris(hydroxymethyl)aminomethane in 900 mL of methanol. Add 25.0 mL of dilute hydrochloric acid (1 in 10), dilute with water to 1 liter, and mix.

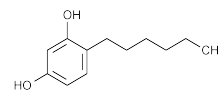
*Standard preparation*—Place about 50 mg of USP Hexachlorophene RS, accurately weighed, in a 100-mL volumetric flask, dissolve in 10 mL of alcohol, and dilute with *Alkaline buffer* to volume. Preserve in a tight container.

*Standard hexachlorophene graph*—To 50-mL volumetric flasks add, by pipet and in duplicate, 2-, 3-, 4-, 5-, 6-, and 7-mL portions of the *Standard preparation*. To one flask of each pair of duplicates, add to volume acidified 90 percent methanol containing, in each 100 mL, 5 mL of acetic acid and 0.3 mL of hydrochloric acid, and mix. To the second flask of each pair, add *Alkaline buffer* to volume.

Arrange the two series of standard hexachlorophene solutions in pairs according to their hexachlorophene content, and determine the absorbances of the alkaline solutions at 312 nm, with a suitable spectrophotometer, using the corresponding acid solution as the blank. Plot the observed absorbance on the ordinate scale against the corresponding concentration of hexachlorophene, in mg per 100 mL, on the abscissa scale.

*Procedure*—Accurately weigh a portion of Soap, containing the equivalent of about 100 mg of hexachlorophene, and transfer to a 100-mL volumetric flask. Add alcohol to volume, and mix. Transfer 25.0 mL of this solution to a 100-mL volumetric flask, add 90 percent methanol to volume, mix, and filter if necessary. Add 10.0 mL of this solution to each of two 50-mL volumetric flasks, and fill one flask to volume with 0.3 M acetic acid in 90 percent methanol containing 0.1% of hydrochloric acid, and mix. Fill the other flask to volume with *Alkaline buffer*, and mix. Determine the absorbance of the alkaline solution at 312 nm with the same spectrophotometer used in preparing the *Standard hexachlorophene graph*, using the control as the blank. From the observed absorbance, calculate the weight of hexachlorophene in the Soap taken.

## Hexylresorcinol



$C_{12}H_{18}O_2$  194.27  
1,3-Benzenediol, 4-hexyl-  
4-Hexylresorcinol [136-77-6].

» Hexylresorcinol, dried over silica gel for 4 hours, contains not less than 98.0 percent and not more than 100.5 percent of  $C_{12}H_{18}O_2$ .

**Caution**—Hexylresorcinol is irritating to the oral mucosa and respiratory tract and to the skin, and its solution in alcohol has vesicant properties.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—

**A:** To 1 mL of a saturated solution of it add 1 mL of nitric acid: a light red color appears.

**B:** To 1 mL of a saturated solution of it add 1 mL of bromine TS: a yellow, flocculent precipitate is formed. Add 2 mL of 6 N ammonium hydroxide: the precipitate dissolves, producing a yellow solution.

**Melting range**, *Class I* (741): between 62° and 67°.

**Acidity**—Dissolve 250 mg in 500 mL of water, add methyl red TS, and titrate with 0.020 N sodium hydroxide: no more than 1.0 mL is required for neutralization.

**Residue on ignition** (281): not more than 0.1%.

**Mercury**—[NOTE—Select all reagents for this test to have as low a content of mercury as practicable, and store all rea-

gent solutions in containers of borosilicate glass. Glassware used in this test shall be specially cleaned by being soaked in warm 8 N nitric acid for 30 minutes and rinsed with water. Keep flasks for this determination separate from other flasks, and use only for mercury determinations.]

**Standard preparation**—Transfer 34.0 mg of mercuric chloride to a 250-mL volumetric flask. Add 1 drop of hydrochloric acid, add water to dissolve, and dilute with water to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume.

**Test preparation**—Transfer 134 mg to a 250-mL beaker, and cautiously add 10 mL of 11 N nitric acid and 10 mL of 18 N sulfuric acid. Digest, with the aid of heat, in a well-ventilated hood until the evolution of brown fumes ceases. Cautiously add an additional 10 mL of 11 N nitric acid, and continue heating until no more fumes are evolved. Cool, transfer to a 200-mL volumetric flask, and dilute with water to volume.

**Procedure**—Transfer 100 mL of *Standard preparation* to a 300-mL mercury analysis reaction vessel, add 2 drops of potassium permanganate solution (1 in 20), and mix (the solution should be purple; add additional permanganate solution dropwise, if necessary). Add 5 mL of 11 N nitric acid, stir, and allow to stand for not less than 15 seconds. Add 5 mL of 18 N sulfuric acid, stir, and allow to stand for not less than 45 seconds. Add 5 mL of hydroxylamine hydrochloride solution (3 in 200), stir, and allow to stand until the solution turns light yellow or colorless. Add 5 mL of stannous chloride solution (1 in 10) [NOTE—Disregard the presence of insoluble matter in this solution; mix prior to use], immediately insert the aerator connected to the air pump, and determine the maximum absorbance of the treated *Standard preparation* at the mercury resonance line of 253.65 nm, with a suitable atomic absorption spectrophotometer equipped with a mercury hollow-cathode lamp and an absorption cell that permits the flameless detection of mercury. Connect in a closed system with a circulating air pump, a calcium chloride drying tube, and an aerator inserted in a 300-mL reaction vessel so that air passed through the treated preparation contained in the reaction vessel evaporates any metallic mercury present. In a similar manner, treat 100 mL of the *Test preparation* and 100 mL of water (reagent blank), and determine the maximum absorbances at the same wavelength [NOTE—Check the zero setting of the instrument frequently]. The absorbance of the solution from the *Test preparation* does not exceed that of the solution from the *Standard preparation* (3 ppm).

**Resorcinol and other phenols**—Shake about 1 g with 50 mL of water for a few minutes, filter, and to the filtrate add 3 drops of ferric chloride TS: no red or blue color is produced.

**Assay**—Dissolve 70 mg to 100 mg of Hexylresorcinol, previously dried over silica gel for 4 hours and accurately weighed, in 10 mL of methanol in a 250-mL iodine flask. Add 30.0 mL of 0.1 N bromine VS, then add quickly 5 mL of hydrochloric acid, and insert the stopper in the flask immediately. Cool the flask under running water to room temperature, shake vigorously for 5 minutes, then set aside for 5 minutes. Add 6 mL of potassium iodide TS around the stopper, cautiously loosen the stopper, again insert the stopper tightly, and swirl gently. Add 1 mL of chloroform, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *Residual Titrations under Titrimetry* (541)). Each mL of 0.1 N bromine is equivalent to 4.857 mg of  $C_{12}H_{18}O_2$ .

## Hexylresorcinol Lozenges

» Hexylresorcinol Lozenges contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{12}H_{18}O_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Hexylresorcinol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Dissolve 3.4 g of monobasic potassium phosphate in about 850 mL of water, adjust with phosphoric acid to a pH of  $3.0 \pm 0.05$ , dilute with water to 1000 mL, mix, and pass through a suitable filter having a 0.5- $\mu$ m or finer porosity. Prepare a mixture of methanol and this solution (650:350), and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Internal standard solution**—Prepare a solution in *Mobile phase* containing about 0.25 mg of hexanophenone per mL.

**Standard preparation**—Transfer about 40 mg of USP Hexylresorcinol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution and 10.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.08 mg of USP Hexylresorcinol RS per mL.

**Assay preparation**—Weigh and pulverize not fewer than 20 Lozenges. Transfer an accurately weighed portion of the powder, equivalent to about 4 mg of hexylresorcinol, to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution* and 20 mL of *Mobile phase*, and shake until dissolved. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a suitable filter of 0.5  $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7 and is maintained at  $37 \pm 2^\circ$ . The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.6 for hexylresorcinol and 1.0 for hexanophenone, the tailing factor is not less than 0.9 and not more than 1.4, the column efficiency is not less than 1500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%. Inject the *Assay preparation*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between the hexylresorcinol peak and the nearest adjacent peak is not less than 1.2.

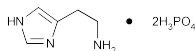
**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{12}H_{18}O_2$  in the portion of Lozenges taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Hexylresorcinol RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the responses of the hexylresorcinol peak

and the hexanophenone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Histamine Phosphate



$C_5H_9N_3 \cdot 2H_3PO_4$  307.14

1*H*-Imidazole-4-ethanamine, phosphate (1:2).

Histamine phosphate (1:2) [51-74-1].

» Histamine Phosphate contains not less than 98.0 percent and not more than 101.0 percent of  $C_5H_9N_3 \cdot 2H_3PO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### Identification—

**A:** Dissolve 0.10 g in a mixture of 7 mL of water and 3 mL of 1 N sodium hydroxide, and add the solution to a mixture of 50 mg of sulfanilic acid, 10 mL of water, 2 drops of hydrochloric acid, and 2 drops of sodium nitrite solution (1 in 10): a deep red color is produced.

**B:** Dissolve 50 mg in 5 mL of hot water, add a hot solution of 50 mg of picrolonic acid in 10 mL of alcohol, and allow to crystallize. Filter the crystals with suction, wash with a small amount of ice-cold water, and dry at 105° for 1 hour: the crystals so obtained melt between 250° and 254°, with decomposition.

**C:** A solution (1 in 10) responds to the tests for *Phosphate* (191).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 3.0% of its weight.

**Assay**—Dissolve about 150 mg, accurately weighed, of Histamine Phosphate in 10 mL of water. Add 5 mL of chloroform and 25 mL of alcohol, then add 10 drops of thymolphthalein TS, and titrate with 0.2 N sodium hydroxide VS. Each mL of 0.2 N sodium hydroxide is equivalent to 15.36 mg of  $C_5H_9N_3 \cdot 2H_3PO_4$ .

## Histamine Phosphate Injection

» Histamine Phosphate Injection is a sterile solution of Histamine Phosphate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_5H_9N_3 \cdot 2H_3PO_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Endotoxin RS

USP Histamine Dihydrochloride RS

### Identification—

**A:** Evaporate a volume of Injection, equivalent to about 2 mg of histamine phosphate, on a steam bath to dryness, dissolve the residue in 0.5 mL of water, and add 0.5 mL of 1 N sodium hydroxide. Add 2 drops of sodium nitrite solution (1 in 10), and add 1 mL of a solution prepared by mixing 50 mg of sulfanilic acid with 10 mL of water containing 2 drops of hydrochloric acid: an orange-red color is produced.

**B:** To 1 mL of Injection, equivalent to not less than 1 mg of histamine phosphate (concentrate a larger volume by evaporation, if necessary), add ammonium molybdate TS dropwise: a yellow precipitate, which is soluble in ammonia TS, is formed.

**Bacterial endotoxins** (85)—It contains not more than 125.0 USP Endotoxin Units per mg of histamine phosphate.

**pH** (791): between 3.0 and 6.0.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Histamine Dihydrochloride RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of 20 µg per mL, equivalent to 33.4 µg of histamine phosphate.

**Assay preparation**—Dilute an accurately measured volume of Injection, equivalent to about 1.65 mg of histamine phosphate, with water in a 50-mL volumetric flask to volume, and mix.

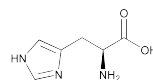
If *phenol* is present, prepare the *Assay preparation* as follows. Dilute an accurately measured volume of Injection, equivalent to about 1.65 mg of histamine phosphate, with water to about 25 mL. Heat the solution on a steam bath until the odor of phenol is no longer perceptible, adding water as required to maintain a volume of about 15 mL. Transfer to a 50-mL volumetric flask, cool, dilute with water to volume, and mix.

**Procedure**—Pipet 5 mL each of the *Standard preparation* and the *Assay preparation* into separate, 10-mL volumetric flasks, to each add 1 mL of sodium borate solution (1 in 100), followed by 1 mL of a freshly prepared solution of 50 mg of β-naphthoquinone-4-sodium sulfonate in 10 mL of water. Place the flasks in boiling water for 10 minutes, then immerse them for 5 minutes in water maintained between 5° and 10°. To each flask, add 1 mL of acid-formaldehyde (made by adding 0.5 mL of formaldehyde TS to a mixture of 45 mL of 1 N hydrochloric acid and 10 mL of glacial acetic acid and diluting with water to 80 mL), mix, add 1 mL of 0.1 N sodium thiosulfate, then dilute with water to volume, and mix. Concomitantly and immediately determine the absorbances of both solutions at the wavelength of maximum absorbance at about 460 nm, with a suitable spectrophotometer, against a reagent blank. Calculate the quantity, in mg, of  $C_5H_9N_3 \cdot 2H_3PO_4$  in each mL of the Injection taken by the formula:

$$C(0.0835 / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Histamine Dihydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Histidine



$C_6H_9N_3O_2$   
L-Histidine [71-00-1].

155.15

### DEFINITION

Histidine contains NLT 98.5% and NMT 101.5% of L-histidine ( $C_6H_9N_3O_2$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K): The *Sample* and USP L-Histidine RS are previously recrystallized from 80% alcohol.

**ASSAY**• **PROCEDURE**

**Sample:** 150 mg of Histidine

**Blank:** Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate very slowly with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of histidine (C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>) in the *Sample* taken:

$$\text{Result} = \{(V_s - V_b) \times N \times F\} / W \times 100$$

$V_s$  = *Titrant* volume consumed by the *Sample* (mL)

$V_b$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 155.2 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.4%
- **CHLORIDE AND SULFATE**, *Chloride* (221)  
**Standard solution:** 0.50 mL of 0.020 N hydrochloric acid  
**Sample:** 0.73 g of Histidine  
**Acceptance criteria:** NMT 0.05%
- **CHLORIDE AND SULFATE**, *Sulfate* (221)  
**Standard solution:** 0.10 mL of 0.020 N sulfuric acid  
**Sample:** 0.33 g of Histidine  
**Acceptance criteria:** NMT 0.03%
- **IRON** (241): NMT 30 ppm
- **HEAVY METALS**, *Method I* (231): NMT 15 ppm
- **RELATED COMPOUNDS**

**System suitability solution:** 0.4 mg/mL each of USP L-Histidine RS and USP L-Proline RS

**Standard solution:** 0.05 mg/mL of USP L-Histidine RS in water. [NOTE—This solution has a concentration equivalent to 0.5% of the *Sample solution*.]

**Sample solution:** 10 mg/mL of Histidine in water

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

**System suitability**

**Suitability requirements:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

**Analysis**

**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*  
After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

**Acceptance criteria:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

**Individual impurities:** NMT 0.5%

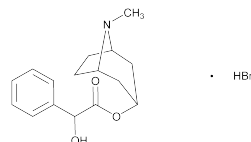
**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S)  
**Sample solution:** 110 mg/mL in 6 N hydrochloric acid  
**Acceptance criteria:** +12.6° to +14.0°
- **pH** (791)  
**Sample solution:** 20 mg/mL solution  
**Acceptance criteria:** 7.0–8.5
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.2% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP L-Histidine RS  
USP L-Proline RS

**Homatropine Hydrobromide**

C<sub>16</sub>H<sub>21</sub>NO<sub>3</sub> · HBr 356.25

Benzeneacetic acid,  $\alpha$ -hydroxy-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, hydrobromide, *endo*-(±)-. 1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol mandelate (ester) hydrobromide [51-56-9].

» Homatropine Hydrobromide contains not less than 98.0 percent and not more than 102.0 percent of C<sub>16</sub>H<sub>21</sub>NO<sub>3</sub> · HBr, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Homatropine Hydrobromide RS  
USP Scopolamine Hydrobromide RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** It responds to the tests for *Bromide* (191).

**pH** (791): between 5.7 and 7.0, in a solution (1 in 50).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281): not more than 0.25%.

**Limit of tropine**—

**Adsorbent:** 0.2-mm layer of chromatographic silica gel mixture.

**Diluent**—Prepare a mixture of methanol and water (9:1).

**Test solution**—Transfer about 0.2 g of Homatropine Hydrobromide to a 5-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Standard solution**—Dilute 0.5 mL of the *Test solution* with *Diluent* to 100.0 mL.

**Tropine reference solution**—Prepare a solution of tropine having a concentration of about 0.4 mg per mL.

**Application volume:** 1  $\mu$ L.

**Developing solvent system:** a mixture of ethyl acetate, anhydrous formic acid, and water (67:16.5:16.5).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621), applying the *Test solu-*

tion, the *Standard solution*, and the *Tropine reference solution*. Spray the plate with Dragendorff's reagent, followed by hydrogen peroxide TS, and immediately cover with a glass plate of the same size. Examine the plate no later than 5 to 10 minutes after spraying. In the chromatogram obtained from the *Test solution*, identify the spot corresponding to the principal spot in the chromatogram of the *Tropine reference solution*: this spot is not more intense than the spot obtained from the *Standard solution*: not more than 0.5% of tropine is found.

#### Chromatographic purity—

*Buffer solution*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the Assay.

*Test solution*—Use the *Assay preparation*, prepared as directed in the Assay.

*Procedure*—Separately inject a volume (about 7  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Continue the elution for 2.2 times the retention time of the homatropine peak. Disregard the peak for the bromide ion, which appears close to the solvent peak. Calculate the percentage of each impurity in the portion of Homatropine Hydrobromide taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  and  $r_s$  are the peak response for each impurity and the sum of all peak responses, respectively, obtained from the *Test solution*. In addition to not exceeding the limits for each impurity in Table 1, not more than 0.1% of any other individual impurity is found; and not more than 1.0% of total impurities is found.

Table 1

Impurity	Relative Retention Time	Limit (%)
Mandelic acid	0.3	0.1
Dehydrohomatropine	0.9	0.5
Scopolamine	1.1	0.1
Atropine	1.9	0.1

#### Assay—

*Buffer solution*—Dissolve 6.8 g of monobasic potassium phosphate and 7.0 g of sodium 1-heptanesulfonate monohydrate in 1000 mL of water, adjust with 3 M phosphoric acid to a pH of 2.7, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (67:33).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Homatropine Hydrobromide RS in *Mobile phase* to obtain a solution having a concentration of about 2 mg per mL.

*System suitability solution*—Prepare a solution of USP Scopolamine Hydrobromide RS having a concentration of about 0.1 mg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 0.5 mL of the *Standard preparation*, and dilute with *Mobile phase* to volume.

*Assay preparation*—Transfer about 100 mg of Homatropine Hydrobromide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  10-cm column that contains 3- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Stan-*

*dard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution between homatropine and scopolamine peaks is not less than 1.5.

*Procedure*—Separately inject equal volumes (about 7  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{16}H_{21}NO_3 \cdot HBr$  in the portion of Homatropine Hydrobromide taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Homatropine Hydrobromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Homatropine Hydrobromide Ophthalmic Solution

» Homatropine Hydrobromide Ophthalmic Solution is a sterile, buffered, aqueous solution of Homatropine Hydrobromide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{16}H_{21}NO_3 \cdot HBr$ . It may contain suitable antimicrobial agents.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—  
USP Homatropine Hydrobromide RS

#### Identification—

**A:** Proceed with Ophthalmic Solution as directed under *Identification*—*Organic Nitrogenous Bases* <181>. The specified results are obtained.

**B:** It responds to the tests for *Bromide* <191>.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 2.5 and 5.0.

#### Assay—

*Standard preparation*—Accurately weigh about 50 mg of USP Homatropine Hydrobromide RS, dissolve in water, and dilute with water in a volumetric flask to 100 mL. Dilute 10.0 mL of this solution with water to 50.0 mL to obtain a solution having a known concentration of about 100  $\mu$ g per mL. Prepare this solution fresh.

*Assay preparation*—Transfer a portion of Ophthalmic Solution, equivalent to 50 mg of homatropine hydrobromide, to a 100-mL volumetric flask, and dilute with water to volume. Dilute 10.0 mL of this solution with water to 50.0 mL.

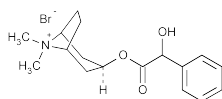
*Procedure*—Transfer duplicate 2-mL portions of the *Standard preparation* and of the *Assay preparation* to separate glass-stoppered, 40-mL centrifuge tubes. To one set of two tubes add 3 mL of water and 1 mL of sodium hydroxide solution (1 in 100). Heat these tubes in a boiling water bath for 20 minutes, and allow to cool to room temperature. To the remaining set of tubes, which serve as blanks for the *Standard preparation* and the *Assay preparation*, respectively, add 4 mL of water. To each tube, add 2 mL of approximately 0.2 M ceric sulfate in diluted sulfuric acid (prepared by dissolving 12.6 g of ceric ammonium sulfate in 50 mL of water and 3 mL of sulfuric acid, and diluting with water to 100 mL) and 20.0 mL of isooctane. Shake by mechanical means for 15 minutes, allow the layers to separate, and remove the isooctane from each tube. Concomitantly deter-

mine the absorbances of the isooctane solutions from the hydrolyzed aliquots in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, against the respective blanks. Calculate the quantity, in mg, of  $C_{16}H_{21}NO_3 \cdot HBr$  in the portion of Ophthalmic Solution taken by the formula:

$$0.5C(A_U / A_S)$$

in which C is the concentration, in  $\mu g$  per mL, of USP Homatropine Hydrobromide RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Homatropine Methylbromide



$C_{17}H_{24}BrNO_3$  370.28  
8-Azoniabicyclo[3.2.1]octane, 3-(hydroxyphenylacetyl)oxy-8,8-dimethyl-, bromide, *endo*-(±)-;  
3 $\alpha$ -Hydroxy-8-methyl-1 $\alpha$ H,5 $\alpha$ H-tropanium bromide mandelate;  
(1*R*,3*S*,5*S*)-3-[[*(2R)*-2-Hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide [80-49-9].

### DEFINITION

Homatropine Methylbromide contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{24}BrNO_3$ , calculated on the dried basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION <197K>

[NOTE—If differences are observed, dissolve the specimen and the Reference Standard separately in methanol, and recrystallize by adding dioxane to each solution.]

#### B. IDENTIFICATION TESTS—GENERAL, Bromide <191>

**Sample solution:** 50 mg/mL in water

**Acceptance criteria:** Meets the requirements

### ASSAY

#### Procedure

**Solution A:** 3.4 g/L of monobasic potassium phosphate and 5 g/L of 1-pentanesulfonic acid sodium salt in water. Adjust with a 330-g/L solution of phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile and *Solution A* (3:2)

**Diluent:** Acetonitrile and *Solution A* (9:41)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
15	30	70
15.1	70	30
20	70	30

**System suitability solution:** 0.01 mg/mL each of USP Homatropine Methylbromide RS and USP Homatropine Hydrobromide RS in *Diluent*

**Standard solution:** 2.0 mg/mL of USP Homatropine Methylbromide RS in *Diluent*

**Sample solution:** 2.0 mg/mL of Homatropine Methylbromide in *Diluent*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu m$  packing L1

**Flow rate:** 1.4 mL/min

**Injection size:** 5  $\mu L$

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for homatropine methylbromide and homatropine hydrobromide are 1.0 and 1.14, respectively.]

### Suitability requirements

**Resolution:** NLT 2.5 between homatropine methylbromide and homatropine hydrobromide, *System suitability solution*

**Tailing factor:** NMT 1.5 for homatropine methylbromide peak, *System suitability solution*

**Relative standard deviation:** NMT 1%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{24}BrNO_3$  in the portion of Homatropine Methylbromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of Homatropine Methylbromide from the *Sample solution*

$r_S$  = peak response of homatropine methylbromide from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

#### Organic Impurities

#### PROCEDURE

**Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and Sample solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.01 mg/mL of USP Homatropine Methylbromide RS in *Diluent*

**Chromatographic system:** Proceed as directed in the *Assay*, except for injection size.

**Injection size:** 10  $\mu L$

### System suitability

**Sample:** *System suitability solution*

### Suitability requirements

**Resolution:** NLT 2.5 between homatropine methylbromide and homatropine hydrobromide

**Tailing factor:** NMT 1.5 for the homatropine methylbromide peak

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Homatropine Methylbromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of homatropine methylbromide from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)  
[NOTE—Reporting level for impurities is 0.05%.]

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.0%

[NOTE—Disregard the peak due to the bromide ion that elutes close to the solvent peak at about 1 min.]

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methyldehydrohomatropine bromide <sup>a</sup>	0.94	0.5
Homatropine methylbromide	1.0	—
Homatropine hydrobromide	1.1	0.5
Any other individual impurity	—	0.1

<sup>a</sup> (1*R*,3*S*,5*S*)-3-[[[(2*R*)-2-Hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]oct-6-ene.

#### SPECIFIC TESTS

##### • pH (791)

**Sample solution:** 50 mg/mL in carbon dioxide-free water

**Acceptance criteria:** 4.5–6.5

##### • Loss on Drying (731):

Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Homatropine Methylbromide RS

USP Homatropine Hydrobromide RS

## Homatropine Methylbromide Tablets

» Homatropine Methylbromide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{24}BrNO_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Homatropine Methylbromide RS

**Identification**—Shake a quantity of finely powdered Tablets, equivalent to about 10 mg of homatropine methylbromide, with 15 mL of a mixture of equal volumes of methanol and water for 10 minutes, and filter. Evaporate the filtrate on a steam bath to dryness, and dry at 105° for 1 hour. The residue of homatropine methylbromide so obtained melts between 190° and 198° (see *Class I* under *Melting Range or Temperature* (741)), the temperature at which distinct liquefaction of the specimen is first observed being taken as the beginning of melting.

#### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{24}BrNO_3$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solu-

tion under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Homatropine Methylbromide RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{24}BrNO_3$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—

**Standard preparation**—Transfer about 25 mg of USP Homatropine Methylbromide RS, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix. The concentration of USP Homatropine Methylbromide RS in the *Standard preparation* is about 100 µg per mL.

**Test preparation**—Transfer 1 finely powdered Tablet to a volumetric flask, suitably sized such that when the specimen is diluted to volume, the concentration is equivalent to about 100 µg of homatropine methylbromide per mL. Add water to about one-half of the volume of the flask, shake for 10 minutes, dilute with water to volume, mix, and filter, discarding the first 10 mL of filtrate. Use the subsequent filtrate as directed in the *Procedure*.

**Procedure**—Transfer 2.0 mL each of the *Standard preparation* and the *Test preparation* to separate glass-stoppered, 50-mL flasks. To each flask, add 0.1 mL of sodium hydroxide solution (1 in 10), and heat in a water bath at 80° for 15 minutes. Cool to room temperature, add 2.0 mL of 0.2 M ceric ammonium sulfate in 1 N sulfuric acid, and mix. To each flask, add 20.0 mL of *n*-hexane, and shake for 15 minutes. Decant the hexane layers into separate 1-cm cells, and concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of  $C_{17}H_{24}BrNO_3$  in the Tablet by the formula:

$$(TC/D)(A_U/A_S)$$

in which *T* is the labeled quantity, in mg, of homatropine methylbromide in the Tablet; *C* is the concentration, in µg per mL, of USP Homatropine Methylbromide RS in the *Standard preparation*; *D* is the concentration, in µg per mL, of the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

#### Assay—

**Standard preparation**—Transfer about 25 mg of USP Homatropine Methylbromide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in water, dilute with water to volume, and mix.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 12.5 mg of homatropine methylbromide, and shake with 10 mL of water at frequent intervals during 30 minutes. Filter under reduced pressure through a sintered-glass crucible into a test tube placed in the suction flask under the filtering funnel, and wash under suction with several small portions of water. Transfer the contents of the test tube to a 25-mL volumetric flask, dilute with water to volume, and mix.

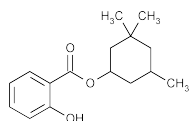
**Procedure**—Transfer 10.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes, to each add 1 mL of 5 N sulfuric acid and 2 mL of ammonium reineckate TS, shake gently but well, and allow to stand for 1 hour. Filter through a sintered-glass crucible with suction, using portions of the filtrate to transfer the precipitate completely to the filter, and wash it with three 2-mL portions of ice-cold water. Completely dissolve the precipitate by pouring over it 1-mL portions of acetone with the application of suction, receiving the solution in a 10-mL volumetric flask,

add acetone to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 525 nm, with a suitable spectrophotometer, using acetone as the blank. Calculate the quantity, in mg, of  $C_{17}H_{24}BrNO_3$  in the portion of Tablets taken by the formula:

$$0.025C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Homatropine Methylbromide RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Homosalate



$C_{16}H_{22}O_3$  262.34  
Benzoic acid, 2-hydroxy, 3,3,5-trimethylcyclohexyl ester;  
3,3,5-Trimethylcyclohexyl salicylate [118-56-9].

### DEFINITION

Homosalate contains NLT 90.0% and NMT 110% of  $C_{16}H_{22}O_3$ , calculated on the as-is basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197F>

### ASSAY

#### • PROCEDURE

**Standard solution:** 5.0 mg/mL of USP Homosalate RS in chloroform

**Sample solution:** 5.0 mg/mL of Homosalate in chloroform

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm  $\times$  30-m column coated with a 1- $\mu\text{m}$  film of G27

**Temperature**

**Injector:** 270°

**Detector:** 270°

**Column:** See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	6	220	5

**Carrier gas:** Hydrogen

**Flow rate:** 6 mL/min

**Injection size:** 1  $\mu\text{L}$

**Split ratio:** 1:10

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the *cis*-isomer and the *trans*-isomer are about 0.98 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between the *cis*-isomer and the *trans*-isomer

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of homosalate ( $C_{16}H_{22}O_3$ ) in the portion of Homosalate taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = sum of the peak responses for the *cis*- and *trans*-isomers from the *Sample solution*

$r_S$  = sum of the peak responses for the *cis*- and *trans*-isomers from the *Standard solution*

$C_S$  = concentration of USP Homosalate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Homosalate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110% on the as-is basis

### SPECIFIC TESTS

- **SPECIFIC GRAVITY** <841>: 1.049–1.053
- **REFRACTIVE INDEX** <831>: 1.516–1.519 at 20°

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** <11>  
USP Homosalate RS

## Hyaluronidase Injection

» Hyaluronidase Injection is a sterile solution of dry, soluble enzyme product, prepared from mammalian testes and capable of hydrolyzing mucopolysaccharides of the type of hyaluronic acid, in Water for Injection. It contains not less than 90.0 percent of the labeled amount of USP Hyaluronidase Units. Hyaluronidase Injection contains not more than 0.25  $\mu\text{g}$  of tyrosine for each USP Hyaluronidase Unit. It may contain suitable stabilizers.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store in a refrigerator.

#### USP Reference standards <11>—

USP Endotoxin RS

USP Hyaluronidase RS

USP L-Tyrosine RS

**Bacterial endotoxins** <85>—It contains not more than 2.30 USP Endotoxin Units per USP Hyaluronidase Unit.

**pH** <791>: between 6.4 and 7.4.

**Limit of tyrosine**—Transfer an accurately measured volume, equivalent to about 120 USP Hyaluronidase Units, to a 15-mL centrifuge tube calibrated at 6 mL, and evaporate at 105° to dryness. Proceed as directed in *Limit of tyrosine under Hyaluronidase for Injection*, beginning with “Add 200  $\mu\text{L}$  of 6 N sodium hydroxide,” except to read Hyaluronidase Injection for Hyaluronidase for Injection in the second paragraph. Calculate the quantity, in  $\mu\text{g}$ , of tyrosine in the volume of Injection taken by the formula:

$$45(A_U / A_S)$$

in which  $A_U$  and  $A_S$  are the absorbances of the solution from the Injection and the Standard solution, respectively: not more than 0.25  $\mu\text{g}$  of tyrosine is found for each USP Hyaluronidase Unit.

**Other requirements**—It meets the requirements under *Injections* <1>.



**Assay—**

*Acetate buffer solution, Phosphate buffer solution, Hydrolyzed gelatin, Diluent for hyaluronidase solutions, Serum stock solution, Serum solution, Potassium hyaluronate stock solution, Hyaluronate solution, and Standard solution*—Prepare as directed in the Assay under *Hyaluronidase for Injection*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with cold *Diluent for hyaluronidase solutions*, on the basis of trial or experience, so that the observed absorbances with the three dilutions of the Injection fall on the upper, linear part of the standard curve prepared as directed in the *Procedure*.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Hyaluronidase for Injection*. The potency, in USP Hyaluronidase Units, of the portion of Injection taken is the average of the six activity values read from the standard curve.

## Hyaluronidase for Injection

» Hyaluronidase for Injection is a sterile, dry, soluble enzyme product prepared from mammalian testes and capable of hydrolyzing mucopolysaccharides of the type of hyaluronic acid. Its potency, in USP Hyaluronidase Units, is not less than the labeled potency. Hyaluronidase for Injection contains not more than 0.25 µg of tyrosine for each USP Hyaluronidase Unit. It may contain a suitable stabilizer.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), preferably of Type I or Type III glass, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Hyaluronidase RS

USP L-Tyrosine RS

**Sterility** (71): meets the requirements.

**Bacterial endotoxins** (85)—It contains not more than 2.30 USP Endotoxin Units per USP Hyaluronidase Unit.

**Limit of tyrosine**—Dissolve the entire contents of 1 or more containers of Hyaluronidase for Injection in sufficient water, accurately measured, to give a concentration of about 60 USP Hyaluronidase Units per mL. Transfer 2.0 mL of the solution to a 15-mL centrifuge tube calibrated at 6 mL, and evaporate at 105° to dryness. Add 200 µL of 6 N sodium hydroxide, and heat with steam under pressure at 121° for 3 hours. Add 300 µL of 7 N sulfuric acid, then add 1.5 mL of water and 1.5 mL of a 15 in 100 solution of mercuric sulfate in 5 N sulfuric acid. Heat on a steam bath for 10 minutes, and cool to room temperature. Add 1 mL of 7 N sulfuric acid and 1 mL of sodium nitrite solution (1 in 500), with shaking. Add water to make 6 mL, mix, centrifuge, and decant the supernatant. Twenty minutes after diluting to 6 mL, determine the absorbance of the supernatant at 540 nm, with a suitable spectrophotometer.

Repeat the preceding test, using the same quantities of the same reagents and in the same manner but omitting the Hyaluronidase for Injection and replacing the 1.5 mL of water with 1.5 mL of a solution of USP L-Tyrosine RS in 0.4 N sulfuric acid containing 30 µg in each mL.

Calculate the quantity, in µg, of tyrosine in the 2-mL aliquot of the solution of Hyaluronidase for Injection taken by the formula:

$$45(A_U / A_S)$$

in which  $A_U$  and  $A_S$  are the absorbances of the solution from Hyaluronidase for Injection and the Standard solution, re-

spectively: not more than 0.25 µg of tyrosine is found for each USP Hyaluronidase Unit.

**Assay—**

*Acetate buffer solution*—Dissolve 14 g of potassium acetate and 20.5 mL of glacial acetic acid in water to make 1000 mL.

*Phosphate buffer solution*—Dissolve 2.5 g of monobasic sodium phosphate, 1.0 g of anhydrous dibasic sodium phosphate, and 8.2 g of sodium chloride in water to make 1000 mL.

*Hydrolyzed gelatin*—Dissolve 50 g of bacteriological gelatin in 1000 mL of water, heat in an autoclave at 121° for 90 minutes, and freeze-dry the solution.

*Diluent for hyaluronidase solutions*—Mix 250 mL of *Phosphate buffer solution* with 250 mL of water and, within 2 hours before use, dissolve 330 mg of *Hydrolyzed gelatin* in the mixture.

*Serum stock solution*—Constitute dried horse serum with water to its original volume, and dilute it with 9 volumes of *Acetate buffer solution*. Adjust with 4 N hydrochloric acid to a pH of 3.1, and allow the solution to stand at room temperature for 18 to 24 hours. Store the solution at 0° to 4°, and use within 30 days.

*Serum solution*—On the day of the assay, dilute 1 volume of *Serum stock solution* with 3 volumes of *Acetate buffer solution*, and adjust to room temperature.

*Potassium hyaluronate stock solution*—Prepare a stock solution to contain, in each mL, 500 µg of potassium hyaluronate, previously dried in vacuum over phosphorus pentoxide for 48 hours. Do not keep the hyaluronate over phosphorus pentoxide indefinitely. The use of a weighing bottle is desirable. Store the solution at a temperature not exceeding 5°, and use within 30 days.

*Hyaluronate solution*—On the day of the assay, dilute 1 volume of *Potassium hyaluronate stock solution* with 1 volume of *Phosphate buffer solution*.

*Standard solution*—Dissolve a suitable quantity of USP Hyaluronidase RS, accurately weighed, in cold *Diluent for hyaluronidase solutions* to obtain a solution having a known concentration of about 1.5 USP Hyaluronidase Units in each mL. Prepare this solution immediately before use in the assay.

*Assay solution*—Dissolve the contents of 1 container of Hyaluronidase for Injection by adding cold *Diluent for hyaluronidase* directly to the container. On the basis of trial or experience, dilute the solution so prepared with cold *Diluent for hyaluronidase* so that the observed absorbances with the three dilutions of Hyaluronidase for Injection fall on the upper, linear part of the standard curve prepared as directed below.

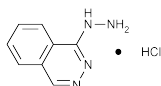
*Procedure*—Prepare a standard concentration-response curve by adding to each of twelve 16- × 100-mm test tubes 500 µL of *Hyaluronate solution*. To each of two of the tubes add, respectively, 500, 400, 300, 200, 100, and 0 µL of *Diluent for hyaluronidase solutions*. If quantities of *Standard solution* other than those indicated below are used, change the above-specified quantities of *Diluent for hyaluronidase solutions* so that the final volume of solution in each tube, after the addition of the *Standard solution*, is 1.00 mL.

At 30-second intervals, accurately timed, add to each of two tubes 0, 100, 200, 300, 400, and 500 µL, respectively, of *Standard solution*. Mix the contents by gentle shaking, and place each tube in a water bath maintained at 37 ± 0.2°. After 30 ± 0.25 minutes, remove each tube, in order, from the water bath at 30-second intervals, and immediately add 4.0 mL of *Serum solution*. Shake the tube, and allow to stand at room temperature for 30 ± 2 minutes. Again shake the tube, and determine the absorbance at 640 nm, with a suitable spectrophotometer. Perform a blank determination but omit the hyaluronate, and make any necessary correction. Plot the average absorbance value for each

level against the hyaluronidase activity expressed in Units, and draw the smooth curve that best fits the plotted points.

Concurrently, to six test tubes add 500  $\mu$ L of *Hyaluronate solution* and sufficient *Diluent for hyaluronidase solutions* so that the final volume, after the addition of the *Assay solution*, is 1.00 mL. Add, at 30-second intervals, sufficient *Assay solution* so that duplicate tubes contain about 0.30, 0.50, and 0.70 Unit, respectively. Shake each tube gently, and treat as directed in the preceding paragraph for the *Standard solution*, beginning with "place each tube in a water bath." Measure the absorbances in the spectrophotometer, and make any necessary correction for the blank. The potency of Hyaluronidase for Injection is the average of the six activity values read from the standard curve.

## Hydralazine Hydrochloride



$C_8H_8N_4 \cdot HCl$  196.64  
Phthalazine, 1-hydrazino-, monohydrochloride.  
1-Hydrazinophthalazine monohydrochloride [304-20-1].

» Hydralazine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_8H_8N_4 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Hydralazine Hydrochloride RS

### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 1 in 100,000.

*Medium:* water.

Absorptivities at 260 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** A solution (1 in 4000) responds to the tests for *Chloride* (191).

**pH** (791): between 3.5 and 4.2, in a solution (1 in 50).

**Loss on drying** (731)—Dry it at 110° for 15 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Water-insoluble substances**—Transfer 2.0 g to a 250-mL conical flask, add 100 mL of water, and shake by mechanical means for about 30 minutes. Filter the solution through a tared sintered-glass crucible, and wash into the crucible any undissolved residue remaining in the flask. Wash the residue with three 10-mL portions of water, dry at 105° for 3 hours, cool, and weigh: the weight of the residue does not exceed 10 mg (0.5%).

**Heavy metals, Method II** (231): 0.002%.

### Limit of hydrazine—

**Benzaldehyde solution**—Transfer 1.0 mL of benzaldehyde to a 100-mL volumetric flask, dilute with a mixture of methanol and water (9:1) to volume, and mix.

**Acetonitrile solution**—Transfer 300 mL of water to a 1000-mL volumetric flask, dilute with acetonitrile to volume, and mix.

**Phosphate buffer**—Dissolve 5.82 g of dibasic sodium phosphate and 3.81 g of monobasic potassium phosphate in

1000 mL of water, and adjust with either 1 N sodium hydroxide or 1 N phosphoric acid to a pH of  $7.0 \pm 0.1$ .

**Mobile phase**—Dissolve 300 mg of edetate disodium in 300 mL of water in a 1000-mL volumetric flask. Dilute with acetonitrile to volume, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 65 mg of hydrazine dihydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.65 mg per mL. Dilute this solution quantitatively, and stepwise if necessary, with water to obtain a *Standard solution* having a known concentration of about 0.325  $\mu$ g of hydrazine dihydrochloride per mL. Transfer 1.0 mL of the *Standard solution* to a 10-mL reaction vessel. Add 4.0 mL of *Benzaldehyde solution*, and shake by mechanical means for 20 minutes. Transfer 2.0 mL of this solution to a 5-mL volumetric flask, dilute with *Acetonitrile solution* to volume, and mix.

**Test solution**—[NOTE—Condition the extraction column specified in this procedure in the following manner. Wash the column with two 2.0-mL portions of hexanes, and dry with the aid of vacuum for two minutes. Wash the column with two 2.0-mL portions of methanol, two 2.0-mL portions of water, and two 2.0-mL portions of pH 7.0 phosphate buffer. At no time after the hexanes wash should the column be allowed to dry out.] Transfer about 20 mg of Hydralazine Hydrochloride, accurately weighed, to a 10-mL reaction vessel, and dissolve in 1.0 mL of water. Add 4.0 mL of *Benzaldehyde solution*, and shake by mechanical means for 20 minutes. Pipet 2.0 mL of this solution into a freshly conditioned solid phase extraction column containing benzenesulfonic acid strong cation-exchange packing with a sorbent-mass to column volume ratio of 500 mg per 3 mL, or equivalent, and elute into a 5-mL volumetric flask. Wash the column with two 1.5-mL portions of *Acetonitrile solution*, collecting the washings with the eluate, dilute with *Acetonitrile solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 310-nm detector and a 4.0-mm  $\times$  25-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for hydralazine derivative and 1.5 for hydrazine derivative; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the solution from the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of hydrazine in the portion of Hydralazine Hydrochloride taken by the formula:

$$(32.05/104.97)(0.1C/W)(r_U/r_S)$$

in which 32.05 and 104.97 are the molecular weights of hydrazine and hydrazine dihydrochloride, respectively; C is the concentration, in  $\mu$ g per mL, of hydrazine dihydrochloride in the *Standard solution*; W is the weight, in mg, of Hydralazine Hydrochloride taken for the *Test solution*; and  $r_U$  and  $r_S$  are the hydrazine peak responses obtained from the *Test solution* and from the solution from the *Standard solution*, respectively: not more than 0.001% of hydrazine is found.

### Chromatographic purity—

**Mobile phase and Resolution solution**—Prepare as directed in the Assay.

**Test solution**—Transfer about 25 mg of Hydralazine Hydrochloride, accurately weighed, to a 50-mL volumetric flask. Add about 30 mL of 0.1 N acetic acid, and sonicate to

dissolve. Cool, dilute with 0.1 N acetic acid to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.0-mm × 25-cm column that contains 10-μm packing L10. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.65 for phthalazine and 1.0 for hydralazine hydrochloride; and the resolution,  $R$ , between the phthalazine peak and the hydralazine peak is not less than 4.0.

**Procedure**—Inject about 20 μL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each peak, other than the solvent peak and the hydralazine peak, in the portion of Hydralazine Hydrochloride taken by the same formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of each peak; and  $r_t$  is the sum of the responses of all the peaks, excluding that of the solvent peak: not more than 1.0% total impurities is found.

#### Assay—

**Mobile phase**—Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, and add 230 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 3.0, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydralazine Hydrochloride RS in 0.1 N acetic acid to obtain a solution having a known concentration of about 0.4 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N acetic acid to volume, and mix to obtain a solution having a known concentration of about 40 μg per mL.

**Resolution solution**—Prepare a solution in 0.1 N acetic acid containing about 0.25 mg of USP Hydralazine Hydrochloride RS and 0.05 mg of phthalazine per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.1 N acetic acid to volume, and mix to obtain a solution containing about 25 μg of USP Hydralazine Hydrochloride RS and 5 μg of phthalazine per mL.

**Assay preparation**—Transfer about 100 mg of Hydralazine Hydrochloride, accurately weighed, to a 250-mL volumetric flask. Dissolve in and dilute with 0.1 N acetic acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N acetic acid to volume, mix, and filter, discarding the first 10 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.0-mm × 25-cm column that contains 10-μm packing L10. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.65 for phthalazine and 1.0 for hydralazine hydrochloride; and the resolution,  $R$ , between the phthalazine and hydralazine peaks is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_8H_8N_4 \cdot HCl$  in the portion of Hydralazine Hydrochloride taken by the formula:

$$(2.5C)(r_U / r_S)$$

in which  $C$  is the concentration, in μg per mL, of USP Hydralazine Hydrochloride RS in the *Standard preparation*;

and  $r_U$  and  $r_S$  are the hydralazine hydrochloride peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydralazine Hydrochloride Injection

» Hydralazine Hydrochloride Injection is a sterile solution of Hydralazine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

#### USP Reference standards <11>—

USP Hydralazine Hydrochloride RS

USP Endotoxin RS

**Identification**—Mix a volume of Injection, equivalent to about 60 mg of hydralazine hydrochloride, with an amount of 1 N hydrochloric acid sufficient to prepare 25 mL of solution. Place 20 mL of this solution in a separator, wash with 10 mL of methylene chloride, and discard the methylene chloride washing. Mix the aqueous solution in the separator with 2 mL of sodium nitrite solution (14 in 1000), add 10 mL of methylene chloride, shake by mechanical means for 5 minutes, and allow the layers to separate. Pass the methylene chloride layer through a filter of anhydrous sodium sulfate that previously has been washed with methylene chloride, and collect the solution in a 50-mL beaker. Evaporate with the aid of gentle heat and a stream of dry nitrogen to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of USP Hydralazine Hydrochloride RS similarly treated and prepared.

**Bacterial endotoxins** <85>—It contains not more than 1.45 USP Endotoxin Units per mg of hydralazine hydrochloride.

**pH** <791>: between 3.4 and 4.4.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—Transfer to a 250-mL iodine flask an accurately measured volume of Injection, equivalent to about 100 mg of hydralazine hydrochloride. Add 20 mL of hydrochloric acid, cool to room temperature, add 5 mL of chloroform, and titrate with 0.02 M potassium iodate VS until the purple color of iodine disappears from the chloroform, adding the last portion of the potassium iodate solution dropwise and agitating the mixture vigorously and continuously. Each mL of 0.02 M potassium iodate is equivalent to 3.933 mg of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ).

## Hydralazine Hydrochloride Oral Solution

#### DEFINITION

Hydralazine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ).

Prepare Hydralazine Hydrochloride Oral Solution of the designated percentage strength as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* <795>).

Hydralazine Hydrochloride	
For 0.1% Oral Solution	100 mg
For 1.0% Oral Solution	1.0 g
Sorbitol Solution (70%)	40 g
Methylparaben	65 mg
Propylparaben	35 mg
Propylene Glycol	10 g
Aspartame	50 mg
Purified Water, a sufficient quantity to make	100 mL

Dissolve the *Hydralazine Hydrochloride* in 30 mL of *Purified Water*, add the *Aspartame*, and shake or stir until the solids have dissolved. Add the *Sorbitol Solution*. In a separate container, dissolve an aliquot portion of an intimate homogeneous mixture of accurately weighed quantities of *Methylparaben* and *Propylparaben* in the *Propylene Glycol*, and, with stirring, add this mixture to the solution containing the *Hydralazine Hydrochloride*. Add sufficient *Purified Water* to make the preparation measure 100 mL, and mix.

[NOTE—Hydralazine reacts with many flavors; do not add flavors when compounding.]

#### SPECIFIC TESTS

- **PH** (791): 3.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a suitable light-resistant glass or plastic bottle, with a child-resistant closure. Store in a refrigerator.
- **LABELING:** Label it to state, as part of the official title, the amount of hydralazine hydrochloride, expressed as a percentage and parenthetically (mg/5 mL). Label it to state that it is to be stored in a refrigerator. The label indicates that patients may mix the appropriate dose with fruit juice or apple sauce just before administration. [Precaution—Phenylketonurics: It contains 1.4 mg/5 mL of phenylalanine.]
- **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded

### Hydralazine Hydrochloride Tablets

» Hydralazine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Hydralazine Hydrochloride RS

#### Identification—

**A:** *Infrared Absorption* (197K)—

*Test specimen*—Transfer a quantity of finely powdered Tablets, equivalent to about 100 mg of hydralazine hydrochloride, to a glass-stoppered flask. Add 40 mL of 1 N hydrochloric acid, shake by mechanical means for 5 minutes, and filter, discarding the first few mL of the filtrate. Place 20 mL of the filtrate in a separator, wash with 10 mL of methylene chloride, and discard the methylene chloride washing. Mix the aqueous solution in the separator with 2 mL of sodium nitrite solution (14 in 1000), add 10 mL of methylene chloride, shake by mechanical means for 5 minutes, and allow the layers to separate. Pass the methylene chloride layer through a filter of anhydrous sodium sulfate that previously has been washed with methylene chloride, and collect the solution in a 50-mL beaker. Evaporate with

the aid of gentle heat and a stream of dry nitrogen to dryness.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_8H_8N_4 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 260 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Hydralazine Hydrochloride RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_8H_8N_4 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Hydralazine Hydrochloride*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of hydralazine hydrochloride, to a 250-mL volumetric flask, dissolve in and dilute with 0.1 N acetic acid to volume, and centrifuge. Pipet 10 mL of the clear liquid into a 100-mL volumetric flask, dilute with 0.1 N acetic acid to volume, mix, and filter.

*Procedure*—Proceed as directed in the *Assay* under *Hydralazine Hydrochloride*. Calculate the quantity, in mg, of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$2.5C(r_U / r_S)$$

in which the terms are as defined therein.

#### Add the following:

### Hydrochloric Acid Injection

#### DEFINITION

Hydrochloric Acid Injection contains NLT 328 mg and NMT 401 mg of hydrochloric acid (HCl) in 100 mL.

Prepare Hydrochloric Acid Injection, 0.1 N, as follows (see *Pharmaceutical Compounding—Sterile Preparations* (797)).

Hydrochloric Acid, 1.0 N	10 mL
Sodium Chloride Injection, 0.45% or 0.9%, a sufficient quantity to make	100 mL

Prepare *1.0 N Hydrochloric Acid* by adding Hydrochloric Acid, NF, to Purified Water in an appropriate volumetric flask with continuous stirring. Allow the solution to cool to room temperature, add sufficient Purified Water to bring to final volume, and continue mixing for 1 min. Pass through a compatible filter of 0.45-μm pore size to reduce particulate matter load. Accurately measure the nonsterile *1.0 N Hydrochloric Acid* with an appropriate glass or plastic device, and accurately dilute to final volume with 0.45% or 0.9% Sodium Chloride Injection. Sterilize the solution by passing through a sterile acid-com-

patible membrane filter of 0.2- $\mu$ m pore size into a sterile intravenous polypropylene container in an ISO Class 5 environment. [NOTE—This is a High-Risk Level Compounded Sterile Preparation (CSP) that shall be prepared according to *Pharmaceutical Compounding—Sterile Preparations* <797>.]

**ASSAY****• PROCEDURE**

**Sample:** 25 mL

**Blank:** 25 mL water

**Titrimetric system**

(See *Titrimetry* <541>.)

**Mode:** Direct titration

**Titrant:** 0.5 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Place *Sample* in a conical flask, add methyl red TS, and titrate with *Titrant*.

Calculate the weight of hydrochloric acid in 100 mL:

$$\text{Result} = [(V_S - V_B) \times N \times F] \times D$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 18.23 mg/mEq

$D$  = dilution factor, 4

**Acceptance criteria:** 328–401 mg in 100 mL

**SPECIFIC TESTS**

**• pH <791>:** 1.0–1.2

**• STERILITY TESTS <71>:** It meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined*, *Membrane Filtration*.

**• BACTERIAL ENDOTOXINS TEST <85>:** NMT 1.5 USP Endotoxin/mL

**ADDITIONAL REQUIREMENTS**

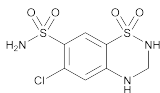
**• PACKAGING AND STORAGE:** Package in polypropylene container or equivalent. Store at controlled room temperature or at controlled cold temperature.

**• LABELING:** Label it Hydrochloric Acid Injection, 0.1 N. Label it to state that this is a single-dose intravenous injection and that it contains no antimicrobial preservatives. Label it to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** If a sterility test and bacterial endotoxin test is passed, NMT 120 days when stored at controlled room temperature or controlled cold temperature. In the absence of passing a sterility test and endotoxin test, the storage periods at controlled room temperature or cold temperature for *High-Risk Level CSPs* applies. (See *CSP Microbial Contamination Risk Levels in Pharmaceutical Compounding—Sterile Preparations* <797>.)

**• USP REFERENCE STANDARDS <11>**

USP Endotoxin RS $\blacktriangle$ USP36

**Hydrochlorothiazide**

$C_7H_8ClN_3O_4S_2$  297.74

2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide;

6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [58-93-5].

**DEFINITION**

Hydrochlorothiazide contains NLT 98.0% and NMT 102.0% of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ), calculated on the dried basis.

**IDENTIFICATION****• A. INFRARED ABSORPTION <197K>**

**Sample:** Potassium bromide–hydrochlorothiazide mixture, previously heated at 105° for 2 h

**Acceptance criteria:** Meets the requirements

**• B. ULTRAVIOLET ABSORPTION <197U>**

**Sample solution:** 10  $\mu$ g/mL in methanol

**Acceptance criteria:** Meets the requirements

**ASSAY****• PROCEDURE**

**Buffer:** 2.76 g of monobasic sodium phosphate in a 1000-mL volumetric flask. Add 990 mL of water. Adjust with phosphoric acid to a pH of  $2.7 \pm 0.1$ , and dilute with water to volume.

**Diluent:** Acetonitrile and *Buffer* (3:7)

**Solution A:** Acetonitrile and methanol (3:1)

**Solution B:** Anhydrous formic acid in water (5 in 1000)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	3	97
5	3	97
14	36	64
18	3	97
20	3	97

**System suitability solution:** 0.32 mg/mL of USP Hydrochlorothiazide RS, 0.0032 mg/mL of USP Chlorothiazide RS, and 0.0032 mg/mL of USP Benzothiadiazine Related Compound A RS in *Diluent*; sonicate if necessary to dissolve. Pass a portion through a filter of 0.45- $\mu$ m or finer pore size.

**Standard solution:** 0.32 mg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonicate if necessary to dissolve. Pass a portion through a filter of 0.45- $\mu$ m or finer pore size.

**Sample solution:** 0.32 mg/mL of Hydrochlorothiazide in *Diluent*, prepared as follows. Transfer 32 mg of Hydrochlorothiazide into a 100-mL volumetric flask. Add 70 mL of *Diluent*, sonicate for 10 min if necessary to dissolve, and allow to cool to ambient temperature. Dilute with *Diluent* to volume. Pass a portion through a filter of 0.45- $\mu$ m or finer pore size.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm

**Column:** 4.6-mm  $\times$  5-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Chromatograph the *Diluent* to check for interference by system-related peaks.

**Suitability requirements**

**Resolution:** NLT 2.0 between benzothiadiazine related compound A and chlorothiazide and NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

**Tailing factor:** NMT 1.5 for the peaks for benzothiadiazine related compound A, chlorothiazide, and hydrochlorothiazide, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Hydrochlorothiazide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Hydrochlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **CHLORIDE AND SULFATE**, *Chloride* (221)

**Sample solution:** Shake 0.50 g with 40 mL of water for 5 min, and filter.

**Acceptance criteria:** 0.035%; the filtrate shows no more chloride than corresponds to 0.25 mL of 0.020 N hydrochloric acid.

• **SELENIUM** (291)

**Sample:** 200 mg

**Acceptance criteria:** NMT 30 ppm

• **HEAVY METALS**, *Method II* (231): NMT 10 ppm

• **ORGANIC IMPURITIES**

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Quantitative limit solution:** 0.16 µg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonicate if necessary to dissolve.

**Sample solution:** 0.32 mg/mL of Hydrochlorothiazide in *Diluent*, prepared as follows. Transfer 32 mg of Hydrochlorothiazide into a 100-mL volumetric flask. Add 70 mL of *Diluent*, sonicate for 10 min if necessary to dissolve, and allow to cool to ambient temperature. Dilute with *Diluent* to volume, mix, and pass a portion through a filter of 0.45-µm or finer pore size.

#### System suitability

**Samples:** *System suitability solution* and *Quantitative limit solution*

[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between benzothiadiazine related compound A and chlorothiazide and NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

**Tailing factor:** NMT 1.5 for the peaks for benzothiadiazine related compound A, chlorothiazide, and hydrochlorothiazide, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for benzothiadiazine related compound A and chlorothiazide, *System suitability solution*; NMT 25% for hydrochlorothiazide, based on 3 replicate injections, *Quantitative limit solution*

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Hydrochlorothiazide taken:

$$\text{Result} = (R_U/R_T) \times 100$$

$R_U$  = ratio of the peak area for each impurity to its response factor

$R_T$  = sum of the ratios of all the peak areas to their respective response factors

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A	0.5	0.54	1.0
Chlorothiazide	0.8	0.63	0.5
Hydrochlorothiazide	1.0	—	—
5-Chlorohydrochlorothiazide	2.1	—	0.5
Hydrochlorothiazide dimer <sup>a</sup>	2.6	—	0.5
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	0.9 <sup>b</sup>

<sup>a</sup> 6-Chloro-N-[(6-chloro-7-sulfamoyl-2,3-dihydro-4H-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.

<sup>b</sup> Excluding benzothiadiazine related compound A.

#### SPECIFIC TESTS

• **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 1 h.

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Benzothiadiazine Related Compound A RS  
4-Amino-6-chloro-1,3-benzenedisulfonamide.

$C_6H_8ClN_3O_4S_2$  285.73

USP Chlorothiazide RS

USP Hydrochlorothiazide RS

## Hydrochlorothiazide Capsules

#### DEFINITION

Hydrochlorothiazide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

#### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 13.8 g/L of monobasic sodium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (10:90). Adjust with 10% (v/v) phosphoric acid to a pH of  $3.0 \pm 0.1$ . Pass through a filter of 0.45-µm pore size.

**System suitability solution:** 0.15 mg/mL each of USP Hydrochlorothiazide RS and USP Chlorothiazide RS in *Mobile phase*. Sonicate to completely dissolve.

**Standard stock solution:** 0.50-mg/mL solution prepared as follows: Dissolve a quantity of USP Hydrochlorothiazide RS in acetonitrile (10% of the volume of the flask), and dilute with *Mobile phase*. Sonicate to completely dissolve.

**Standard solution:** 50-µg/mL solution in *Mobile phase* from the *Standard stock solution*. Sonicate to completely dissolve.

**Sample stock solution:** 0.25 mg/mL of hydrochlorothiazide solution prepared as follows: Transfer a number of

Capsules into a suitable volumetric flask. Add water, 10% of the volume of the flask, and sonicate for 10 min with vigorous shaking. Add *Buffer*, 20% of the volume of the flask, and again sonicate for 10 min. Add acetonitrile up to 40% of the volume of the flask, and sonicate for 30 min. Dilute with *Buffer* to volume, and pass through a suitable filter of 0.45- $\mu$ m pore size.

**Sample solution:** 50  $\mu$ g/mL of hydrochlorothiazide in *Mobile phase* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 272 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 2.0 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of hydrochlorothiazide in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION <711>

##### Test 1

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution:** 6.75- $\mu$ g/mL solution prepared as follows: Dissolve a quantity of USP Hydrochlorothiazide RS in acetonitrile (10% of the volume of the flask), and dilute with *Medium*. Sonicate to completely dissolve.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Dilute with *Medium* to a concentration similar to the *Standard solution*.

**Analytical wavelength:** UV 272 nm

**Pathlength:** 1 cm

**Blank:** *Medium*

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of hydrochlorothiazide in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$D$  = dilution for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard stock solution:** 0.35 mg/mL of USP Hydrochlorothiazide RS in *Medium*. An amount of acetonitrile, not exceeding 25% of the final volume, may be used to help solubilize hydrochlorothiazide.

**Standard solution:** ( $L/900$ ) mg/mL of hydrochlorothiazide in *Medium*, from the *Standard stock solution*, where  $L$  is the Capsule label claim in mg

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Empty capsules solution:** Place 10 Capsules into a 900-mL volumetric flask. Slowly add 800 mL of *Medium* pre-heated to 37°, and stir until dissolved. Cool to room temperature, and dilute with *Medium* to volume.

**Analytical wavelength:** UV 272 nm

**Pathlength:** 1 cm

**Blank:** *Medium*

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = [(A_U - A_{EC})/A_S] \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_{EC}$  = absorbance of the *Empty capsules solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of hydrochlorothiazide in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 0.25-mg/mL solution prepared as follows: Dissolve a quantity of USP Hydrochlorothiazide RS in acetonitrile (10% of the volume of the flask), and dilute with *Mobile phase*.

**Standard solution:** 0.25  $\mu$ g/mL of USP Hydrochlorothiazide RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** Use the *Sample stock solution* as prepared in the *Assay*.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

- $r_s$  = peak response of hydrochlorothiazide from the *Standard solution*  
 $C_s$  = concentration of hydrochlorothiazide in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of hydrochlorothiazide in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = relative response factor (see *Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A	0.65	0.61	1.0
Chlorothiazide	0.80	—	— <sup>a</sup>
Hydrochlorothiazide	1.0	1.0	—
5-Chlorohydrochlorothiazide	2.88	—	— <sup>a</sup>
Any other individual unspecified degradant	—	1.0	0.2
Total impurities <sup>b</sup>	—	—	1.5

<sup>a</sup> Process related impurity. The relative retention time is given for identification.

<sup>b</sup> Total impurities include benzothiadiazine related compound A and all unknown degradation impurities. Disregard any peak less than 0.05%.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Chlorothiazide RS
  - 2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.
  - $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$  295.72
  - USP Hydrochlorothiazide RS

**Hydrochlorothiazide Tablets**

» Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrochlorothiazide ( $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Hydrochlorothiazide RS  
 USP Benzothiadiazine Related Compound A RS  
 4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $\text{C}_6\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$  285.73

**Identification**—

**A:** Transfer a portion of finely powdered Tablets, equivalent to about 50 mg of hydrochlorothiazide, to a 50-mL volumetric flask. Add about 20 mL of sodium hydroxide solution (1 in 125), and shake vigorously for 15 minutes. Dilute with the same solvent to volume, mix, and filter, discarding the first few mL of the filtrate. Transfer 5 mL of the filtrate to a 125-mL separator, and add 5 mL of dilute hydrochloric acid (1 in 10). Extract with 50 mL of ether, filter the ether extract through a small, dry, folded filter paper, and evaporate to dryness. Add 5 mL of alcohol, and again evaporate

to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydrochlorothiazide RS previously dissolved in alcohol and recovered by evaporating the solution to dryness.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* 0.1 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 60 minutes.

*Procedure*—Determine the amount of  $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 272 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Hydrochlorothiazide RS in the same *Medium*.

*Tolerances*—Not less than 60% (Q) of the labeled amount of  $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—

*Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Proceed as directed for *Assay preparation* in the *Assay*.

*Standard solution*—[NOTE—A volume of acetonitrile not exceeding 10% of the total volume of the solution may be used to dissolve the USP Reference Standard.] Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 1.5  $\mu\text{g}$  per mL.

*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of benzothiadiazine related compound A in the portion of tablets taken by the formula:

$$0.2C(r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution*; and  $r_u$  and  $r_s$  are the peak responses for benzothiadiazine related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is present.

**Assay**—

*Mobile phase*—Prepare a degassed mixture of 0.1 M monobasic sodium phosphate and acetonitrile (9:1), adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—[NOTE—A volume of acetonitrile not exceeding 10% of the total volume of solution may be used to dissolve the USP Reference Standards.] Dissolve accurately weighed quantities of chlorothiazide and USP Hydrochlorothiazide RS in *Mobile phase* to obtain a solution containing about 0.15 mg of each per mL.

*Standard preparation*—[NOTE—A volume of acetonitrile not exceeding 10% of the total volume of the solution may be used to dissolve the USP Reference Standard.] Dissolve an accurately weighed quantity of USP Hydrochlorothiazide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of



the powder, equivalent to about 30 mg of hydrochlorothiazide, to a 200-mL volumetric flask. Add about 20 mL of *Mobile phase*, sonicate for 5 minutes, and add about 20 mL of acetonitrile. Sonicate for 5 minutes, add about 50 mL of *Mobile phase*, and shake by mechanical means for 10 minutes. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 10 mL of the filtrate.

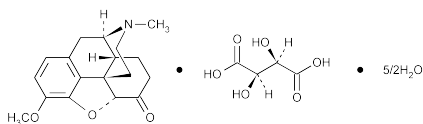
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for chlorothiazide and 1.0 for hydrochlorothiazide; and the resolution, *R*, between chlorothiazide and hydrochlorothiazide is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Tablets taken by the formula:

$$200C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocodone Bitartrate



C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> · 2½H<sub>2</sub>O 494.49  
Morphinan-6-one, 4,5-epoxy-3-methoxy-17-methyl-, (5α)-, [R-(R\*,R\*)]-2,3-dihydroxybutanedioate (1:1), hydrate (2:5).  
4,5α-Epoxy-3-methoxy-17-methylmorphinan-6-one tartrate (1:1) hydrate (2:5) [34195-34-1; 6190-38-1].  
Anhydrous 449.46 [143-71-5].

» Hydrocodone Bitartrate, dried in vacuum at 105° for 2 hours, contains not less than 98.0 percent and not more than 102.0 percent of C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards <11>—

USP Dihydrocodeine Bitartrate RS

USP Hydrocodone Bitartrate RS

USP Hydrocodone Bitartrate Related Compound A RS

### Identification—

**A:** *Infrared Absorption* <197M>.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 100 µg per mL.

*Medium:* 0.1 N sulfuric acid.

**Specific rotation** <781S>: between −79° and −84°.

*Test solution:* 20 mg, undried, per mL, in water. Calculate the result on the basis of the undried aliquot.

**pH** <791>: between 3.2 and 3.8, in a solution (1 in 50).

**Loss on drying**—Dry it in vacuum at 105° for 2 hours [NOTE—See the *Note* in the *Assay* for precautions regarding handling of the dried material.]: it loses not less than 7.5% and not more than 12.0% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Chloride**—To 10 mL of a solution (1 in 100), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

**Assay**—[NOTE—Dry both the USP Hydrocodone Bitartrate RS and the Hydrocodone Bitartrate materials in vacuum at 105° for 2 hours. Immediately transfer the dried materials to a desiccator containing phosphorus pentoxide. Weigh each dried material individually within 1 minute, and proceed with the *Assay*.]

**Mobile phase**—Prepare a mixture of acetonitrile, water, and diethylamine (800:4:1). Prepare a filtered and degassed mixture of this solution and methanol (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer about 10 mg of previously dried USP Hydrocodone Bitartrate RS, accurately weighed, to a 10-mL volumetric flask, add 5 mL of water, and mix to dissolve. Dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of previously dried Hydrocodone Bitartrate, equivalent to about 100 mg of hydrocodone bitartrate, C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>, to a 100-mL volumetric flask, add 50 mL of water, and mix to dissolve. Dilute with methanol to volume, and mix.

**Resolution solution**—Prepare a solution in methanol containing about 0.4 mg of USP Dihydrocodeine Bitartrate RS and 0.6 mg of USP Hydrocodone Bitartrate RS per mL. Prepare a mixture of this solution and water (1:1).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for hydrocodone and 1.0 for dihydrocodeine; and the resolution, *R*, between hydrocodone and dihydrocodeine is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> in the portion of Hydrocodone Bitartrate taken by the formula:

$$(100C)(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocodone Bitartrate RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocodone Bitartrate Tablets

» Hydrocodone Bitartrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocodone bi-

tartrate disesquihydrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hydrocodone Bitartrate RS

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**Dissolution** (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

**Phosphate buffer–bromothymol blue solution**—Dissolve 3.40 g of monobasic sodium phosphate in about 50 mL of water, add 18 mL of 0.1 N sodium hydroxide, dilute with water to 500 mL, and mix. Adjust the solution, if necessary, with 1 N sodium hydroxide or 1 N phosphoric acid (*Buffer solution*) to a pH of  $5.8 \pm 0.1$ . Mix 31.2 mg of bromothymol blue with 1.0 mL of 0.1 N sodium hydroxide, add *Buffer solution* to obtain 500 mL of solution, and mix.

**Standard solution**—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

**Test solution**—Pipet into a 125-mL separator a volume of a filtered portion of the solution under test that is estimated to contain about 0.4 mg of hydrocodone bitartrate. Add 2 drops of 6 N ammonium hydroxide, and extract with three 25-mL portions of chloroform. Filter the extracts through about 3 g of anhydrous sodium sulfate supported on filter paper, and combine the filtered extracts in a 100-mL volumetric flask. Add chloroform through the filter to volume, and mix.

**Procedure**—Pipet 10 mL of the *Standard solution* into a 125-mL separator, and add 25.0 mL of chloroform. Pipet 25 mL of the *Test solution* into a second 125-mL separator, and add 10.0 mL of water. Pipet 25 mL of chloroform and 10 mL of water into a third 125-mL separator to provide a blank. Treat each mixture as follows. Add 30 mL of *Phosphate buffer–bromothymol blue solution*, and shake vigorously for not less than 15 minutes. Allow the layers to separate, and pass the chloroform layer through filter paper, discarding the first 10 mL of the filtrate. Determine the absorbances of the clear filtrates in 3-cm cells at the wavelength of maximum absorbance at about 415 nm, using the solution from the blank to set the spectrometer. Calculate the amount of  $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$  dissolved by the formula:

$$(494.49 / 449.46)100(C_S / V_U)(A_U / A_S)$$

in which 494.49 and 449.46 are the molecular weights of the hydrated and anhydrous forms of hydrocodone bitartrate, respectively;  $C_S$  is the concentration, in  $\mu$ g per mL, of USP Hydrocodone Bitartrate RS in the *Standard solution*;  $V_U$  is the volume of the filtered solution under test that is estimated to contain 0.4 mg of hydrocodone bitartrate; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 50-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Filter if necessary, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution and a solution of USP Hydrocodone Bitartrate RS in the same medium, having a known concentration of about 100  $\mu$ g per mL, in 1-cm cells at the

wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of  $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$  in the Tablet taken by the formula:

$$(494.49 / 449.46)(TC / D)(A_U / A_S)$$

in which 494.49 and 449.46 are the molecular weights of the hydrated and anhydrous forms of hydrocodone bitartrate, respectively;  $T$  is the labeled quantity, in mg, of hydrocodone bitartrate in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Hydrocodone Bitartrate RS in the *Standard solution*;  $D$  is the concentration, in  $\mu$ g per mL, of the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the *Standard solution*, respectively.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of hydrocodone bitartrate, to a 250-mL separator with the aid of 10 mL of water. Add a small piece of red litmus paper, then add, dropwise, 6 N ammonium hydroxide until the litmus paper turns blue (about 3 drops). Extract with 25-, 25-, 20-, 20-, 15-, and 15-mL portions of chloroform, and filter the chloroform extracts through a small pledget of cotton into a 250-mL conical flask. Evaporate the combined chloroform extracts almost to dryness, remove the flask from the steam bath, and evaporate the remainder of the chloroform with the aid of a current of air. Dissolve the residue in 80 mL of glacial acetic acid, warming, if necessary. Cool, and titrate with 0.02 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.02 N perchloric acid is equivalent to 9.890 mg of  $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ .

## Hydrocodone Bitartrate and Acetaminophen Tablets

» Hydrocodone Bitartrate and Acetaminophen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocodone bitartrate disesquihydrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ) and acetaminophen ( $C_8H_9NO_2$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—The labeling indicates the *Dissolution* test with which the product complies.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Hydrocodone Bitartrate RS

**Identification**—

**A:** Finely powder 1 Tablet, and transfer about half of the powder to a test tube. Add 1 mL of 1 N sodium hydroxide and 10 mL of water, and centrifuge. Add 5 or 6 drops of ferric chloride TS: a deep blue color develops, and almost immediately a gray-black precipitate forms (*presence of acetaminophen*).

**B:** The retention times of the hydrocodone bitartrate peak and the acetaminophen peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

TEST 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** pH 5.8 ± 0.05 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Proceed as directed in the *Assay*, making any necessary modifications.

**Tolerances**—Not less than 80% (Q) each of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and hydrocodone bitartrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ) are dissolved in 30 minutes.

**TEST 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus, Time, and Procedure**—Proceed as directed under *Test 1*.

**Tolerances**—Not less than 80% (Q) each of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and hydrocodone bitartrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Buffer solution**—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (85:15), and add 0.2 mL of triethylamine per L of *Mobile phase*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Hydrocodone bitartrate standard stock preparation**—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 35 µg per mL.

**Standard preparation**—Transfer about 38 mg of USP Acetaminophen RS, accurately weighed, to a 50-mL volumetric flask. Add an accurately measured volume of *Hydrocodone bitartrate standard stock preparation* containing about 38000 µg of USP Hydrocodone Bitartrate RS, *J* being the ratio of the labeled amount, in mg, of hydrocodone bitartrate to that of acetaminophen per Tablet, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.76 mg of USP Acetaminophen RS per mL and about 760 µg of USP Hydrocodone Bitartrate RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 76 mg of acetaminophen, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pass a portion of this mixture through a membrane having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector set at 210 nm for hydrocodone bitartrate and 295 nm for acetaminophen and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for acetaminophen and 2.0 for hydrocodone; the resolution, *R*, between hydrocodone and acetaminophen is not less than 5.0; the tailing factor for the hydrocodone peak is not more than 1.6; and the relative standard deviation for replicate injections determined from both acetaminophen and hydrocodone is not more than 2.0% each.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. From the responses obtained at 210 nm, calculate the quantity, in mg, of hy-

drocodone bitartrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(494.49/449.46)(0.1C)(r_U / r_S)$$

in which 494.49 is the molecular weight of hydrocodone bitartrate disesquihydrate; 449.46 is the molecular weight of anhydrous hydrocodone bitartrate; *C* is the concentration, in µg per mL, of USP Hydrocodone Bitartrate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. From the responses obtained at 295 nm, calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocodone Bitartrate and Homatropine Methylbromide Tablets

» Hydrocodone Bitartrate and Homatropine Methylbromide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocodone bitartrate disesquihydrate ( $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\frac{1}{2}H_2O$ ) and homatropine methylbromide ( $C_{17}H_{24}BrNO_3$ ). NOTE—Use of silanized autosampler vials such as dimethyldichlorosilane vials\* is required for the *Dissolution* test, the *Limit* tests, and the *Assay* to prevent drug degradation.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### USP Reference standards (11)—

USP Dihydrocodeine Bitartrate RS  
USP Homatropine Methylbromide RS  
USP Hydrocodone Bitartrate RS

#### Identification—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

**Solution A**—Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

**Solution B**—Dissolve 8 g of potassium iodide in 20 mL of water.

**Stock solution:** a mixture of *Solution A* and *Solution B* (1:1).

**Solvent:** a mixture of methanol and water (9:1).

**Spray reagent 1**—[NOTE—Prepare immediately before use.] Prepare a mixture of water, glacial acetic acid, and *Stock solution* (50:10:5).

**Spray reagent 2:** hydrogen peroxide TS.

**Standard solution 1**—Transfer an accurately weighed quantity of about 30 mg of USP Homatropine Methylbromide RS to a 100-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

**Standard solution 2**—Transfer an accurately weighed quantity of about 25 mg of USP Hydrocodone Bitartrate RS

\*A suitable grade is available from Analytical Research and Testing, Somerville, NJ; Fax: 908-725-8848.

to a 25-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

**Test solution**—Transfer a portion of 20 finely powdered Tablets, equivalent to the average Tablet weight, to a centrifuge tube, add 5.0 mL of *Solvent*, centrifuge, and use the supernatant.

**Developing solvent system**: a mixture of ethyl acetate, water, and formic acid (134:33:33).

**Procedure**—Apply 50  $\mu$ L of *Standard solution 1*, *Standard solution 2*, and the *Test solution*, and proceed as directed in the chapter. Remove the plate, and dry at 105°. Spray the plate with *Spray reagent 1* and then with *Spray reagent 2*: the  $R_f$  values for the principal spots in the chromatogram of the *Test solution* correspond to those of the *Standard solutions*.

**B**: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution <711>—

TEST 1—

**Medium**: water; 900 mL, deaerated.

**Apparatus 2**: 50 rpm.

**Time**: 30 minutes.

Determine the amounts of hydrocodone bitartrate and homatropine bromide dissolved employing the following method.

**Buffer solution and Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS and USP Homatropine Methylbromide RS in *Medium*, and dilute quantitatively with *Medium* to obtain a solution having known concentrations of about 0.0055 mg per mL and 0.00165 mg per mL, respectively.

**Test solution**—Use the solution under test passed through a suitable 0.45- $\mu$ m filter.

**Chromatographic system**—Proceed as directed in the *Assay*, using the *Standard solution*: the resolution,  $R$ , between homatropine methylbromide and hydrocodone bitartrate is not less than 13; and the relative standard deviation for replicate injections is not more than 3.0% for each analyte.

**Procedure**—Separately inject equal volumes (about 250  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of hydrocodone bitartrate and homatropine methylbromide dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak areas of each drug substance in the *Test solution* and in the *Standard solution*, respectively;  $C_S$  is the concentration of each drug substance, in mg per mL, in the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $LC$  is the tablet label claim for each drug substance, in mg.

**Tolerances**—Not less than 80% (Q) of hydrocodone bitartrate and homatropine methylbromide is dissolved in 30 minutes.

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium**: water, 500 mL.

**Apparatus 2**: 50 rpm.

**Time**: 45 minutes.

Determine the amounts of hydrocodone bitartrate and homatropine bromide dissolved employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (3:1) that contains 1.4 g of octanesulfonic acid sodium salt and 1.0 mL of phosphoric acid per 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Hydrocodone bitartrate standard solution**—Transfer about 50 mg, accurately weighed, of USP Hydrocodone Bitartrate RS to a 100-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.

**Homatropine methylbromide standard solution**—Transfer about 37.5 mg, accurately weighed, of USP Homatropine Methylbromide RS to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume.

**System suitability solution**—Transfer 10.0 mL of *Hydrocodone bitartrate standard solution* and *Homatropine methylbromide standard solution* to a 500-mL volumetric flask, add 105 mL of *Mobile phase*. Dilute with *Medium* to volume.

**Test solution**—Pass a portion of 20 mL of the solution under test through a suitable 0.45- $\mu$ m filter, discarding the first 2 to 3 mL. Mix thoroughly 15.0 mL of the filtrate with 5.0 mL of *Mobile phase*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 212-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *System suitability solution*, and record the chromatogram as directed for *Procedure*: the tailing factor for each drug substance is not more than 1.5; the resolution,  $R$ , between homatropine methylbromide and hydrocodone bitartrate is not less than 2.2; and the relative standard deviation for replicate injections is not more than 3.0% for homatropine methylbromide and not more than 2.0% for hydrocodone bitartrate.

**Procedure**—Separately inject equal volumes (about 200  $\mu$ L) of the appropriate *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each drug substance dissolved by the formula:

$$\frac{r_U \times C_S \times D_U \times 500 \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak responses of each drug substance in the *Test solution* and in the correspondent *Standard solution*, respectively;  $C_S$  is the concentration of each drug substance, in mg per mL, in the correspondent *Standard solution*;  $D_U$  is the dilution factor of the *Test solution*; 500 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $LC$  is the tablet label claim for each drug substance, in mg.

**Tolerances**—Not less than 75% (Q) of hydrocodone bitartrate and homatropine methylbromide is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Limit of dihydrocodeine bitartrate, hydrocodone diol, and related substances—

**Ion-pair solution**—Prepare 0.005 M sodium 1-octanesulfonate, and adjust with glacial acetic acid to a pH of  $2.5 \pm 0.1$ .

**Mobile phase**—Prepare a filtered and degassed mixture of *Ion-pair solution* and methanol (6:4). Add 0.5 mL of triethylamine per L. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve about 2 mg each of hydrocodone diol and USP Dihydrocodeine Bitartrate RS in 35 mL of *Mobile phase* in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute this solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of each of about 0.1  $\mu$ g per mL.

**Standard solution**—Use the *Standard preparation*, prepared as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.67 for hydrocodone diol, 0.75 for dihydrocodeine bitartrate, and 1.0 for hydrocodone bitartrate; the resolution, *R*, between hydrocodone diol and dihydrocodeine bitartrate is not less than 2.0; and the relative standard deviation for replicate injections of each of these compounds is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 200 μL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentages of hydrocodone diol and dihydrocodeine bitartrate in the portion of Tablets taken by the formula:

$$100(r_D / r_S)$$

in which  $r_D$  is the individual peak response of either hydrocodone diol or dihydrocodeine in the chromatogram obtained from the *Test solution*; and  $r_S$  is the peak response of hydrocodone bitartrate in the chromatogram obtained from the *Standard solution*: not more than 0.5% of hydrocodone diol is found, and not more than 1.0% of dihydrocodeine bitartrate is found. Calculate the percentage of each other related substance in the portion of Tablets taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for any individual related substance with a retention time greater than 5 minutes; and  $r_S$  is the sum of the responses of all the peaks: not more than 0.5% of any individual related substance is found. The sum of all impurities is not more than 1.5%.

#### Limit of homatropine hydrobromide and related substances—

**Buffer solution**—Prepare a solution of 0.005 M dibasic potassium phosphate, and adjust with phosphoric acid to a pH of  $6.4 \pm 0.1$ .

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (17:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of homatropine hydrobromide in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 0.6 μg per mL.

**Test solution**—Use the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of homatropine hydrobromide in the portion of Tablets taken by the formula:

$$100(r_H / r_S)$$

in which  $r_H$  is the individual peak response of homatropine hydrobromide in the chromatogram obtained from the *Test*

*solution*; and  $r_S$  is the peak response for homatropine methylbromide in the chromatogram obtained from the *Standard solution*: not more than 0.5% of homatropine hydrobromide is found. Calculate the percentage of each other related substance in the portion of Tablets taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for any individual related substance with a relative retention time less than 0.44 in relation to the retention time of hydrocodone bitartrate; and  $r_S$  is the sum of the responses of all the peaks: not more than 0.5% of any individual related substance is found. The sum of all impurities is not more than 1.5%.

#### Limit of tropine—

**Adsorbent**: 0.25-mm layer of chromatographic silica gel.

**Diluent**: diethyl ether.

**Test solution**—Finely powder 25 Tablets, and add to a centrifuge tube. Pipet 5.0 mL of diethyl ether into the centrifuge tube, mix on a vortex mixer for 5 minutes, centrifuge, and use the supernatant.

**Standard stock solution**—Dissolve an accurately weighed quantity of tropine in *Diluent*, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 150 μg per mL.

**Stock solutions**—Transfer 5.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume quantitatively, and stepwise if necessary, to obtain *Standard solutions A, B, C, and D* having known concentrations of about 75 μg per mL, 37.5 μg per mL, 18.75 μg per mL, and 9.38 μg per mL, respectively.

**Spray reagent**—Dissolve 300 mg of platinic acid in 3 mL of diluted hydrochloric acid, add 97 mL of water and 100 mL of 6% potassium iodide in water, and mix.

**Developing solvent system**: a mixture of alcohol and ammonium hydroxide (400:100).

**Procedure**—Apply equal volumes (about 500 μL) of the *Standard stock solution*, *Standard solutions A, B, C, and D*, and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* (621)), and proceed as directed in the chapter. After the plate has dried, position it in a chamber saturated with iodine vapor for about 30 minutes, then place it in a hood to allow the iodine to sublime from the plate, and spray the plate with *Spray reagent* until spots appear. Any spot from the *Test solution* occurring at an  $R_f$  value corresponding to tropine is not greater in size or intensity than the corresponding spot obtained from *Standard solution B* (0.5%): not more than 0.5% of tropine is found.

#### Assay—

**Buffer solution**—Prepare a solution of 0.005 M dibasic potassium phosphate, and adjust with phosphoric acid to a pH of  $6.4 \pm 0.01$ .

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (17:3).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS and USP Homatropine Methylbromide RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having known concentrations of about 0.2 mg per mL and 0.06 mg per mL, respectively.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of hydrocodone bitartrate and about 1.5 mg of homatropine methylbromide, to a 25-mL volumetric flask. Pipet 15 mL of the *Mobile phase* into the volumetric flask, sonicate for 15 minutes, and then shake with a wrist-action shaker for 15 additional minutes. Pipet an additional 10 mL of *Mobile phase* into the volumetric flask, and mix. Pass the solution through a filter having a 0.45-μm porosity prior to injection into the chromatograph.

**Chromatographic system**—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.44 for homatropine methylbromide and 1.0 for hydrocodone bitartrate; the resolution, *R*, between hydrocodone bitartrate and homatropine methylbromide is not less than 2.5; and the relative standard deviation for replicate injections is not more than 3.0% for each analyte.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the amount, in mg, of homatropine methylbromide (C<sub>17</sub>H<sub>24</sub>BrNO<sub>3</sub>) in the portion of Tablets taken by the formula:

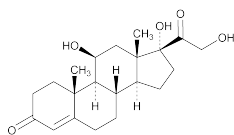
$$(LC_S / C_U)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of homatropine methylbromide in each Tablet; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Homatropine Methylbromide RS in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of homatropine methylbromide in the *Assay preparation*, based on the labeled amount per Tablet and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the homatropine methylbromide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the amount, in mg, of hydrocodone bitartrate disesquihydrate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> · 2½ H<sub>2</sub>O) in the portion of Tablets taken by the formula:

$$(494.50/449.46)(LC_S / C_U)(r_U / r_S)$$

in which 494.50 and 449.46 are the molecular weights of hydrocodone bitartrate disesquihydrate and anhydrous hydrocodone bitartrate, respectively; *L* is the labeled amount, in mg, of hydrocodone bitartrate disesquihydrate in each Tablet; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Hydrocodone Bitartrate RS in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of hydrocodone bitartrate disesquihydrate in the *Assay preparation*, based on the labeled amount per Tablet and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone



C<sub>21</sub>H<sub>30</sub>O<sub>5</sub> 362.46  
Pregn-4-ene-3,20-dione, 11,17,21-trihydroxy-, (11β)-;  
Cortisol [50-23-7].

### DEFINITION

Hydrocortisone contains NLT 97.0% and NMT 102.0% of C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>, calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197M>
- **B. ULTRAVIOLET ABSORPTION** <197U>  
Analytical wavelength: 242 nm  
Sample solution: 10 μg/mL in methanol  
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.5%.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, and water (25:25:50)

**Diluent:** Methanol and water (1:1)

**Internal standard solution:** 1 mg/mL of propylparaben in methanol

**Standard stock solution:** 1 mg/mL of USP Hydrocortisone RS in methanol

**Standard solution:** 2.0 mL of *Standard stock solution* and 2.0 mL of *Internal standard solution*. Dilute with *Diluent* to 50 mL.

**Sample stock solution:** 1 mg/mL of Hydrocortisone in methanol

**Sample solution:** 2.0 mL of *Sample stock solution* and 2.0 mL of *Internal standard solution*. Dilute with *Diluent* to 50 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for hydrocortisone and propylparaben are about 1.0 and 1.8, respectively.]

#### Suitability requirements

**Resolution:** NLT 9.0 between the hydrocortisone and propylparaben peaks

**Column efficiency:** NLT 3000 theoretical plates for hydrocortisone

**Tailing factor:** NMT 1.2

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) in the portion of Hydrocortisone taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times 100$$

*R<sub>U</sub>* = peak response ratio of hydrocortisone to the internal standard from the *Sample solution*

*R<sub>S</sub>* = peak response ratio of hydrocortisone to the internal standard from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of Hydrocortisone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

#### • RESIDUE ON IGNITION <281>

**Sample:** 100 mg

**Acceptance criteria:** NMT 0.5%

#### • ORGANIC IMPURITIES

**Mobile phase:** Butyl chloride, tetrahydrofuran, methanol, glacial acetic acid, and water (890: 56: 28: 24: 0.4)

**Diluent:** Butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (81.5: 10: 8: 0.5)

**Standard solution:** 40 μg/mL of USP Hydrocortisone RS in *Diluent*. [NOTE—Sonicate for 5 min.]

**Sample solution:** 2 mg/mL of Hydrocortisone in *Diluent*. [NOTE—Sonicate for 5 min.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L3

**Flow rate:** 1.5 mL/min

**Injection size:** 5 μL

**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 5%**Analysis****Samples:** *Standard solution*, *Sample solution*, and *Diluent*. [NOTE—Ignores artifact peaks.]

Calculate the percentage of impurities in the portion of Hydrocortisone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = individual impurity peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Hydrocortisone in the *Sample solution* (mg/mL)**Acceptance criteria****Individual impurities:** NMT 0.5%**Total impurities:** NMT 2.0%**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S)  
**Sample solution:** 10 mg/mL, in dioxane  
**Acceptance criteria:** +150° to +156°
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Hydrocortisone RS

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**Hydrocortisone Cream**

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**DEFINITION**Hydrocortisone Cream is Hydrocortisone in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>).**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Sample solution:** Transfer a portion of Cream, equivalent to 5 mg of hydrocortisone, to a flask. Add 5 mL of alcohol, and heat on a steam bath for 5 min, with frequent shaking. Cool, and filter. Use the filtrate.**Analysis:** Proceed as directed in the chapter.**Acceptance criteria:** Meets the requirements**ASSAY**

- **PROCEDURE**

**Diluent:** Dilute methanol (1 in 2)**Mobile phase:** Acetonitrile and water (25:75)**Standard stock solution:** 500 µg/mL of USP Hydrocortisone RS in methanol**Standard solution:** 50 µg/mL of USP Hydrocortisone RS prepared by mixing *Standard stock solution* and *Diluent* (1:9). [NOTE—If methanol is used in the final dilution of the *Sample solution*, similarly use methanol instead of aqueous methanol in the final dilution of the *Standard solution*.]**Sample solution:** Transfer a quantity of Cream, nominally equivalent to 10 mg of hydrocortisone, to a 150-mL beaker. Add 40 mL of methanol, and heat on a steam bath while stirring to melt and disperse the Cream. Cool to room temperature, and filter through glass wool into a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of methanol, com-bining the filtrates in the 100-mL volumetric flask. Add methanol to volume, and mix. Quantitatively dilute one volume of this solution with an equal volume of water, and pass through a membrane filter of 5-µm pore size. If precipitation occurs on dilution with water and the solution is still cloudy after filtration, dilute the initial *Sample solution* with methanol instead of water. Pass this solution through a membrane filter of 5-µm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; packing L1**Injection volume:** 10–25 µL**System suitability**[NOTE—Adjust the composition of the *Mobile phase* so that the retention time of hydrocortisone is about 10 min.]**Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 3.0% for five replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (µg/mL) $C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (µg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Hydrocortisone RS

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**Hydrocortisone Gel**

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» Hydrocortisone Gel is Hydrocortisone in a suitable hydroalcoholic gel base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>).**Packaging and storage**—Preserve in tight containers.**USP Reference standards** (11)—

USP Hydrocortisone RS

**Identification**—Proceed with Gel as directed under *Hydrocortisone Cream*. The specified result is obtained.**Minimum fill** (755): meets the requirements.**Assay**—**Mobile solvent**—Prepare a mixture of 2 volumes of methanol, 2 volumes of acetonitrile, and 6 volumes of water.

**Standard preparation**—Dissolve a suitable quantity of USP Hydrocortisone RS, accurately weighed, in alcohol and dichloromethane (75:25) to obtain a solution having a known concentration of about 0.1 mg per mL. Dilute this solution with alcohol to a concentration of 10 µg per mL.

**Assay preparation**—Weigh accurately an aliquot of Gel, equivalent to about 10 mg of hydrocortisone, and dilute with alcohol and dichloromethane (75:25) to obtain a solution having a concentration of about 0.1 mg per mL. Dilute this solution with alcohol to a concentration of 10 µg per mL.

**Procedure**—Introduce equal volumes (between 5 µL and 15 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* <621>) operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the *Standard preparation* is about 0.6 full-scale. Typically, the apparatus is fitted with a 4-mm × 30-cm column that contains packing L1 and is equipped with an UV detector capable of monitoring absorption at 254 nm, and a suitable recorder, and is capable of operating at a column pressure of up to 6000 psi. In a suitable chromatogram, the coefficient of variation for five replicate injections of the *Standard preparation* is not more than 3.0%. Determine the ratios of the peak heights, at equivalent retention times, obtained from the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) in the portion of Gel taken by the formula:

$$C(H_U / H_S)$$

in which C is the concentration, in µg per mL, of USP Hydrocortisone RS in the *Standard preparation*; and  $H_U$  and  $H_S$  are the peak heights of the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone Lotion

### DEFINITION

Hydrocortisone Lotion is Hydrocortisone in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST <201>

**Standard solution:** 500 µg/mL of USP Hydrocortisone RS in methanol

**Sample solution:** Transfer a quantity of Lotion, nominally equivalent to 5 mg of hydrocortisone, to a separator containing 10 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Filter the methylene chloride extract onto a suitable chromatographic column that has been packed with 2 g of activated magnesium silicate. Wash the column with 25 mL of methylene chloride with the aid of slight air pressure, discarding the washings, and elute the hydrocortisone with 10 mL of methanol.

#### Chromatographic system

**Developing solvent system:** Chloroform, methanol, and water (180:15:1)

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, and water (21:21:58)

**Standard stock solution:** 0.1 mg/mL of USP Hydrocortisone RS in alcohol

**Standard solution:** 0.05 mg/mL of USP Hydrocortisone RS from *Standard stock solution* in water

**System suitability stock solution:** 0.05 mg/mL of propylparaben in alcohol

**System suitability solution:** 5 µg/mL of propylparaben and 45 µg/mL of USP Hydrocortisone RS, prepared by diluting 1 mL of the *System suitability stock solution* with *Standard solution* to 10 mL

**Sample stock solution:** Nominally 0.1 mg/mL of hydrocortisone, prepared as follows. Shake a 100-mL portion of Lotion to ensure homogeneity, and allow to stand until the entrapped air rises. Invert carefully, and transfer a quantity of this Lotion, freshly mixed but free from air bubbles, nominally equivalent to 10 mg of hydrocortisone, to a 40-mL beaker, and add 30 mL of alcohol. Warm gently until the Lotion is dispersed, and cool to room temperature. Filter the mixture into a 100-mL volumetric flask through a pledget of cotton previously moistened with alcohol. Rinse the beaker with two 20-mL portions of alcohol, and collect the washings in the same volumetric flask. Dilute with alcohol to volume.

**Sample solution:** Nominally 0.05 mg/mL of hydrocortisone from *Sample stock solution* in water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between propylparaben and hydrocortisone, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates for hydrocortisone, *System suitability solution*

**Tailing factor:** NMT 1.2 for hydrocortisone, *System suitability solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **MINIMUM FILL <755>:** Meets the requirements

### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>:** It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS <11>**  
USP Hydrocortisone RS



## Hydrocortisone Ointment

### DEFINITION

Hydrocortisone Ointment is Hydrocortisone in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Sample solution:** Transfer a portion of Ointment, equivalent to 5 mg of hydrocortisone, to a flask. Add 10 mL of methanol, and heat on a steam bath for 5 min with frequent shaking. Cool to solidify the ointment base, and filter. Use the filtrate.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Diluent:** Dilute alcohol (1 in 2)

**Mobile phase:** Acetonitrile and water (25:75)

**Standard stock solution:** 500  $\mu\text{g/mL}$  of USP Hydrocortisone RS in alcohol

**Standard solution:** 50  $\mu\text{g/mL}$  of USP Hydrocortisone RS prepared by mixing *Standard stock solution* and *Diluent* (1:9). [NOTE—If alcohol is used in the final dilution of the *Sample solution*, similarly use alcohol instead of aqueous alcohol in the final dilution of the *Standard solution*.]

**Sample solution:** Transfer a quantity of Ointment, nominally equivalent to 10 mg of hydrocortisone, to a 150-mL beaker. Add 40 mL of alcohol, and heat on a steam bath while stirring to melt and disperse the Ointment. Cool to room temperature, and filter through glass wool into a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the filtrates in the 100-mL volumetric flask. Add alcohol to volume, and mix. Quantitatively dilute one volume of this solution with an equal volume of water, and pass through a membrane filter of 5- $\mu\text{m}$  pore size. If precipitation occurs on dilution with water, and the solution is still cloudy after filtration, dilute the initial *Sample solution* with alcohol instead of water. Pass this solution through a membrane filter of 5- $\mu\text{m}$  pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Injection volume:** 10–25  $\mu\text{L}$

#### System suitability

[NOTE—Adjust the composition of the *Mobile phase* so that the retention time of hydrocortisone is about 10 min.]

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0% for 5 replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of hydrocortisone in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED ORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Hydrocortisone RS

## Hydrocortisone Injectable Suspension

» Hydrocortisone Injectable Suspension is a sterile suspension of Hydrocortisone in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ).

**Packaging and storage—**Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Hydrocortisone RS

USP Endotoxin RS

**Identification—**It responds to the *Identification* test under *Hydrocortisone Lotion*.

**Bacterial endotoxins (85)—**It contains not more than 1.25 USP Endotoxin Units per mg of hydrocortisone.

**pH (791):** between 5.0 and 7.0.

**Other requirements—**It meets the requirements under *Injections* (1).

#### Assay—

**Standard preparation—**Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Hydrocortisone RS.

**Assay preparation—**Transfer to a separator an accurately weighed quantity of Injectable Suspension, equivalent to about 50 mg of hydrocortisone, using a total of 25 mL of water to effect the transfer. Extract with four 40-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 200-mL volumetric flask. Add chloroform to volume, and mix. Pipet 20 mL of this solution into a 100-mL volumetric flask, add chloroform to volume, and mix. Pipet 10 mL of the resulting solution into a glass-stoppered, 100-mL conical flask, evaporate on a steam bath just to dryness, cool, and dissolve the residue in 50.0 mL of alcohol.

**Procedure—**Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{21}H_{30}O_5$  in the portion of Injectable Suspension taken by the formula:

$$5C(A_U / A_S).$$

## Hydrocortisone Rectal Suspension

» Hydrocortisone Rectal Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydrocortisone RS

**Identification**, *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution*—Use the *Assay preparation*, except to omit addition of the *Internal standard solution*.

**pH** (791): between 5.5 and 7.0.

**Assay**—

*Mobile phase*—Mix 55 mL of a solution of water in methanol (5 in 100) with 1.0 mL of glacial acetic acid, dilute with water-washed 1,2-dichloroethane to 1000 mL, and mix. Degass before using. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Dissolve 200 mg of acetaminophen in 4 mL of methanol, dilute with water-washed 1,2-dichloroethane to 200 mL, and mix. Keep the solution tightly stoppered and protected from light.

*Standard preparation*—Accurately weigh about 8 mg of USP Hydrocortisone RS, add 4 mL of methanol and 4.0 mL of *Internal standard solution*, dilute with chloroform to 100.0 mL, and mix to obtain a solution having a known concentration of about 0.08 mg of USP Hydrocortisone RS per mL.

*Assay preparation*—Transfer an accurately weighed quantity of Rectal Suspension, equivalent to about 8 mg of hydrocortisone, to a separator. Extract with four 20-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Add 4 mL of methanol and 4.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix. Pass the extract through a 0.5- $\mu$ m porosity polytetrafluoroethylene filter, discarding the first 20 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.3 for acetaminophen and 1.0 for hydrocortisone; the resolution,  $R$ , between the analyte and internal standard is not less than 2.5; the column efficiency determined from the analyte peak is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Rectal Suspension taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone Tablets

### DEFINITION

Hydrocortisone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197M)

**Sample:** Powder a number of Tablets, equivalent to 50 mg of hydrocortisone, and digest with 15 mL of solvent hexane for 15 min. Decant the solvent hexane as completely as possible, and extract the residue first with 10 mL of solvent hexane, then with 10 mL of peroxide-free ether in the same manner as before, and discard the extracts. Digest the final residue with 25 mL of dehydrated alcohol for 15 min with frequent agitation. Filter, and evaporate the alcohol extract on a steam bath to dryness. Use the residue.

**Acceptance criteria:** Meet the requirements

### ASSAY

#### • PROCEDURE

**Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

**Internal standard solution:** 0.06 mg/mL of USP Prednisone RS in water-saturated chloroform

**Standard solution:** 0.1 mg/mL of USP Hydrocortisone RS in *Internal standard solution*

**Sample solution:** Nominally 0.1 mg/mL of hydrocortisone from NLT 10 finely powdered Tablets, prepared as follows. Transfer a portion of the powder, equivalent to 5 mg of hydrocortisone, to a suitable container, and add 50.0 mL of the *Internal standard solution*. Shake vigorously for 30 min, and centrifuge a portion. Use the clear supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L3

**Flow rate:** 0.9 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between hydrocortisone and prednisone

**Relative standard deviation:** NMT 2.0% for four replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times 100$$

$R_U$  = peak response ratio of hydrocortisone to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of hydrocortisone to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION 〈711〉

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Hydrocortisone RS at a known concentration in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with Medium if necessary.

### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 〈851〉.)

Mode: UV

Analytical wavelength: Maximum absorbance at about 248 nm

### Analysis

Samples: Standard solution and Sample solution  
Determine the amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) dissolved.

Tolerances: NLT 70% (Q) of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905)

#### Procedure for content uniformity

Mobile phase, Internal standard solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Sample solution: Nominally 0.1 mg/mL of hydrocortisone, prepared as follows. Transfer 1 Tablet to a suitable container, and add 0.3 mL of water directly onto the Tablet. Allow the Tablet to stand for about 5 min. Shake the container to break up the Tablet, and sonicate briefly to ensure complete disintegration. Add a few small glass beads and 50.0 mL of the Internal standard solution to the container. Shake the container for about 30 min. Dilute an accurately measured volume of the clear supernatant with a known, accurately measured volume of the Internal standard solution to obtain the desired concentration. Shake the contents of the container to mix.

### Analysis

Samples: Standard solution and Sample solution  
Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of hydrocortisone to the internal standard from the Sample solution

$R_S$  = peak response ratio of hydrocortisone to the internal standard from the Standard solution

$C_S$  = concentration of USP Hydrocortisone RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of hydrocortisone in the Sample solution (mg/mL)

Acceptance criteria: Meet the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** 〈11〉

USP Hydrocortisone RS

USP Prednisone RS

## Hydrocortisone and Acetic Acid Otic Solution

### DEFINITION

Hydrocortisone and Acetic Acid Otic Solution is a solution of Hydrocortisone and Glacial Acetic Acid in a suitable non-aqueous solvent. It contains NLT 90.0% and NMT

120.0% of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ), and NLT 85.0% and NMT 130.0% of the labeled amount of acetic acid ( $C_2H_4O_2$ ).

## IDENTIFICATION

### • A.

Analysis: Dilute 5 mL of Otic Solution with 10 mL of water, and adjust with 1 N sodium hydroxide to a pH of about 7. Add ferric chloride TS.

Acceptance criteria: A deep red color is produced, and it is destroyed by the addition of hydrochloric acid.

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, both relative to the internal standard, as obtained in the Assay for Acetic Acid.

• **C.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay for Hydrocortisone.

## ASSAY

### • ACETIC ACID

Internal standard solution: Dilute 2.0 mL of anisole with methanol to 100 mL.

Standard stock solution: 20 mg/mL of glacial acetic acid in methanol

Standard solution: 10 mg/mL of glacial acetic acid in methanol, prepared as follows. Transfer a sufficient volume of the Standard stock solution to a volumetric flask of suitable size, add 20% of the flask volume of the Internal standard solution, and dilute with methanol to volume.

Sample solution: Nominally 10 mg/mL of glacial acetic acid, prepared as follows. Transfer a sufficient volume of Otic Solution to a volumetric flask of suitable size, add 20% of the flask volume of the Internal standard solution, and dilute with methanol to volume.

### Chromatographic system

(See *Chromatography* 〈621〉, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 20% liquid phase G35 on support STA

Carrier gas: Nitrogen

Flow rate: 25 mL/min

Temperatures

Injection port: 180°

Detector: 220°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
115	0	115	12
115	35	190	3

Injection volume: 4 µL

### System suitability

Sample: Standard solution

[NOTE—The relative retention times for anisole and acetic acid are 1.0 and 1.5, respectively.]

### Suitability requirements

Resolution: NLT 1.5 between anisole and acetic acid

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetic acid ( $C_2H_4O_2$ ) in the portion of Otic Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of acetic acid to the internal standard from the Sample solution

- $R_S$  = peak response ratio of acetic acid to the internal standard from the *Standard solution*  
 $C_S$  = concentration of glacial acetic acid in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acetic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 85.0%–130.0%

#### • HYDROCORTISONE

**Diluent:** Dilute alcohol (1 in 2)  
**Mobile phase:** Acetonitrile and water (30:70)  
**Standard solution:** 0.5 mg/mL of USP Hydrocortisone RS in *Diluent*  
**Sample solution:** Nominally equivalent to 0.5 mg/mL of hydrocortisone from Otic Solution in *Diluent*  
**Chromatographic system**  
 (See *Chromatography* <621>, *System Suitability*).  
**Mode:** LC  
**Detector:** UV 254 nm  
**Column:** 4-mm × 30-cm; packing L1  
**Flow rate:** 2 mL/min  
**Injection volume:** 20 µL  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Relative standard deviation:** NMT 2.0% for four replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–120.0%

#### SPECIFIC TESTS

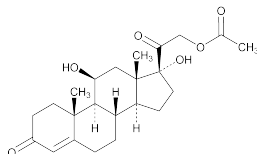
##### • pH <791>

**Sample solution:** Otic Solution and water (1:1)  
**Acceptance criteria:** 2.0–4.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** <11>  
 USP Hydrocortisone RS

## Hydrocortisone Acetate



$C_{23}H_{32}O_6$  404.50  
 Pregn-4-ene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11 $\beta$ )-;  
 Cortisol 21-acetate [50-03-3].

#### DEFINITION

Hydrocortisone Acetate contains NLT 97.0% and NMT 102.0% of  $C_{23}H_{32}O_6$ , calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** <197M>
- **B. ULTRAVIOLET ABSORPTION** <197U>  
**Analytical wavelength:** 242 nm  
**Sample solution:** 10 µg/mL in methanol  
**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 2.5%.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)  
**Standard solution:** 0.10 mg/mL of USP Hydrocortisone Acetate RS in *Mobile phase*  
**Sample solution:** 0.10 mg/mL of Hydrocortisone Acetate in *Mobile phase*  
**Chromatographic system**  
 (See *Chromatography* <621>, *System Suitability*).  
**Mode:** LC  
**Detector:** UV 254 nm  
**Column:** 3.9-mm × 30-cm; 10-µm packing L3  
**Flow rate:** 1 mL/min  
**Injection size:** 10 µL  
**System suitability**  
**Sample:** *Standard solution*  
**Suitability requirements**  
**Tailing factor:** NMT 2.0  
**Relative standard deviation:** NMT 2.0%  
**Analysis**  
**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in the portion of Hydrocortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of hydrocortisone acetate from the *Sample solution*  
 $r_S$  = peak response of hydrocortisone acetate from the *Standard solution*  
 $C_S$  = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Hydrocortisone Acetate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** <281>  
**Sample:** 100 mg  
**Acceptance criteria:** NMT 0.5%
- **ORGANIC IMPURITIES**  
**Solution A:** Acetonitrile and water (20:80)  
**Solution B:** Acetonitrile and water (70:30)  
**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
25	10	90
30	10	90
35	90	10
40	90	10

**Diluent:** Acetonitrile, glacial acetic acid, and water (700:1:300)  
**Standard solution:** 5 µg/mL of USP Hydrocortisone Acetate RS in *Diluent*  
**Sample solution:** 1 mg/mL of Hydrocortisone Acetate in *Diluent*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 3-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Hydrocortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Hydrocortisone Acetate in the *Sample solution* (mg/mL)**Acceptance criteria****Individual impurities:** NMT 1.0%**Total impurities:** NMT 2.0%**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* <781S>**Sample solution:** 10 mg/mL in dioxane**Acceptance criteria:** Between +158° and +167° at 20°• **LOSS ON DRYING** <731>: Dry a sample in vacuum at 60° for 3 h: it loses NMT 1.0% of its weight.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** <11>

USP Hydrocortisone Acetate RS

Pregn-4-ene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-.

C<sub>23</sub>H<sub>32</sub>O<sub>6</sub> 404.50

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**Hydrocortisone Acetate Cream**

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**DEFINITION**Hydrocortisone Acetate Cream is Hydrocortisone Acetate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate (C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>).**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>**Standard solution:** 250 μg/mL of USP Hydrocortisone Acetate RS in methanol**Sample solution:** To 1 g of Cream add 40.0 mL of a 35% solution of acetonitrile in methanol, and shake until dissolved. To 20.0 mL of this solution add 10.0 mL of isooctane, and mix. Allow the layers to separate, and use the bottom layer.**Developing solvent system:** Ethyl acetate, toluene, and acetone (140:40:13)**Analysis:** Heat the prepared chromatographic plate at 105° for 10 min, and cool. Develop in the *Developing solvent system* in a paper-lined chromatographic chamber equilibrated in an atmosphere of ammonia vapor.**Acceptance criteria:** Meets the requirements**ASSAY**• **PROCEDURE****Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)**Standard solution:** 0.10 mg/mL of USP Hydrocortisone Acetate RS in *Mobile phase***Sample solution:** Transfer a quantity of Cream, nominally equivalent to 25 mg of hydrocortisone acetate, to a suitable container, add 100.0 mL of tetrahydrofuran, and shake until the Cream dissolves. Transfer 10.0 mL of the resulting solution to another container, and add 15.0 mL of *Mobile phase*.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; 10-μm packing L3**Flow rate:** 1 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of hydrocortisone acetate (C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **MINIMUM FILL** <755>: Meets the requirements**SPECIFIC TESTS**• **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** <11>

USP Hydrocortisone Acetate RS

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**Hydrocortisone Acetate Lotion**

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» Hydrocortisone Acetate Lotion is Hydrocortisone Acetate in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate (C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>).**Packaging and storage**—Preserve in tight containers.**USP Reference standards** <11>—

USP Hydrocortisone Acetate RS

**Identification**—It responds to the *Identification* test under *Hydrocortisone Acetate Ointment*.

**Minimum fill** (755): meets the requirements.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Hydrocortisone Acetate RS in water-saturated chloroform to obtain a solution having a known concentration of about 0.10 mg per mL.

*Assay preparation*—Transfer an accurately weighed quantity of Lotion, equivalent to about 2.5 mg of hydrocortisone acetate, to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 minutes. Centrifuge, and use the clear, lower chloroform layer.

*Procedure*—Introduce equal volumes of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph fitted with a 254-nm detector. Typically the apparatus is fitted with a 4-mm × 30-cm column containing packing L3 and operated at room temperature. Six replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0%. Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in the portion of Lotion taken by the formula:

$$25C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the hydrocortisone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone Acetate Ointment

» Hydrocortisone Acetate Ointment is Hydrocortisone Acetate in a suitable ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{23}H_{32}O_6$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Hydrocortisone Acetate RS

**Identification**—Transfer a quantity of Ointment, equivalent to about 5 mg of hydrocortisone acetate, to a flask, add 5 mL of methanol, and heat on a steam bath for 5 minutes with frequent mixing. Cool to solidify the ointment base, and filter. Using the filtrate as the test solution, proceed as directed under *Thin-layer Chromatographic Identification Test* (201). Locate the spots by spraying the dried plate with a 70% methanolic sulfuric acid solution. Heat the plate for 20 to 30 minutes at 90°, allow to cool, and view under long-wavelength UV light: the  $R_f$  value and fluorescence of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—Proceed with Ointment as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

## Hydrocortisone Acetate Ophthalmic Ointment

» Hydrocortisone Acetate Ophthalmic Ointment is Hydrocortisone Acetate in a suitable ophthalmic ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of total steroids, calculated as  $C_{23}H_{32}O_6$ . It is sterile.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**USP Reference standards** (11)—

USP Hydrocortisone Acetate RS

**Identification**—It responds to the *Identification* test under *Hydrocortisone Acetate Ointment*.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Particulate matter**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

**Assay—**

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Hydrocortisone Acetate RS.

*Assay preparation*—Transfer to a suitable flask an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 10 mg of hydrocortisone acetate, and add 30 mL of alcohol. Heat on a steam bath to melt the ointment base, and mix. Cool to solidify the ointment base, and filter the alcohol solution into a 100-mL volumetric flask. Repeat the extraction with three 20-mL portions of alcohol, add alcohol to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, add alcohol to volume, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.

*Procedure*—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_6$  in the portion of Ophthalmic Ointment taken by the formula:

$$C(A_u / A_s)$$

in which the terms are as defined therein.

## Hydrocortisone Acetate Injectable Suspension

» Hydrocortisone Acetate Injectable Suspension is a sterile suspension of Hydrocortisone Acetate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of total steroids, calculated as hydrocortisone acetate ( $C_{23}H_{32}O_6$ ). It may contain suitable buffers and suspending agents.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Hydrocortisone Acetate RS

**Identification**—Extract a volume of Injectable Suspension, equivalent to about 50 mg of hydrocortisone acetate, with two 10-mL portions of peroxide-free ether, and discard the ether extracts. Filter with suction, wash with small portions

of water, and dry at 105° for 1 hour: the hydrocortisone acetate so obtained responds to *Identification test A* under *Hydrocortisone Acetate*.

**pH** (791): between 5.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Hydrocortisone Acetate RS.

*Assay preparation*—Transfer to a separator an accurately measured volume of Injectable Suspension, equivalent to about 50 mg of hydrocortisone acetate, and dilute with water to about 15 mL. Extract with four 25-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 250-mL volumetric flask. Add chloroform to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, add chloroform to volume, and mix. Pipet 10 mL of the resulting solution into a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol.

*Procedure*—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in each mL of the Injectable Suspension taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which *V* is the volume, in mL, of Injectable Suspension taken; and the other terms are as defined therein.

## Hydrocortisone Acetate Ophthalmic Suspension

» Hydrocortisone Acetate Ophthalmic Suspension is a sterile suspension of Hydrocortisone Acetate in an aqueous medium containing a suitable antimicrobial agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of total steroids, calculated as hydrocortisone acetate ( $C_{23}H_{32}O_6$ ). It may contain suitable buffers and suspending agents.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydrocortisone Acetate RS

**Identification**—Evaporate 50 mL of the *Assay preparation* prepared as directed in the *Assay* on a steam bath just to dryness, dissolve the residue in 1 mL of chloroform, and proceed as directed under *Thin-layer Chromatographic Identification Test* (201).

**Sterility** (71): meets the requirements.

**pH** (791): between 6.0 and 8.0.

**Assay**—Proceed as directed in the *Assay* under *Hydrocortisone Acetate Injectable Suspension*, except to read Ophthalmic Suspension in place of Injectable Suspension.

## Hydrocortisone Butyrate

$C_{25}H_{36}O_6$  432.55  
Pregn-4-ene-3,20-dione, 11,21-dihydroxy-17-  
(1-oxobutoxy)-, (11 $\beta$ )-;  
Cortisol 17-butyrate;

11 $\beta$ ,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butyrate  
[13609-67-1].

### DEFINITION

Hydrocortisone Butyrate contains NLT 97.0% and NMT 102.0% of hydrocortisone butyrate ( $C_{25}H_{36}O_6$ ), calculated on the dried basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 10  $\mu$ g/mL in methanol

Analytical wavelength: 242 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile, glacial acetic acid, and water (76:1:124)

**Diluent A:** Tetrahydrofuran and glacial acetic acid (1000:1)

**Diluent B:** Methanol, water, and glacial acetic acid (500:500:1)

**System suitability stock solution:** 0.1 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent A*

**System suitability solution:** 0.02 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent B*, from *System suitability stock solution*

**Standard stock solution:** 0.1 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent A*

**Standard solution:** 0.02 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent B*, from *Standard stock solution*

**Sample stock solution:** 0.1 mg/mL of Hydrocortisone Butyrate in *Diluent A*

**Sample solution:** 0.02 mg/mL of Hydrocortisone Butyrate in *Diluent B*, from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.0-mm  $\times$  10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 5  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for propyl 4-hydroxybenzoate and hydrocortisone butyrate are 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4.0 between propyl 4-hydroxybenzoate and hydrocortisone butyrate, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.6, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of hydrocortisone butyrate ( $C_{25}H_{36}O_6$ ) in the portion of Hydrocortisone Butyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone Butyrate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Hydrocortisone Butyrate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

## IMPURITIES

### • ORGANIC IMPURITIES

**Solution A:** 1 g of monobasic potassium phosphate in 1000 mL of water, adjusted with 45% potassium hydroxide to a pH of 5.5

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
12.5	35	65
15.5	35	65
20.5	80	20
22.5	80	20

**Diluent:** Acetonitrile and Solution A (80:20)

**Standard solution:** 0.5 mg/mL of USP Hydrocortisone Butyrate RS in Diluent

**Sample solution:** 1 mg/mL of Hydrocortisone Butyrate in Diluent

### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 10-cm; 3-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 5 μL

### System suitability

**Samples:** Standard solution and Sample solution

### Suitability requirements

**Resolution:** NLT 1.0 between hydrocortisone butyrate and any impurity

**Column efficiency:** NLT 10,000 theoretical plates

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Hydrocortisone Butyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of hydrocortisone butyrate from the Standard solution

$C_S$  = concentration of USP Hydrocortisone Butyrate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Hydrocortisone Butyrate in the Sample solution (mg/mL)

### Acceptance criteria

Disregard any peak having a percentage of 0.05% or less.

**Any individual impurity:** NMT 1.0%

**Total impurities:** NMT 2.0%

## SPECIFIC TESTS

### • OPTICAL ROTATION, Specific Rotation <781S>

**Sample solution:** 10 mg/mL, in chloroform

**Acceptance criteria:** +47° to +54° at 20°

### • LOSS ON DRYING <731>

**Sample:** Dry Hydrocortisone Butyrate under vacuum at 78° for 3 h.

**Acceptance criteria:** NMT 1.0%

### • COMPLETENESS OF SOLUTION <641>

**Sample solution:** 1 g in 10 mL of dichloromethane

**Acceptance criteria:** The Sample solution is clear.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## • USP REFERENCE STANDARDS <11>

USP Hydrocortisone Butyrate RS

## Hydrocortisone Butyrate Cream

### DEFINITION

Hydrocortisone Butyrate Cream is Hydrocortisone Butyrate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone butyrate ( $C_{25}H_{36}O_6$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, glacial acetic acid, and water (76:1:124)

**Diluent A:** Tetrahydrofuran and glacial acetic acid (1000:1)

**Diluent B:** Methanol, water, and glacial acetic acid (500:500:1)

**System suitability stock solution:** 0.1 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in Diluent A

**System suitability solution:** 0.02 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in Diluent B, from System suitability stock solution

**Standard stock solution:** 0.1 mg/mL of USP Hydrocortisone Butyrate RS in Diluent A

**Standard solution:** 0.02 mg/mL of USP Hydrocortisone Butyrate RS in Diluent B, from Standard stock solution

**Sample solution:** Nominally 0.02 mg/mL of hydrocortisone butyrate, prepared as follows. Transfer a sufficient quantity of Cream to a volumetric flask of suitable size, add 20% of the flask volume of Diluent A, shake by mechanical means for 30 min, and dilute with Diluent B to volume.

### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.0-mm × 10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 5 μL

### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—The relative retention times for propyl 4-hydroxybenzoate and hydrocortisone butyrate are 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 4.0 between propyl 4-hydroxybenzoate and hydrocortisone butyrate, System suitability solution

**Column efficiency:** NLT 4000 theoretical plates, Standard solution

**Tailing factor:** NMT 1.6, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of hydrocortisone butyrate ( $C_{25}H_{36}O_6$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution



- $C_s$  = concentration of USP Hydrocortisone Butyrate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of hydrocortisone butyrate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PH** (791): 3.5–4.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Hydrocortisone Butyrate RS

**Hydrocortisone Hemisuccinate**

$C_{25}H_{34}O_8 \cdot H_2O$  480.55  
 Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, (11 $\beta$ )-, monohydrate.  
 Cortisol 21-(hydrogen succinate) monohydrate  
 [83784-20-7].  
 Anhydrous 462.54 [2203-97-6].

» Hydrocortisone Hemisuccinate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{25}H_{34}O_8$ , calculated on the dried basis. It contains one molecule of water of hydration or is anhydrous.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether it is hydrous or anhydrous.

**USP Reference standards** (11)—  
 USP Hydrocortisone Hemisuccinate RS  
 USP Fluorometholone RS

**Identification**—

- A:** *Infrared Absorption* (197M).  
**B:** *Ultraviolet Absorption* (197U)—  
*Solution:* 20  $\mu$ g per mL.  
*Medium:* alcohol.

Absorptivities at 242 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +124° and +134°.

*Test solution:* 10 mg per mL, in acetone.

**Loss on drying** (731)—Dry it at 105° for 3 hours: the anhydrous form loses not more than 1.0% of its weight, and the hydrous form loses not more than 4.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (700:285:15). Add 3.0 mL of glacial acetic acid per Liter of this solution. Mix thoroughly. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Diluting solution*—Prepare a mixture of water, acetonitrile, tetrahydrofuran, and glacial acetic acid (500:250:250:1). Mix thoroughly.

*Standard solution*—Dissolve an accurately weighed quantity of USP Hydrocortisone Hemisuccinate RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary,

with *Diluting solution* to obtain a solution having a known concentration of about 6.6  $\mu$ g per mL.

*Test solution*—Transfer about 6.6 mg of Hydrocortisone Hemisuccinate, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix. [NOTE—Samples should be maintained at 5° or colder during analysis.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.

*Procedure*—Inject a volume (about 15  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Hydrocortisone Hemisuccinate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found. Disregard any peak representing less than 0.05%.

**Assay**—

*Internal standard solution*—Prepare a solution of USP Fluorometholone RS in tetrahydrofuran containing about 3 mg per mL.

*Mobile phase*—Prepare a filtered mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer an accurately weighed amount of USP Hydrocortisone Hemisuccinate RS to a suitable container to obtain a solution containing 0.6 mg per mL. Add an accurately measured volume of *Internal standard solution* so that the *Standard preparation* contains 10% *Internal standard solution*. Dilute with chloroform containing 3% glacial acetic acid to volume.

*Assay preparation*—Transfer about 30 mg of Hydrocortisone Hemisuccinate, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with chloroform containing 3% glacial acetic acid to volume.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between hydrocortisone hemisuccinate and the internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 6  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{25}H_{34}O_8$  in the portion of Hydrocortisone Hemisuccinate taken by the formula:

$$50C(R_u / R_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Hydrocortisone Hemisuccinate RS in the *Standard preparation*; and  $R_u$  and  $R_s$  are the peak area ratios of hydrocortisone hemisuccinate to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone Sodium Phosphate

$C_{21}H_{29}Na_2O_8P$  486.40

Pregn-4-ene-3,20-dione, 11,17-dihydroxy-21-(phosphonoxy)-, disodium salt, (11 $\beta$ )-.

Cortisol 21-(disodium phosphate) [6000-74-4].

» Hydrocortisone Sodium Phosphate contains not less than 96.0 percent and not more than 102.0 percent of  $C_{21}H_{29}Na_2O_8P$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydrocortisone RS

USP Hydrocortisone Phosphate Triethylamine RS

$C_{21}H_{31}O_8P \cdot C_6H_{15}N$  543.64

**Identification**—

**A:** Evaporate 15 mL of a methylene chloride solution of it, prepared as directed under *Procedure* in the *Assay*, on a steam bath to dryness, and dissolve the residue in 1 mL of methylene chloride. Proceed as directed in *Identification* test B under *Hydrocortisone Sodium Phosphate Injection*, beginning with "Apply 5  $\mu$ L of this solution."

**B:** The residue from the ignition of about 20 mg of it responds to the tests for *Phosphate* (191) and for *Sodium* (191).

**Phosphate ions**—

*Standard phosphate solution*—Dissolve 143.3 mg of dried monobasic potassium phosphate,  $KH_2PO_4$ , in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate ( $PO_4$ ) in each mL.

*Phosphate reagent A*—Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.

*Phosphate reagent B*—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.

*Procedure*—Dissolve about 50 mg of Hydrocortisone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25 mL, mix, and allow to stand at room temperature for 30 minutes. Similarly and concomitantly, prepare a standard solution, using 5.0 mL of *Standard phosphate solution* instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the standard solution. The limit is 1.0% of phosphate ( $PO_4$ ).

**Chloride (as NaCl)**—Dissolve about 3 g, accurately weighed, in 75 mL of water, add 1 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a glass silver-silver chloride electrode system. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl. Not more than 1.00% of NaCl is found.

**Specific rotation, pH, and free hydrocortisone**—Place about 2.5 g in a tared 50-mL flask, and weigh accurately ( $W_U$ ). Add 25 mL of carbon dioxide-free water, and again weigh ( $W_S$ ). Calculate the quantity, in mg, of anhydrous hydrocortisone sodium phosphate in each g of solution taken by the formula:

$$1000W_U(1 - L/100) / W_S$$

in which  $W_U$  is the weight of Hydrocortisone Sodium Phosphate taken,  $L$  is the average percentage of *Loss on drying*, and  $W_S$  is the weight of the solution in carbon dioxide-free water. Use this as the *Test preparation* for the following tests.

*Specific rotation* (781S): between +121° and +129°, determined in a solution prepared by weighing accurately 5.0 mL of *Test preparation* and diluting with pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to 50.0 mL.

*pH* (791): between 7.5 and 10.5, in a solution prepared by diluting a portion of *Test preparation* with 9 volumes of carbon dioxide-free water.

*Free hydrocortisone*—Dilute 1 mL of *Test preparation* with carbon dioxide-free water to 100 mL. Pipet 5 mL of this solution into a glass-stoppered, 50-mL tube, add 25.0 mL of methylene chloride, insert the stopper, and mix by gentle shaking. Prepare a 1 in 500,000 solution of USP Hydrocortisone RS in methylene chloride. Similarly, shake 25 mL of this solution with 5 mL of water. Allow to stand until the methylene chloride layers are clear (about 5 minutes). Determine the absorbances of the methylene chloride solutions in 1-cm cells at 239 nm, with a suitable spectrophotometer, using methylene chloride as the blank. The absorbance of the *Test preparation* does not exceed that of the Standard solution (1.0%).

**Loss on drying** (731)—Dry it in vacuum at 80° for 5 hours: the average percentage weight loss for two determinations ( $L$ ) does not exceed 5.0%.

**Heavy metals, Method II** (231): 0.004%.

**Assay**—

*pH 9 buffer with magnesium*—Mix 3.1 g of boric acid and 500 mL of water in a 1-liter volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.

*Alkaline phosphatase solution*—Transfer 250 mg of alkaline phosphatase enzyme to a 25-mL volumetric flask, and dissolve by diluting with *pH 9 buffer with magnesium* to volume. Prepare this solution fresh daily.

*Standard preparation*—Dissolve about 50 mg of USP Hydrocortisone Phosphate Triethylamine RS, accurately weighed, in carbon dioxide-free water to make 25.0 mL.

*Assay preparation*—Weigh accurately, in g, 2.0 mL of the *Test preparation*, prepared as directed under *Specific rotation*, *pH*, and *Free hydrocortisone*, into a tared 100-mL volumetric flask, and dilute with carbon dioxide-free water that has been saturated with methylene chloride to volume. Pipet 10 mL of this solution into a 125-mL separator, and extract with two 25-mL portions of water-washed methylene chloride, discarding the washings.

*Procedure*—Weigh accurately 1.0 mL each of the *Standard preparation* ( $W_S$ ) and the *Assay preparation* ( $W_A$ ) into separate tared 100-mL volumetric flasks. To each flask, and to a similar flask containing 1.0 mL of water to provide a blank, add 1.0 mL of *Alkaline phosphatase solution* and then 50 mL of methylene chloride, and insert the stopper. Allow the flasks to stand at room temperature (not below 25°) for 2 hours with gentle mixing about every 15 minutes. Add 1 mL of dilute hydrochloric acid (1 in 10) to each flask, and mix gently. Add methylene chloride to each flask until the interfaces are at the 100-mL marks, and mix gently. Remove the aqueous layers by aspiration. Determine the absorbances of the methylene chloride solutions obtained from the *Standard preparation* and the *Assay preparation* at 239 nm, with a suitable spectrophotometer, using the methylene chloride solution blank to set the instrument. Calculate the percentage of  $C_{21}H_{29}Na_2O_8P$ , on the dried basis, taken by the formula:

$$100(A_U / A_S)(C_S / C_A)0.895(W_S / W_A)$$

in which  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively;  $C_A$  and  $C_S$  are the corresponding concentrations, in mg per mL, of those preparations; and 0.895 is the ratio of the molecular weight of hydrocortisone sodium phosphate to that of hydrocortisone phosphate triethylamine.

## Hydrocortisone Sodium Phosphate Injection

» Hydrocortisone Sodium Phosphate Injection is a sterile, buffered solution of Hydrocortisone Sodium Phosphate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

### USP Reference standards (11)—

USP Endotoxin RS

USP Hydrocortisone RS

USP Hydrocortisone Phosphate Triethylamine RS

$C_{21}H_{31}O_8P \cdot C_6H_{15}N$  543.64

### Identification—

**A:** *Ultraviolet Absorption* (197U)—

**Solution:** 20 µg per mL, USP Hydrocortisone Phosphate Triethylamine RS being used to prepare the Standard solution.

**Medium:** water.

**B:** Place 5 mL of the *Assay preparation*, obtained as directed in the *Assay*, in a glass-stoppered, 50-mL tube, and add 5 mL of a solution prepared by dissolving 50 mg of alkaline phosphatase enzyme in 50 mL of pH 9 buffer with magnesium prepared as directed in the *Assay* under *Hydrocortisone Sodium Phosphate*. Allow to stand at room temperature for 2 hours, with occasional mixing, and extract with 25 mL of methylene chloride. Evaporate 15 mL of the methylene chloride extract on a steam bath to dryness, and dissolve the residue in 0.5 mL of methylene chloride. Apply 5 µL of this solution and 5 µL of a solution of USP Hydrocortisone RS in methylene chloride containing 300 µg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a tank completely lined with filter paper, using a solvent system consisting of a mixture of 50 parts of chloroform, 50 parts of acetone, and 1 part of water, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, and dry. Spray the plate with dilute sulfuric acid (1 in 2), and heat at 105° until brown or black spots appear: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Bacterial endotoxins** (85)—It contains not more than 1.25 USP Endotoxin Units per mg of hydrocortisone.

**pH** (791): between 7.5 and 8.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Phenylhydrazine hydrochloride solution**—Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of dilute sulfuric acid (3 in 5), add 50 mL of isopropyl alcohol, and mix. Prepare this solution fresh daily.

**Standard preparation**—Dissolve a suitable quantity of USP Hydrocortisone Phosphate Triethylamine RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 110 µg per mL.

**Assay preparation**—Pipet a volume of Injection, equivalent to about 100 mg of hydrocortisone sodium phosphate, into a 100-mL volumetric flask, and dilute with water to volume.

Pipet 10 mL of this solution into a separator, wash the solution with two 25-mL portions of methylene chloride, and discard the washings. Transfer the aqueous layer to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Pipet 2 mL each of the *Standard preparation* and the *Assay preparation* into separate glass-stoppered, 50-mL conical flasks. To each flask, and to a similar flask containing 2.0 mL of water to provide a blank, add 10.0 mL of *Phenylhydrazine hydrochloride solution*, and mix. Place the flasks in a water bath maintained at a temperature of 60° for 2 hours, then cool the solutions to room temperature. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of  $C_{21}H_{30}O_5$  in each mL of the Injection taken by the formula:

$$0.667(C/V)(A_U/A_S)$$

in which 0.667 is the ratio of the molecular weight of hydrocortisone to that of hydrocortisone phosphate triethylamine; C is the concentration, in µg per mL, of USP Hydrocortisone Phosphate Triethylamine RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrocortisone Sodium Succinate

$C_{25}H_{33}NaO_8$  484.51  
Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-  
11,17-dihydroxy-, monosodium salt, (11β)-;  
Cortisol 21-(sodium succinate) [125-04-2].

### DEFINITION

Hydrocortisone Sodium Succinate contains NLT 97.0% and NMT 102.0% of total steroids, calculated as  $C_{25}H_{33}NaO_8$ , on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION

**Sample:** Transfer 100 mg of Hydrocortisone Sodium Succinate to a suitable container, and dissolve in 10 mL of water. In rapid succession, add 1 mL of 3 N hydrochloric acid, shake briefly, immediately decant the aqueous layer, and wash the precipitate with two additional 10-mL portions of water, each time removing the water by decanting. Remove as much of the water as possible, spread the precipitate in a suitable container, and dry under vacuum at 60° for 3 h.

**Acceptance criteria:** The IR spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydrocortisone Hemisuccinate RS.

#### • B. ULTRAVIOLET ABSORPTION (197U)

**Sample solution:** 20 µg/mL in methanol

**Analytical wavelength:** 242 nm

**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

#### • C. IDENTIFICATION TESTS—GENERAL, Sodium (191):

It meets the requirements of the flame test.

### ASSAY

#### • ASSAY FOR STEROIDS (351)

**Blue tetrazolium solution:** 5 mg/mL of blue tetrazolium in alcohol

**Tetramethylammonium hydroxide solution:** Tetramethylammonium hydroxide TS in alcohol (1 in 10)

**Standard preparation:** Prepare as directed in the chapter for the *Standard Preparation*, using USP Hydrocortisone Hemisuccinate RS, but dilute the solution with alcohol to a concentration of 12.5 µg/mL.

**Assay preparation:** 12.5 µg/mL of Hydrocortisone Sodium Succinate in alcohol

**Blank solution:** Alcohol

#### Analysis

**Samples:** *Standard preparation*, *Assay preparation*, and *Blank solution*

Transfer 20.0-mL aliquots of the *Samples* to separate glass-stoppered, 50-mL conical flasks. To each flask add 2.0 mL of *Blue tetrazolium solution*, mix, and add 4.0 mL of *Tetramethylammonium hydroxide solution*. Allow to stand in the dark for 90 min, add 1.0 mL of glacial acetic acid, and proceed as directed in the *Procedure*, beginning with "Concomitantly determine the absorbances..."

Calculate the percentage of total steroids, as  $C_{25}H_{33}NaO_8$ , in the portion of Hydrocortisone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Assay preparation*

$A_S$  = absorbance of the *Standard preparation*

$C_S$  = concentration of USP Hydrocortisone Hemisuccinate RS in the *Standard preparation* (µg/mL)

$C_U$  = concentration of hydrocortisone sodium succinate in the *Assay preparation* (µg/mL)

$M_{r1}$  = molecular weight of hydrocortisone sodium succinate, 484.51

$M_{r2}$  = molecular weight of hydrocortisone hemisuccinate, 462.53

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### SPECIFIC TESTS

##### • SODIUM CONTENT

**Sample:** 1 g

**Analysis:** Dissolve the *Sample*, with gentle heating, in 75 mL of glacial acetic acid. Add 20 mL of dioxane, then add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of sodium (Na).

**Acceptance criteria:** 4.60%–4.84% on the dried basis

##### • OPTICAL ROTATION, Specific Rotation (7815)

**Sample solution:** 10 mg/mL in alcohol

**Acceptance criteria:** +140° to +150°

##### • LOSS ON DRYING (731)

**Sample:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 2.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Hydrocortisone Hemisuccinate RS

## Hydrocortisone Sodium Succinate for Injection

#### DEFINITION

Hydrocortisone Sodium Succinate for Injection is a sterile mixture of Hydrocortisone Sodium Succinate and suitable buffers. It may be prepared from Hydrocortisone Sodium Succinate, or from Hydrocortisone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in sin-

gle-compartment containers, or in the volume of solution designated on the label of containers that are constructed to hold, in separate compartments, the Hydrocortisone Sodium Succinate for Injection and a solvent.

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION

**Sample:** Transfer a quantity of Hydrocortisone Sodium Succinate for Injection, equivalent to 100 mg of hydrocortisone sodium succinate, to a suitable container, and dissolve in 10 mL of water. In rapid succession, add 1 mL of 3 N hydrochloric acid, shake briefly, immediately decant the aqueous layer, and wash the precipitate with two additional 10-mL portions of water, each time removing the water by decanting. Remove as much of the water as possible, spread the precipitate in a suitable container, and dry under vacuum at 60° for 3 h.

**Acceptance criteria:** The IR spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydrocortisone Hemisuccinate RS.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

**Internal standard solution:** 3 mg/mL of USP Fluorometholone RS in tetrahydrofuran

**Diluent:** Glacial acetic acid in chloroform (3 in 100)

**Solution A:** 0.30 mg/mL of USP Hydrocortisone RS in *Diluent*

**Standard solution:** 0.65 mg/mL of USP Hydrocortisone Hemisuccinate RS, prepared as follows. Transfer 32.5 mg of USP Hydrocortisone Hemisuccinate RS to a 50-mL volumetric flask. Add by pipet 5.0 mL of *Internal standard solution* and 5.0 mL of *Solution A*. Dilute with *Diluent* to volume.

**Sample solution:** Mix the constituted solutions prepared from the contents of 10 vials of Hydrocortisone Sodium Succinate for Injection. Transfer a volume, equivalent to 50 mg of hydrocortisone from the resulting constituted solution, to a suitable flask containing 10.0 mL of *Internal standard solution*, and dilute with *Diluent* to 100.0 mL. Shake thoroughly for 5 min, then allow the phases to separate, discarding the upper phase.

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L3

**Flow rate:** 1.0 mL/min

**Injection volume:** 6 µL

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Resolution:** NLT 2.0 between hydrocortisone hemisuccinate and the internal standard

**Relative standard deviation:** NMT 2.0%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The order of elution of peaks is that from the internal standard, hydrocortisone hemisuccinate, and successive smaller peaks representing free hydrocortisone and hydrocortisone 17-hemisuccinate, the relative retention times of which are about 1.0, 1.5, 2.0, and 2.5, respectively.]

Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Hydrocortisone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $R_U$  = ratio of the summation of the peak areas of hydrocortisone hemisuccinate and hydrocortisone 17-hemisuccinate to the peak area of the internal standard from the *Sample solution*
- $R_S$  = ratio of the summation of the peak areas of hydrocortisone hemisuccinate and hydrocortisone 17-hemisuccinate to the peak area of the internal standard from the *Standard solution*
- $C_S$  = concentration of USP Hydrocortisone Hemisuccinate RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of the *Sample solution* (mg/mL)
- $M_{r1}$  = molecular weight of hydrocortisone, 362.46
- $M_{r2}$  = molecular weight of hydrocortisone hemisuccinate, 462.53
- To this percentage add the percentage of free hydrocortisone found in the test for *Free Hydrocortisone*.
- Acceptance criteria:** 90.0%–110.0%

**OTHER COMPONENTS****• FREE HYDROCORTISONE**

**Analysis:** Using the chromatograms obtained in the *Assay*, measure the areas of the peaks from the internal standard and free hydrocortisone. Calculate the percentage of free hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Hydrocortisone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = peak area ratio of the free hydrocortisone to the internal standard from the *Sample solution*
- $R_S$  = peak area ratio of the free hydrocortisone to the internal standard from the *Standard solution*
- $C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of the *Sample solution* (mg/mL)
- Acceptance criteria:** NMT 6.7% of the labeled amount of hydrocortisone

**PERFORMANCE TESTS**

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**SPECIFIC TESTS**

- pH (791)**  
**Sample solution:** A solution containing the equivalent of 50 mg/mL of hydrocortisone  
**Acceptance criteria:** 7.0–8.0
- LOSS ON DRYING (731)**  
**Sample:** Dry at 105° for 3 h.  
**Acceptance criteria:** NMT 2.0%
- BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 1.25 USP Endotoxin Units/mg of hydrocortisone
- PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections (1)*, *Constituted Solutions*.
- STERILITY TESTS (71):** Meets the requirements
- OTHER REQUIREMENTS:** It meets the requirements in *Injections (1)*, *Labeling*.

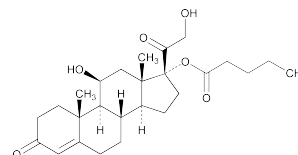
**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve as described in *Injections (1)*, *Containers for Sterile Solids*.
- LABELING:** Label it to indicate that the constituted solution prepared from Hydrocortisone Sodium Succinate for Injection is suitable for use only if it is clear, and that the

solution is to be discarded after 3 days. Label it to indicate that it was prepared by freeze-drying, having been filled into its container in the form of a true solution.

**• USP REFERENCE STANDARDS (11)**

- USP Endotoxin RS
- USP Fluorometholone RS
- USP Hydrocortisone RS
- USP Hydrocortisone Hemisuccinate RS

**Hydrocortisone Valerate**

$C_{26}H_{38}O_6$  446.58  
Pregn-4-ene-3,20-dione, 11,21-dihydroxy-17-[(1-oxopentyl)oxy]-, (11 $\beta$ )-;  
Cortisol 17-valerate;  
11 $\beta$ ,17,21-Trihydroxypregn-4-ene-3,20-dione 17-valerate  
[57524-89-7].

**DEFINITION**

Hydrocortisone Valerate contains NLT 97.0% and NMT 102.0% of hydrocortisone valerate ( $C_{26}H_{38}O_6$ ), calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION (197K)**

**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile and water (45:55)

**Internal standard solution:** 2.0 mg/mL of ethyl benzoate in methanol

**Standard stock solution:** 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.

**Standard solution:** 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.

**Sample stock solution:** 1 mg/mL of Hydrocortisone Valerate in methanol

**Sample solution:** 0.1 mg/mL of Hydrocortisone Valerate in methanol, prepared as follows. Pipet 1 mL of the *Sample stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between ethyl benzoate and hydrocortisone valerate

Relative standard deviation: NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of hydrocortisone valerate ( $C_{26}H_{38}O_6$ ) in the portion of Hydrocortisone Valerate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Hydrocortisone Valerate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Sample:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 1.0%

##### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample:** 10 mg/mL in dioxane

**Acceptance criteria:** +37° to +43°

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Hydrocortisone Valerate RS

## Hydrocortisone Valerate Cream

#### DEFINITION

Hydrocortisone Valerate Cream is Hydrocortisone Valerate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone valerate ( $C_{26}H_{38}O_6$ ).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Samples:** *Standard solution* and *Sample solution* from the Assay

**Acceptance criteria:** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and water (45:55)

**Internal standard solution:** 2.0 mg/mL of ethyl benzoate in methanol

**Standard stock solution:** 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.

**Standard solution:** 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.

**Sample solution:** Nominally 0.1 mg/mL of hydrocortisone valerate, prepared as follows. Transfer a quantity of Cream, equivalent to 1 mg of hydrocortisone valerate, to a screw-capped tube. Add 8.0 mL of a mixture of methanol and water (3:1), and swirl to disperse. Heat at 80° for 1 min, swirl again, and allow to cool to room temperature. Add 2.0 mL of the *Internal standard solution*. Centrifuge for 5 min, and filter, if necessary, to obtain a clear supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between ethyl benzoate and hydrocortisone valerate

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone valerate ( $C_{26}H_{38}O_6$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone valerate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Hydrocortisone Valerate RS

## Hydrocortisone Valerate Ointment

#### DEFINITION

Hydrocortisone Valerate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone valerate ( $C_{26}H_{38}O_6$ ) in a suitable ointment base.

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Samples:** *Standard solution* and *Sample solution* from the Assay

**Acceptance criteria:** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and water (45:55)

**Internal standard solution:** 2.0 mg/mL of ethyl benzoate in methanol

**Standard stock solution:** 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.

**Standard solution:** 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.

**Sample solution:** Nominally 0.1 mg/mL of hydrocortisone valerate, prepared as follows. Transfer a quantity of Ointment, equivalent to 1 mg of hydrocortisone valerate, to a screw-capped tube. Add 8.0 mL of a mixture of methanol and water (3:1), and swirl to disperse. Heat in a steam bath until melted (about 30 s), swirl again, and allow to cool to room temperature. Add 2.0 mL of the *Internal standard solution*. Centrifuge for 5 min, and filter the supernatant, if necessary, to obtain a clear solution.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between ethyl benzoate and hydrocortisone valerate

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone valerate (C<sub>26</sub>H<sub>38</sub>O<sub>6</sub>) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone valerate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** <755>: Meets the requirements

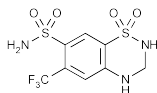
#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total microbial count does not exceed 10<sup>2</sup> cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>  
USP Hydrocortisone Valerate RS

## Hydroflumethiazide



C<sub>8</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 331.29

2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3,4-dihydro-6-(trifluoromethyl)-, 1,1-dioxide.

3,4-Dihydro-6-(trifluoromethyl)-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [135-09-1].

» Hydroflumethiazide contains not less than 98.0 percent and not more than 102.0 percent of C<sub>8</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP 2,4-Disulfamyl-5-trifluoromethylaniline RS

C<sub>7</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 319.29

USP Hydroflumethiazide RS

#### Identification—

**A:** *Infrared Absorption* <197K>, previously dried over silica gel for 4 hours.

**B:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

*Medium:* methanol.

**Melting range, Class I** <741>: between 270° and 275°.

**pH** <791>: between 4.5 and 7.5, in a 1-in-100 dispersion in water.

**Water, Method I** <921>: not more than 1.0%.

**Residue on ignition** <281>: not more than 1.0%.

**Heavy metals, Method II** <31>: 0.002%.

**Selenium** <291>: 0.003%.

#### Diazotizable substances—

**Standard preparation**—Transfer 10.0 mg of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS to a 50-mL volumetric flask, dissolve in and dilute with acetone to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test preparation**—Transfer 100 mg to a 50-mL volumetric flask, dissolve in 5 mL of acetone, dilute with water to volume, and mix.

**Procedure**—Transfer 5.0 mL each of the *Standard preparation*, the *Test preparation*, and a solution of acetone in water (1 in 10) to provide the blank, to separate 25-mL volumetric flasks. To each flask add 2.0 mL of dilute hydrochloric acid (1 in 5), and immediately add 1 mL of freshly prepared sodium nitrite solution (1 in 100). Mix, and allow to stand for 5 minutes. Add 1 mL of freshly prepared ammonium sulfamate solution (1 in 10) to each flask, mix, and allow to stand for 1 minute, with frequent swirling. Add 1 mL of a freshly prepared solution of N-(1-naphthyl)ethylenediamine dihydrochloride (1 in 1000), and mix. After 1 minute, dilute with water to volume, and mix. Concomitantly, and within 5 minutes after mixing, taking care to establish the same elapsed time for each solution, determine the absorbances of the solutions in 1-cm cells at 518 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance of the solution from the *Test preparation* does not exceed that of the solution from the *Standard preparation*, corresponding to not more than 1.0% of diazotizable substances.

**Assay**—Transfer about 50 mg of Hydroflumethiazide, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Hydroflumethiazide RS in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of C<sub>8</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> in the Hydroflumethiazide taken by the formula:

$$5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Hydroflumethiazide RS in the Standard solution; and  $A_U$  and

$A_S$  are the absorbances of the solution of Hydroflumethiazide and the Standard solution, respectively.

## Hydroflumethiazide Tablets

» Hydroflumethiazide Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of hydroflumethiazide ( $C_8H_8F_3N_3O_4S_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydroflumethiazide RS

**Identification**—Finely powder a number of Tablets, equivalent to about 100 mg of hydroflumethiazide, and place the powder in a 35-mL, screw-capped centrifuge tube. Add 30 mL of acetone, cap the tube, and allow it to stand for 30 minutes, with occasional shaking. Centrifuge, and decant the supernatant into a 100-mL beaker. Evaporate on a steam bath to dryness, add 10 mL of sodium hydroxide solution (1 in 250) to the residue, and mix. Transfer the liquid to a 125-mL separator. Rinse the beaker with 5 mL of water, and add the rinsing to the main portion. Add 50 mL of anhydrous ethyl ether to the separator, insert the stopper, shake vigorously for 2 minutes, releasing pressure as necessary, and allow the phases to separate. Draw off the lower phase, retaining any emulsion in the separator, and pass it through a membrane filter having a 0.2- to 2- $\mu$ m porosity. Add dilute hydrochloric acid (1 in 10) dropwise to the filtrate in a 50-mL beaker, stirring well and checking the pH with wide-range test paper after each drop. [NOTE—Crystallization begins at about pH 5. Rubbing the bottom of the beaker with a glass stirring rod helps to initiate crystallization.] When precipitation is complete, decant and discard the supernatant, and wash the precipitate with 5 mL of water. Decant and discard the wash water, and dry the precipitate at 105° for 30 minutes: the IR spectrum of a potassium bromide dispersion of the dried material exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroflumethiazide RS.

**Dissolution** (711)—

*Medium:* dilute hydrochloric acid (1 in 100); 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 60 minutes.

**Procedure**—Determine the amount of  $C_8H_8F_3N_3O_4S_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroflumethiazide RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_8H_8F_3N_3O_4S_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Crush 1 Tablet and quantitatively transfer to a 100-mL volumetric flask, add about 50-mL of methanol, and shake until disintegration is complete. Dilute with methanol to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate with methanol to obtain a solution containing approximately 10  $\mu$ g of hydroflumethiazide per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Hydroflumethiazide RS, in the same medium having a known concentration of about 10  $\mu$ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the

quantity, in mg, of  $C_8H_8F_3N_3O_4S_2$  in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of hydroflumethiazide in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of USP Hydroflumethiazide RS in the Standard solution;  $D$  is the concentration, in  $\mu$ g per mL, of hydroflumethiazide in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—

**Standard preparation**—Transfer about 30 mg of USP Hydroflumethiazide RS, accurately weighed, to a 100-mL volumetric flask, add sodium hydroxide solution (1 in 100) to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with sodium hydroxide solution (1 in 100) to volume, and mix. The concentration of USP Hydroflumethiazide RS in the *Standard preparation* is about 15  $\mu$ g per mL.

**Chromatographic column**—Proceed as directed for *Column Partition Chromatography* under *Chromatography* (621), packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 1 mL of sodium hydroxide solution (1 in 100), and the upper segment is a mixture prepared as directed under *Assay preparation*.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of hydroflumethiazide, to a 50-mL volumetric flask, add about 35 mL of sodium hydroxide solution (1 in 100), shake vigorously, dilute with sodium hydroxide solution (1 in 100) to volume, and mix. Mix 2.0 mL of this solution with 3 g of *Solid Support* as directed under *Chromatographic column*, and transfer to the column. Wash the column with 50 mL of water-saturated chloroform, then with 50 mL of water-saturated ether, and discard the eluates. Elute the hydroflumethiazide from the column with 100 mL of glacial acetic acid in ether (1 in 1000), collecting the eluate in a 250-mL separator. Add 100 mL of a 1 in 1000 solution of glacial acetic acid in ether to a second 250-mL separator to provide a blank, and treat each as follows: Add 60 mL of isooctane to each separator, mix, and extract the resulting solution with three 50-mL portions of sodium hydroxide solution (1 in 100), collecting the extracts in a 200-mL volumetric flask. Dilute with sodium hydroxide solution (1 in 100) to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using the blank. Calculate the quantity, in mg, of hydroflumethiazide ( $C_8H_8F_3N_3O_4S_2$ ) in the portion of Tablets taken by the formula:

$$5C(A_U / A_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Hydroflumethiazide RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrogen Peroxide Concentrate

$H_2O_2$  34.01

Hydrogen peroxide.

Hydrogen peroxide [7722-84-1].

» Hydrogen Peroxide Concentrate contains not less than 29.0 percent and not more than



32.0 percent, by weight, of  $\text{H}_2\text{O}_2$ . It contains not more than 0.05 percent of a suitable preservative or preservatives.

**Caution**—Hydrogen Peroxide Concentrate is a strong oxidant.

**Packaging and storage**—Preserve in partially-filled containers having a small vent in the closure, and store in a cool place.

**Labeling**—Label it to indicate the name and amount of any added preservative. The label states that this article is not intended for direct administration to humans or animals.

**Acidity**—Dilute 25 g with water to 250 mL, and mix thoroughly. Take 25 mL of the solution, add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 2.5 mL is required for neutralization.

**Chloride** (221): 1.5 g diluted with water to 25 mL shows no more chloride than 0.10 mL of 0.020 N hydrochloric acid (0.005%).

**Other requirements**—It responds to the *Identification* test and meets the requirements of the tests for *Nonvolatile residue*, *Heavy metals*, and *Limit of preservative* (90 mL of it being used) under *Hydrogen Peroxide Topical Solution*.

**Assay**—Accurately weigh about 1 mL of Concentrate in a tared 100-mL volumetric flask, dilute with water to volume, and mix. To 20.0 mL of this solution add 20 mL of 2 N sulfuric acid, and titrate with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate is equivalent to 1.701 mg of  $\text{H}_2\text{O}_2$ .

## Hydrogen Peroxide Topical Solution

$\text{H}_2\text{O}_2$  34.01

Hydrogen peroxide.

Hydrogen peroxide [7722-84-1].

» Hydrogen Peroxide Topical Solution contains, in each 100 mL, not less than 2.5 g and not more than 3.5 g of  $\text{H}_2\text{O}_2$ . It contains not more than 0.05 percent of a suitable preservative or preservatives.

**Packaging and storage**—Preserve in tight, light-resistant containers, at controlled room temperature.

**Identification**—Shake 1 mL with 10 mL of water containing 1 drop of 2 N sulfuric acid, and add 2 mL of ether: the subsequent addition of a drop of potassium dichromate TS produces an evanescent blue color in the water layer which upon agitation and standing passes into the ether layer.

**Acidity**—To 25 mL add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 2.5 mL is required for neutralization.

**Barium**—To 10 mL add two drops of 2 N sulfuric acid: no turbidity or precipitate is produced within 10 minutes.

**Heavy metals** (231)—Dilute 4 mL, previously shaken, with 20 mL of water, add 2 mL of 6 N ammonium hydroxide, and gently boil the solution until the volume is reduced to about 5 mL. Dilute with water to 25 mL: the limit is 5 ppm.

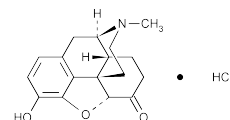
**Limit of nonvolatile residue**—Evaporate 20 mL, previously shaken, on a steam bath to dryness, and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 30 mg.

**Limit of preservative**—Extract 100 mL of well-mixed Topical Solution in a separator with a mixture of 3 volumes of chloroform and 2 volumes of ether, using 50 mL, 25 mL, and 25 mL, respectively. Evaporate the combined extracts at room temperature in a tared glass dish to dryness, and dry

over silica gel for 2 hours: the residue, if any, weighs not more than 50 mg (0.05%).

**Assay**—Pipet 2 mL of Topical Solution into a suitable flask containing 20 mL of water. Add 20 mL of 2 N sulfuric acid, and titrate with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate is equivalent to 1.701 mg of  $\text{H}_2\text{O}_2$ .

## Hydromorphone Hydrochloride



$\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl}$  321.80  
Morphinan-6-one, 4,5-epoxy-3-hydroxy-17-methyl-, hydrochloride, (5 $\alpha$ )-;  
4,5 $\alpha$ -Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride [71-68-1].

### DEFINITION

Hydromorphone Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 101.0% of  $\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl}$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Sample solution: 100  $\mu\text{g}/\text{mL}$   
Analytical wavelength: 280 nm  
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C. IDENTIFICATION TESTS—GENERAL**, Chloride (191)  
Sample solution: 1 in 20  
Acceptance criteria: Meets the requirements

### ASSAY

- **PROCEDURE**  
Sample: 225 mg, previously dried  
Analysis: Transfer the Sample to a 250-mL conical flask. Dissolve in 80 mL of glacial acetic acid, warming, if necessary. Cool, and add 5 mL of acetic anhydride and 10 mL of mercuric acetate TS. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 32.18 mg of  $\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl}$ .  
Acceptance criteria: 98.0%–101.0%, dried at 105° for 2 h

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.3%
- **SULFATE**

Sample solution: 100 mg in 5 mL of water

Analysis: To the Sample solution add 0.5 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS.

Acceptance criteria: No turbidity is produced.

**Organic Impurities** [NOTE—If (5 $\alpha$ )-7-[(5 $\alpha$ )-3,6-dihydroxy-17-methyl-4,5-epoxymorphinan-6-yl]-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one (hydromorphone aldol dimer) or (5 $\alpha$ )-3-hydroxy-8-[(5 $\alpha$ )-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-7-yl]-17-methyl-4,5-epoxymorphinan-6-one (7,8'-bishydromorphone) are potential impurities, Procedure 2 is recommended.]

#### PROCEDURE 1

Diluent: Phosphoric acid and water (1:1000)

Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:9).

Add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

**Solution B:** 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:1). Add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

**System suitability solution:** 0.8 mg/mL each of USP Hydromorphone Hydrochloride RS and USP Hydromorphone Related Compound A RS in *Diluent*. [NOTE—The solution should be kept in a cool place protected from light.]

**Standard solution:** 4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

**Sample solution:** 0.8 mg/mL of Hydromorphone Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 3.9-mm × 15-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.0 between the hydromorphone related compound A and hydromorphone peaks, *System suitability solution*

**Tailing factor:** NMT 1.5 for the hydromorphone peak, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 1/F \times 100$$

$r_u$  = peak response for each degradation found, including those in *Impurity Table 1*, from the *Sample solution*

$r_s$  = peak response of hydromorphone from the *Standard solution*

$C_s$  = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for the corresponding individual specified or unspecified impurity from *Impurity Table 1*

**Acceptance criteria:** See *Impurity Table 1*. [NOTE—Disregard peaks corresponding to those obtained from the *Diluent*.]

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
8-Hydroxyhydromorphone <sup>a</sup>	0.50	1.0	0.15
Dihydromorphone (DHM) <sup>b</sup>	0.61	1.0	0.5
Morphine <sup>c</sup>	0.65	1.8	0.15
Hydromorphone N-oxide <sup>d</sup>	0.79	1.0	0.15
Hydromorphone related compound A <sup>e</sup>	0.93	1.4	0.1
Hydromorphone	1.0	—	—
8,14-Dihydrooripavine*	1.66	1.0	0.15
6β-Te-tetrahydrooripavine*	1.71	1.0	0.15
2,2'-Bis hydromorphone <sup>f</sup>	2.02	1.7	0.15
Individual unspecified impurities	—	1.0	0.1
Total impurities	—	—	1.0

<sup>a</sup> 4,5α-Epoxy-17-methylmorphinan-3,8-diol-6-one.

<sup>b</sup> 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.

<sup>c</sup> 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.

<sup>d</sup> 4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one N-oxide.

<sup>e</sup> 7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one.

<sup>f</sup> (5β)-3-Hydroxy-2-[(5α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.

\* 8,14-Dihydrooripavine and 6β-tetrahydrooripavine are process impurities from another process and are controlled only if present.

#### • PROCEDURE 2

**Diluent:** Phosphoric acid and water (1:100)

**Solution A:** Mix 3520 mL of water, 18.40 g of monobasic ammonium phosphate, and 4.32 g of sodium 1-octanesulfonate. Add 4.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.90, and add 480 mL of acetonitrile.

**Solution B:** Acetonitrile and water (1600:400)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
21	100	0
30	100	0

**System suitability solution:** 0.15 mg/mL of USP Hydromorphone Hydrochloride RS and 0.1 mg/mL of USP Morphine RS in *Diluent*. [NOTE—The solution should be kept in a cool place protected from light.]

**Standard solution:** 15 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

**Sample solution:** 3 mg/mL of Hydromorphone Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 15-cm; 5-µm packing L7

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

**Run time:** 30 min

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 4.0 between the morphine and hydromorphone peaks, *System suitability solution*

**Tailing factor:** NMT 1.5 for the hydromorphone peak, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

$r_U$  = peak response for each peak found, including those in *Impurity Table 2*, from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for the corresponding individual specified or unspecified impurity from *Impurity Table 2*

**Acceptance criteria:** See *Impurity Table 2*. [NOTE—Disregard peaks corresponding to those obtained from the *Diluent*.]

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dihydromorphone (DHM) <sup>a</sup>	0.63	1.0	0.5
Morphine <sup>b</sup>	0.70	1.0	0.5
Hydromorphone	1.0	—	—
2,2'-Bishydromorphone <sup>c</sup>	1.82	2.0	0.5
Hydromorphone Hydrochloride Aldol Dimer <sup>d</sup>	2.12	1.0	0.5
7,8-Bishydromorphone <sup>e</sup>	2.32	1.0	0.5
Individual unspecified impurities	—	1.0	0.10
Total impurities	—	—	2.0

<sup>a</sup> 4,5 $\alpha$ -Epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>b</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>c</sup> (5 $\beta$ )-3-Hydroxy-2-[(5 $\alpha$ )-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.

<sup>d</sup> (5 $\alpha$ )-7-[(5 $\alpha$ )-3,6-Dihydroxy-17-methyl-4,5-epoxymorphinan-6-yl]-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one.

<sup>e</sup> (5 $\alpha$ )-3-Hydroxy-8-[(5 $\alpha$ )-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-7-yl]-17-methyl-4,5-epoxymorphinan-6-one.

**SPECIFIC TESTS**

- OPTICAL ROTATION** (781S)

**Sample solution:** 50 mg/mL

**Acceptance criteria:** Between  $-136^\circ$  and  $-139^\circ$

- ACIDITY**

**Sample:** 300 mg

**Analysis:** Dissolve the *Sample* in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide VS.

**Acceptance criteria:** NMT 0.30 mL is required to produce a yellow color.

- LOSS ON DRYING** (731): Dry a sample at  $105^\circ$  for 2 h: it loses NMT 1.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at  $25^\circ$ , excursions permitted between  $15^\circ$  and  $30^\circ$ .

- USP REFERENCE STANDARDS** (11)

USP Hydromorphone Hydrochloride RS

USP Hydromorphone Related Compound A RS

## Hydromorphone Hydrochloride Injection

» Hydromorphone Hydrochloride Injection is a sterile solution of Hydromorphone Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of hydromorphone hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Hydromorphone Hydrochloride RS

**Identification**—Place a volume of Injection, equivalent to about 10 mg of hydromorphone hydrochloride, in a separator. Extract with four 10-mL portions of chloroform, and discard the extracts. Add 1 mL of sodium carbonate TS, and extract with three 10-mL portions of chloroform. Filter the chloroform extracts into a glass-stoppered, 50-mL flask, and evaporate on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 1 mL of chloroform: the IR absorption spectrum of the solution so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydromorphone Hydrochloride RS.

**Bacterial endotoxins** (85)—It contains not more than 88.0 USP Endotoxin Units per mg of hydromorphone hydrochloride.

**pH** (791): between 3.5 and 5.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Standard preparation**—Using an accurately weighed quantity of USP Hydromorphone Hydrochloride RS, prepare a solution in water having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Injection, if necessary, with water to obtain a solution containing about 0.2 mg per mL.

**Procedure**—Transfer 20.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 50-mL volumetric flasks. To each flask add, with mixing, 1.0 mL of hydrochloric acid and 1.0 mL of sodium nitrite solution (1 in 20). Insert the stoppers, allow to stand for 40 to 45 minutes, with occasional swirling, then add 2 mL of ammonium hydroxide, and mix. Allow to stand for 2 minutes, then dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 440 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of hydromorphone hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$(V_A / V_i)(C)(A_U / A_S)$$

in which  $V_A$  is the volume, in mL, of the *Assay preparation*;  $V_i$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*;  $C$  is the concentration, in mg per mL, of USP Hydromorphone Hydrochloride RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydromorphone Hydrochloride Oral Solution

### DEFINITION

Hydromorphone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydromorphone hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ). It may contain suitable preservatives.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Diluent:** Phosphoric acid and water (1:1000)

**Solution A:** 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:9). To each liter of this solution add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

**Solution B:** 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:1). To each liter of this solution add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ .

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
24	5	95
25	85	15
30	85	15

[NOTE—The *Standard solution* and *Sample solution* should be kept in a cool place, protected from light.]

**Standard solution:** 0.08 mg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

**Sample solution:** Equivalent to 0.08 mg/mL of hydromorphone hydrochloride by diluting a suitable volume of Oral Solution in *Diluent*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  5-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5 for the hydromorphone peak

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydromorphone hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### IMPURITIES

#### ORGANIC IMPURITIES

**Diluent, Solution A, and Solution B:** Prepare as directed in the Assay.

**Mobile phase:** See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

[NOTE—The *System suitability solution*, *Quantitation limit solution*, *Standard solution*, and *Sample solution* should be kept in a cool place, protected from light.]

**System suitability solution:** 0.8 mg/mL of USP Hydromorphone Hydrochloride RS and 0.8  $\mu$ g/mL of USP Hydromorphone Related Compound A RS in *Diluent*

**Quantitation limit solution:** 0.4  $\mu$ g/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

**Standard solution:** 4  $\mu$ g/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

**Sample solution:** Equivalent to 0.4 mg/mL of hydromorphone hydrochloride in *Diluent*

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 3.9-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Samples:** *System suitability solution*, *Quantitation limit solution*, and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.0 between the hydromorphone related compound A and hydromorphone peaks, *System suitability solution*

**Signal-to-noise ratio:** 10:1, *Quantitation limit solution*

**Tailing factor:** NMT 1.5 for the hydromorphone peak, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Diluent, Standard solution, and Sample solution*  
Calculate the percentage of any specified or unspecified impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each degradation product found, including those in *Table 3*, from the *Sample solution*

$r_S$  = peak response of hydromorphone from the *Standard solution*

$C_S$  = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for the corresponding individual specified or unspecified impurity (see *Table 3*)

Calculate the total degradation products by summing the percentage of all individual specified and unspecified degradation products determined to be at a level of 0.1% or greater, excluding the known process impurities, as indicated in *Table 3*.

**Acceptance criteria:** See *Table 3*.

[NOTE—Disregard peaks corresponding to those from the *Diluent*, peaks that elute before a relative retention time of about 0.50, except for any peak with a relative retention time of about 0.34, and peaks that elute at the relative retention times of the process-related substances designated in *Table 3*.]

**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Unknown degradation product	0.34	1.0	0.2
8-Hydroxy-hydromorphone** <sup>a</sup>	0.50	—	—
Dihydromorphine (DHM)** <sup>b</sup>	0.61	—	—
Morphine** <sup>c</sup>	0.65	—	—
Hydromorphone <i>N</i> -oxide* <sup>d</sup>	0.79	0.87	0.2
Hydromorphone	1.0	—	—
2,2'-Bishydromorphone dihydrochloride* <sup>e</sup>	2.02	1.7	0.2

\* Degradation product.

\*\* Process impurity.

<sup>a</sup> 4,5 $\alpha$ -Epoxy-17-methylmorphinan-3,8-diol-6-one.

<sup>b</sup> 4,5 $\alpha$ -Epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>c</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>d</sup> 4,5 $\alpha$ -Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.

<sup>e</sup> 2,2'-Bihydromorphone.

**Table 3 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Individual unspecified degradation products	—	1.0	0.2
Total degradation products	—	—	1.0

\* Degradation product.

\*\* Process impurity.

<sup>a</sup> 4,5 $\alpha$ -Epoxy-17-methylmorphinan-3,8-diol-6-one.

<sup>b</sup> 4,5 $\alpha$ -Epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>c</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-17-methylmorphinan-3,6 $\alpha$ -diol.

<sup>d</sup> 4,5 $\alpha$ -Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.

<sup>e</sup> 2,2'-Bihydromorphone.

**SPECIFIC TESTS**

• **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic microbial count does not exceed 10<sup>2</sup> cfu/mL, and the total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements of the test for the absence of *Escherichia coli*.

• **PH** <791>: 4.5–6.5

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.

• **LABELING:** Identify in the product labeling any preservative used in the Oral Solution.

• **USP REFERENCE STANDARDS** <11>  
USP Hydromorphone Hydrochloride RS  
USP Hydromorphone Related Compound A RS

**Hydromorphone Hydrochloride Tablets**

» Hydromorphone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydromorphone hydrochloride (C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub> · HCl).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Hydromorphone Hydrochloride RS

USP Morphine Sulfate RS

**Identification**—Place a quantity of finely powdered Tablets, equivalent to about 10 mg of hydromorphone hydrochloride, in a separator, and proceed as directed in the *Identification* test under *Hydromorphone Hydrochloride Injection*, beginning with "Extract with four 10-mL portions of chloroform".

**Dissolution** <711>—

*Medium:* water; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

Determine the amount of C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub> · HCl dissolved by employing the following method.

*Buffer solution, Mobile phase, and System suitability solution*—Proceed as directed in the *Assay*.

*Test solution*—Withdraw a 15-mL portion of the solution under test, filter, and discard the first few mL of the filtrate.

**Standard solution**—Dissolve an accurately weighed quantity of USP Hydromorphone Hydrochloride RS in water at a concentration similar to that of the *Test solution*.

**Chromatographic system**—Proceed as directed in the *Assay*, except to inject the *Standard solution* instead of the *Standard preparation* to obtain the relative standard deviation for replicate injections of not more than 5.0%.

**Procedure**—Proceed as directed in the *Assay*, except to use an injection volume of about 200  $\mu$ L.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{19}NO_3 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Buffer solution**—Dissolve 10 g of sodium dodecyl sulfate and 20 mL of glacial acetic acid in 1.2 liters of water, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (33:17). Make adjustments if necessary.

**System suitability solution**—Dissolve suitable quantities of USP Morphine Sulfate RS and USP Hydromorphone Hydrochloride RS in water to obtain a solution containing about 30 and 40  $\mu$ g of each per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydromorphone Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 40  $\mu$ g per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer a portion of the powder, equivalent to about 4 mg of hydromorphone hydrochloride, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, sonicate if necessary, and mix. Filter a portion of the solution using a glass fiber filter, and discard the first 5 mL.

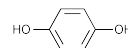
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between morphine and hydromorphone is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the hydromorphone hydrochloride peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydromorphone hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$0.1 C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Hydromorphone Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroquinone



$C_6H_6O_2$  110.11  
1,4-Benzenediol.  
Hydroquinone [123-31-9].

» Hydroquinone contains not less than 99.0 percent and not more than 100.5 percent of  $C_6H_6O_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hydroquinone RS

#### Identification—

**A: Infrared Absorption** (197K).

**B:** Prepare a solution of it in methanol containing approximately 1 mg per mL, and prepare a similar solution of USP Hydroquinone RS. Apply 5  $\mu$ L of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of equal volumes of methanol and chloroform until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Heat on a hot plate or under a lamp until spots appear: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the *Standard solution*.

**C:** A 1 in 40,000 solution in methanol exhibits an absorbance maximum at  $293 \pm 2$  nm.

**Melting range** (741): between  $172^\circ$  and  $174^\circ$ .

**Water, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.5%.

**Assay**—Dissolve about 250 mg of Hydroquinone, accurately weighed, in a mixture of 100 mL of water and 10 mL of 0.1 N sulfuric acid, add 3 drops of diphenylamine TS, and titrate with 0.1 N ceric sulfate VS until a red-violet endpoint is reached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 5.506 mg of  $C_6H_6O_2$ .

## Hydroquinone Cream

» Hydroquinone Cream contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of hydroquinone ( $C_6H_6O_2$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Hydroquinone RS

**Identification**—Dissolve a portion of Cream equivalent to about 50 mg of hydroquinone, in a mixture of equal volumes of methanol and chloroform to make 50 mL: a 5- $\mu$ L portion of this solution responds to *Identification test B* under *Hydroquinone*.

**Minimum fill** (755): meets the requirements.

#### Assay—

**Standard preparation**—Dissolve a suitable quantity of USP Hydroquinone RS in methanol, and dilute quantitatively and

stepwise with methanol to obtain a solution having a known concentration of about 10 µg per mL.

**Assay preparation**—Transfer an accurately weighed portion of Cream, equivalent to about 20 mg of hydroquinone, to a 100-mL beaker. Triturate the Cream with 50 mL of methanol, and pass the liquid through folded filter paper, previously washed with methanol, into a 500-mL volumetric flask. Repeat the trituration and filtration. Dilute, by washing the contents of the filter paper with methanol through the paper into the volumetric flask, to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add methanol to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 293 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of hydroquinone (C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>) in each g of the Cream taken by the formula:

$$2000(C / W)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Hydroquinone RS in the *Standard preparation*; W is the weight, in g, of Cream taken; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroquinone Topical Solution

» Hydroquinone Topical Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of hydroquinone (C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Hydroquinone RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** (791): between 3.0 and 4.2.

### Assay—

**Mobile phase**—Mix 55 volumes of methanol and 45 volumes of water.

**Standard preparation**—Transfer about 250 mg of USP Hydroquinone RS, accurately weighed, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Topical Solution, equivalent to about 30 mg of hydroquinone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 3.0%.

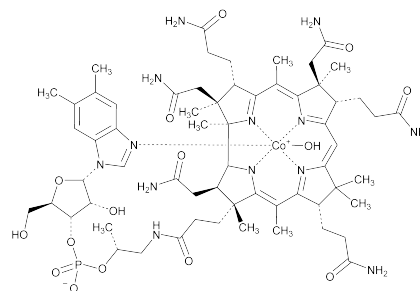
**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention time is

about 4 minutes for hydroquinone. Calculate the quantity, in mg, of hydroquinone (C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>) in each mL of the Topical Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroquinone RS in the *Standard preparation*; V is the volume, in mL, of Topical Solution taken; and r<sub>U</sub> and r<sub>S</sub> are the peak responses of hydroquinone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxocobalamin



C<sub>62</sub>H<sub>89</sub>CoN<sub>13</sub>O<sub>15</sub>P

1346.36

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3'-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1H-benzimidazole;

Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3'-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole [13422-51-0].

### DEFINITION

Hydroxocobalamin contains NLT 95.0% and NMT 102.0% of hydroxocobalamin (C<sub>62</sub>H<sub>89</sub>CoN<sub>13</sub>O<sub>15</sub>P), calculated on the dried basis.

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

**Wavelength range:** 400–700 nm

**Sample solution:** Use the *Sample solution* as directed in *pH-dependent Cobalamins*.

**Acceptance criteria:** Meets the requirements in the chapter. The visible absorption spectrum of the *Sample solution* exhibits maxima at 426 ± 2, 516 ± 2, and 550 ± 2 nm.

#### • B. COBALT

**Sample:** 1 mg of Hydroxocobalamin

**Analysis:** Fuse the *Sample* with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and boil until dissolved. Add 1 drop of phenolphthalein TS, and add 2 N sodium hydroxide dropwise until a pink color appears. Add 0.5 g of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of a 10-mg/mL solution of nitroso R salt. Add 0.5 mL of hydrochloric acid, and boil for 1 min.

**Acceptance criteria:** A red or orange-red color appears immediately after the addition of nitroso R salt. The red or orange-red color persists after boiling with the addition of hydrochloric acid.

### ASSAY

#### • PROCEDURE

Cyanocobalamin tracer reagent, Cresol-carbon tetrachloride solution, Phosphate-cyanide solution, Butanol-benzalkonium chloride solution, and Alumina-resin column: Prepare as directed in *Cobalamin Radiotracer Assay* (371).

**Standard solution:** Use *Standardization* as directed in *Cobalamin Radiotracer Assay* (371).

**Sample solution:** Transfer 40 mg of Hydroxocobalamin to a 2000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 25.0 mL of this solution to a beaker. Add 5.0 mL of *Cyanocobalamin tracer reagent*, and proceed as directed for *Assay preparation* in *Cobalamin Radiotracer Assay* (371), beginning with "Add, while working under a hood, 5 mg of sodium nitrite..."

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Proceed as directed for *Procedure* in *Cobalamin Radiotracer Assay* (371).

Calculate the percentage of hydroxocobalamin ( $C_{62}H_{89}Co-N_{13}O_{15}P$ ) in the portion of Hydroxocobalamin taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (R_S/R_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution* at 361 nm  
 $A_S$  = absorbance of the *Standard solution* at 361 nm  
 $C_S$  = concentration of USP Cyanocobalamin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Hydroxocobalamin in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $R_S$  = corrected average radioactivity values of the *Standard solution* (counts/min/mL)  
 $R_U$  = corrected average radioactivity values of the *Sample solution* (counts/min/mL)  
 $M_{r1}$  = molecular weight of hydroxocobalamin, 1346.36  
 $M_{r2}$  = molecular weight of cyanocobalamin, 1355.37  
**Acceptance criteria:** 95.0%–102.0% on the dried basis

#### IMPURITIES

##### • LIMIT OF CYANOCOBALAMIN

**Cyanocobalamin tracer reagent, Cresol-carbon tetrachloride solution, Butanol-benzalkonium chloride solution, and Alumina-resin column:** Prepare as directed in *Cobalamin Radiotracer Assay* (371).

**Standard solution:** Use *Standardization* as directed in *Cobalamin Radiotracer Assay* (371).

**Sample solution:** 50 mg of Hydroxocobalamin in 25 mL of water

**Analysis:** Transfer 5.0 mL of the *Sample solution* to a glass-stoppered, 50-mL centrifuge tube, and add 5.0 mL of *Cyanocobalamin tracer reagent* and 15 mL of *Cresol-carbon tetrachloride solution*. Insert the stopper, shake gently, centrifuge, carefully remove the upper, aqueous layer by aspiration, and discard the aspirated liquid. Add 25 mL of 5 N sulfuric acid, insert the stopper, shake gently, centrifuge, and remove and discard the upper, aqueous layer. Repeat the washing with additional 25-mL portions of the 5 N sulfuric acid until the acid wash is colorless (6–8 washings), and discard the acid washings. Add *Cresol-carbon tetrachloride solution* as necessary during the acid washings to maintain the volume of this phase at NLT 10 mL. Wash this solution successively with two 10-mL portions of saturated dibasic sodium phosphate solution and one 10-mL portion of water, and discard all of the aqueous washings. Proceed as directed for *Procedure* in *Cobalamin Radiotracer Assay* (371), beginning with "To the washed extract add 30 mL of a mixture of 2 volumes of *Butanol-benzalkonium chloride solution* and 1 volume of *carbon tetrachloride*".

Repeat the same procedure for the *Standard solution*. Calculate the percentage of cyanocobalamin in the portion of Hydroxocobalamin taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (R_S/R_U) \times 100$$

$A_U$  = absorbance of the *Sample solution* at 361 nm  
 $A_S$  = absorbance of the *Standard solution* at 361 nm

$C_S$  = concentration of USP Cyanocobalamin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Hydroxocobalamin in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $R_S$  = corrected average radioactivity values of the *Standard solution* (counts/min/mL)  
 $R_U$  = corrected average radioactivity values of the *Sample solution* (counts/min/mL)

**Acceptance criteria:** NMT 5.0% on the dried basis

#### SPECIFIC TESTS

##### • pH (791)

**Sample solution:** 20 mg/mL of solution

**Acceptance criteria:** 8.0–10.0

##### • LOSS ON DRYING (731):

Dry a sample at a pressure below 5 mm of mercury at 100° for 2 h: it loses 14.0%–18.0% of its weight.

##### • PH-DEPENDENT COBALAMINS

[NOTE—Perform the test in subdued light.]

**Buffer A:** Dissolve 23.8 g of sodium borate and 402 mg of boric acid in 1500 mL of water. The pH is 9.3.

**Buffer B:** Dissolve 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid, and dilute with water to 1500 mL. The pH is 4.0.

**Sample stock solution:** Transfer 40 mg of Hydroxocobalamin into a 25-mL volumetric flask. Dissolve and dilute with carbon dioxide-free water to volume.

**Sample solution A:** Transfer 1.0-mL aliquot of the *Sample stock solution* to a glass-stoppered test tube. Add 3.0 mL of *Buffer A* and mix.

**Sample solution B:** Transfer 1.0-mL aliquot of the *Sample stock solution* to a glass-stoppered test tube. Add 3.0 mL of *Buffer B* and mix.

#### Instrumental conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** Visible

**Analytical wavelength:** 550 nm

**Cell:** 1 cm

#### Analysis

**Samples:** *Sample solution A* and *Sample solution B*  
Determine the absorbance of *Sample solution A* against that of *Sample solution B*. Calculate the percentage of pH-dependent cobalamins, as hydroxocobalamin, in the portion of Hydroxocobalamin taken:

$$\text{Result} = A/(F \times C)$$

$A$  = pH corrected absorbance of *Sample solution A*  
 $F$  = coefficient of extinction ( $E_{1\%}^{1\text{cm}}$ ) of pure hydroxocobalamin in pH 9.3 buffer ( $100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ ), 19.66  
 $C$  = concentration of Hydroxocobalamin in *Sample solution A* (g/mL)

**Acceptance criteria:** 95.0%–102.0% on the dried basis

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

• **USP REFERENCE STANDARDS (11)**  
USP Cyanocobalamin RS

## Hydroxocobalamin Injection

» Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injection. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of hydroxocobalamin ( $C_{62}H_{89}CoN_{13}O_{15}P$ ).



**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** <11>—

USP Cyanocobalamin RS  
USP Endotoxin RS

**Identification**—Dilute 3.0 mL of Injection with pH 4.0 buffer (prepared by dissolving 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid and sufficient water to make 1500 mL of solution) to 100 mL: the UV-visible absorption spectrum of this solution exhibits maxima at  $352 \pm 2$  nm and  $525 \pm 2$  nm. The ratio  $A_{352}/A_{525}$  is between 2.7 and 3.3.

**Bacterial endotoxins** <85>—It contains not more than 0.4 USP Endotoxin Unit per  $\mu$ g of hydroxocobalamin.

**pH** <791>: between 3.5 and 5.0.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

**pH 9.3 Buffer**—Dissolve 23.8 g of sodium borate and 402 mg of boric acid in sufficient water to make 1500 mL of solution, and mix.

**Standard preparation**—Dissolve a suitable quantity of USP Cyanocobalamin RS, accurately weighed, in pH 9.3 Buffer and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 30  $\mu$ g per mL.

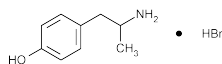
**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of hydroxocobalamin, to a 50-mL volumetric flask containing about 25 mL of pH 9.3 Buffer. Add 5.0 mL of potassium cyanide solution (1 in 10,000), allow to stand at room temperature for 30 minutes, dilute with pH 9.3 Buffer to volume, and mix. Transfer 15.0 mL of this solution to a second 50-mL volumetric flask, dilute with pH 9.3 Buffer to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using pH 9.3 Buffer as the blank. Calculate the quantity, in mg, of hydroxocobalamin ( $C_{62}H_{89}CoN_{13}O_{15}P$ ) in each mL of the Injection taken by the formula:

$$(1346.36 / 1355.37)(0.1667C / V)(A_U / A_S)$$

in which 1346.36 and 1355.37 are the molecular weights of hydroxocobalamin and cyanocobalamin, respectively; C is the concentration, in  $\mu$ g per mL, of USP Cyanocobalamin RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyamphetamine Hydrobromide



$C_9H_{13}NO \cdot HBr$  232.12

Phenol, 4-(2-aminopropyl)-, hydrobromide.  
( $\pm$ )-p-(2-Aminopropyl)phenol hydrobromide [306-21-8].

» Hydroxyamphetamine Hydrobromide contains not less than 98.0 percent and not more than 101.5 percent of  $C_9H_{13}NO \cdot HBr$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** <11>—

USP Hydroxyamphetamine Hydrobromide RS

**Identification**—

**A: Infrared Absorption** <197K>.

**B:** Dissolve about 500 mg of ammonium molybdate in 10 mL of sulfuric acid, and add to this solution about 2 mg of Hydroxyamphetamine Hydrobromide: an intense blue color is produced (*distinction from similar amino compounds such as amphetamine and methamphetamine, which, lacking a phenolic hydroxyl, do not undergo this reaction*).

**C:** Dissolve about 200 mg in 2 mL of water, and add a solution of 500 mg of potassium carbonate in 2 mL of water. Extract with two 10-mL portions of ether, allow the clear ether solution to evaporate to dryness, and dry at about 80°: the hydroxyamphetamine so obtained melts between 124° and 127° (see *Class I* under *Melting Range or Temperature* <741>).

**D:** To a solution of about 10 mg of it in 10 mL of water add 1 mL of 2 N nitric acid, then add silver nitrate TS: a pale yellow precipitate is formed, and it is slightly soluble in 6 N ammonium hydroxide.

**Melting range** <741>: between 189° and 192°.

**Loss on drying** <731>—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Bromide content**—Accurately weigh about 400 mg, and dissolve in 50 mL of water. Add 50 mL of methanol and 10 mL of glacial acetic acid, then add eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 7.990 mg of Br: the content of Br, calculated on the dried basis, is between 33.6% and 35.2%.

**Ordinary impurities** <466>—

**Test solution:** methanol.

**Standard solution:** methanol.

**Eluant:** a mixture of toluene, methanol, and ammonium hydroxide (10:4:0.25).

**Visualization:** 1.

**Assay**—Dissolve about 400 mg of Hydroxyamphetamine Hydrobromide, accurately weighed, in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly, if necessary, to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.21 mg of  $C_9H_{13}NO \cdot HBr$ .

## Hydroxyamphetamine Hydrobromide Ophthalmic Solution

» Hydroxyamphetamine Hydrobromide Ophthalmic Solution is a sterile, buffered, aqueous solution of Hydroxyamphetamine Hydrobromide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_9H_{13}NO \cdot HBr$ . It contains a suitable antimicrobial agent.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Hydroxyamphetamine Hydrobromide RS

**Identification—**

**A:** Dissolve about 500 mg of ammonium molybdate in 10 mL of sulfuric acid, and add 0.2 mL of Ophthalmic Solution: an intense blue color is produced (*distinction from similar amino compounds such as amphetamine and methamphetamine, which, lacking a phenolic hydroxyl, do not undergo this reaction*).

**B:** The dried diacetylhydroxyamphetamine obtained in the Assay melts between 96° and 100° (see *Class I* under *Melting Range or Temperature* <741>), but the range between beginning and end of melting does not exceed 2.0°.

**C:** It responds to *Identification test D* under *Hydroxyamphetamine Hydrobromide*.

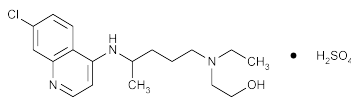
**D:** Dilute a volume of Ophthalmic Solution, equivalent to about 50 mg of hydroxyamphetamine hydrobromide, with 0.01 N hydrochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* <181>, using sodium carbonate TS in place of 1 N sodium hydroxide, beginning with "Transfer the liquid to a separator": the Ophthalmic Solution meets the requirements of the test.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 4.2 and 6.0.

**Assay—**Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 100 mg of hydroxyamphetamine hydrobromide, to a 125-mL separator. Wash the solution with 15 mL of chloroform, and discard the washing. Rinse the stopper and the mouth of the separator with a few drops of water. Add 1.05 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe, rapidly inject 0.5 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with five 10-mL portions of chloroform, filtering each extract through a pledget of cotton, previously washed with chloroform, into a tared 100-mL beaker. Evaporate the combined chloroform extracts on a steam bath in a current of air or stream of nitrogen to dryness. Dry the residue at 80° for 90 minutes, cool in a desiccator, and weigh. The weight of the diacetylhydroxyamphetamine so obtained, multiplied by 0.9866, represents the weight of  $C_9H_{13}NO \cdot HBr$  in the volume of Ophthalmic Solution taken.

## Hydroxychloroquine Sulfate



$C_{18}H_{26}ClN_3O \cdot H_2SO_4$  433.95

Ethanol, 2-[[4-[(7-chloro-4-quinolyl)amino]pentyl]ethylamino]-, (±)-, sulfate (1:1) (salt).

(±)-2-[[4-[(7-chloro-4-quinolyl)amino]pentyl]ethylamino]ethanol sulfate (1:1) (salt) [747-36-4].

» Hydroxychloroquine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ , calculated on the dried basis.

**Packaging and storage—**Preserve in well-closed, light-resistant containers.

**USP Reference standards** <11>—  
USP Hydroxychloroquine Sulfate RS

**Identification—**

**A:** *Ultraviolet Absorption* <197U>—

*Solution:* 10 µg per mL.

*Medium:* dilute hydrochloric acid (1 in 100).

**B:** *Infrared Absorption* <197K>.

**C:** A solution (1 in 100) responds to the tests for *Sulfate* <191>.

**Loss on drying** <731>—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

**Ordinary impurities** <466>—

*Test solution:* 10% water in methanol.

*Standard solution:* 10% water in methanol.

*Eluant:* a mixture of alcohol, water, and ammonium hydroxide (80:16:4).

*Visualization:* 1.

**Assay—**Dissolve about 100 mg of Hydroxychloroquine Sulfate, accurately weighed, in about 5 mL of water, and dilute quantitatively and stepwise with dilute hydrochloric acid (1 in 100) to obtain a solution containing about 10 µg per mL. Similarly prepare a Standard solution of USP Hydroxychloroquine Sulfate RS. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 343 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of  $C_{18}H_{26}ClN_3O \cdot H_2SO_4$  in the portion of Hydroxychloroquine Sulfate taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Hydroxychloroquine Sulfate RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Hydroxychloroquine Sulfate and the Standard solution, respectively.

## Hydroxychloroquine Sulfate Tablets

» Hydroxychloroquine Sulfate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of hydroxychloroquine sulfate ( $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ ).

**Packaging and storage—**Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—  
USP Hydroxychloroquine Sulfate RS

**Identification—**

**A:** Triturate a quantity of finely powdered Tablets, equivalent to about 1 g of hydroxychloroquine sulfate, with 50 mL of water, and filter (retain the remainder of the filtrate for *Identification test B*): the clear filtrate so obtained meets the requirements under —*Identification—Organic Nitrogenous Bases* <181>.

**B:** The clear filtrate obtained from *Identification test A* meets the requirements of the tests for *Sulfate* <191>.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution** <711>—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

**Procedure**—Determine the amount of  $C_{18}H_{26}ClN_3O \cdot H_2SO_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 343 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroxychloroquine Sulfate RS in the same medium.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{18}H_{26}ClN_3O \cdot H_2SO_4$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Assay**—

**Mobile phase**—To 800 mL of water, add 100 mL of methanol, 100 mL of acetonitrile, 2.0 mL of phosphoric acid, and 96 mg of sodium 1-pentanesulfonate, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Solvent mixture**—Prepare a mixture of methanol and water (1:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydroxychloroquine Sulfate RS in *Solvent mixture*, dilute quantitatively with *Solvent mixture*, and mix to obtain *Solution A* having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain the *Standard preparation* having a known concentration of about 0.05 mg per mL.

**Resolution solution**—Prepare a solution of chloroquine phosphate in methanol having a concentration of 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Solution A*, dilute with *Mobile phase* to volume, and mix.

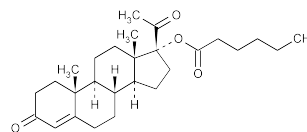
**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of hydroxychloroquine sulfate to a 200-mL volumetric flask, add about 150 mL of *Solvent mixture*, and mix. Sonicate, with intermittent shaking, for about 15 minutes, and cool to room temperature. Dilute with *Solvent mixture* to volume, mix, and filter. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 1.0 mL per minute. Chromatograph about 20  $\mu$ L of the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between chloroquine and hydroxychloroquine is not less than 1.8. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydroxychloroquine sulfate ( $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ ) in the portion of Tablets taken by the formula:

$$4000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroxychloroquine Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Hydroxyprogesterone Caproate**

$C_{27}H_{40}O_4$  428.60

Pregn-4-ene-3,20-dione, 17-[(1-oxohexyl)oxy]-.

17-Hydroxypregn-4-ene-3,20-dione hexanoate [630-56-8].

» Hydroxyprogesterone Caproate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{40}O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** <11>—

USP Hydroxyprogesterone Caproate RS

**Identification, Infrared Absorption** <197K>.

**Melting range** <741>: between 120° and 124°.

**Specific rotation** <781S>: between +58° and +64°.

*Test solution*: 10 mg per mL, in chloroform.

**Water, Method I** <921>: not more than 0.1%.

**Free n-caproic acid**—Dissolve 0.20 g in 25 mL of alcohol that previously has been neutralized to a faint pink color following the addition of 2 or 3 drops of phenolphthalein TS. Promptly titrate with 0.020 N sodium hydroxide: not more than 0.50 mL of 0.020 N sodium hydroxide is required (0.58%).

**Ordinary impurities** <466>—

*Test solution*: chloroform.

*Standard solution*: chloroform.

*Eluant*: a mixture of chloroform and ethyl acetate (3:1).

*Visualization*: 5; then view under long-wavelength UV light.

**Assay**—Transfer about 50 mg of Hydroxyprogesterone Caproate, accurately weighed, to a 100-mL volumetric flask, add alcohol to volume, and mix. Dilute 2.0 mL of this solution with alcohol to volume in a second 100-mL volumetric flask, and mix. Dissolve in alcohol a suitable quantity of USP Hydroxyprogesterone Caproate RS, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, using alcohol as the blank. Calculate the quantity, in mg, of  $C_{27}H_{40}O_4$  in the Hydroxyprogesterone Caproate taken by the formula:

$$5C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Hydroxyprogesterone Caproate RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Hydroxyprogesterone Caproate and the Standard solution, respectively.

## Hydroxyprogesterone Caproate Injection

» Hydroxyprogesterone Caproate Injection is a sterile solution of Hydroxyprogesterone Caproate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyprogesterone caproate ( $C_{27}H_{40}O_4$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.

**USP Reference standards** (11)—  
USP Hydroxyprogesterone Caproate RS

### Identification—

**A:** Transfer a volume of Injection, equivalent to 125 mg of hydroxyprogesterone caproate, to a 60-mL separator containing 10 mL of solvent hexane, 8 mL of methanol, and 2 mL of water. Insert the stopper, shake for 2 minutes, and allow the phases to separate. To 3 mL of the lower layer add sulfuric acid dropwise until a color develops, then add 3 mL of methanol: a purple color develops, and the solution, when viewed under long-wavelength UV light, exhibits a pale yellow fluorescence.

**B:** Evaporate 4 mL of the *Assay preparation*, obtained as directed in the *Assay*, on a water bath to dryness, and dissolve the residue in 0.5 mL of chloroform. Apply 10  $\mu$ L of this solution and 10  $\mu$ L of a solution of USP Hydroxyprogesterone Caproate RS in chloroform, containing 400  $\mu$ g per mL, to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, on a line about 2.5 cm from the bottom edge and about 2 cm apart. Place the plate in a developing chamber that contains and that has been equilibrated with a mixture of 3 volumes of chloroform and 1 volume of ethyl acetate. Develop the plate until the solvent front has moved to about 10 cm above the points of application. Remove the plate, mark the solvent front, and dry. Spray the plate with a mixture of 1 volume of sulfuric acid and 3 volumes of alcohol, and heat in an oven at 105° for 5 minutes: the  $R_f$  value of the principal yellowish green spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Water, Method I** (921): not more than 0.2%.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Isoniazid reagent**—Dissolve 375 mg of isoniazid and 0.47 mL of hydrochloric acid in 500 mL of methanol.

**Standard preparation**—Dissolve a suitable quantity of USP Hydroxyprogesterone Caproate RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 50  $\mu$ g per mL.

**Assay preparation**—Transfer to a 250-mL volumetric flask an accurately measured volume of Injection, equivalent to about 250 mg of hydroxyprogesterone caproate, add methanol to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, add methanol to volume, and mix.

**Procedure**—Pipet 5 mL of *Assay preparation* into a glass-stoppered, 50-mL conical flask. Pipet 5 mL of *Standard preparation* into a similar flask. To each flask, add 10.0 mL of *Isoniazid reagent*, mix, and allow to stand in a water bath at 30° for about 45 minutes. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 380 nm, with a suitable spectrophotometer, using as a blank a mixture of 5 mL of methanol and 10 mL of *Isoniazid reagent*. Calculate the quantity, in

mg, of hydroxyprogesterone caproate ( $C_{27}H_{40}O_4$ ) in each mL of the Injection taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Hydroxyprogesterone Caproate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxypropyl Cellulose Ocular System

### DEFINITION

Hydroxypropyl Cellulose Ocular System contains NLT 85.0% and NMT 115.0% of the labeled amount of Hydroxypropyl Cellulose. It contains no other substance. It is sterile.

### IDENTIFICATION

#### • INFRARED ABSORPTION

**Sample solution:** A 10-mg/mL solution in methanol, based on the labeled amount of Hydroxypropyl Cellulose. Evaporate 2 drops of the solution on a silver chloride plate so that it forms a thin film.

**Acceptance criteria:** The infrared absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroxypropyl Cellulose RS.

### ASSAY

#### • PROCEDURE

**Standard stock solution:** 0.25 mg/mL of USP Hydroxypropyl Cellulose RS prepared as follows: Weigh 25 mg of USP Hydroxypropyl Cellulose RS into a 100-mL volumetric flask. Dissolve in 80 mL of water. Mix well by agitating on a mechanical shaker until completely dissolved. Add one drop of methanol to dispel the foam and dilute with water to volume. [NOTE—Stirring overnight before diluting to volume is recommended.]

**Standard solution:** 0.05 mg/mL of hydroxypropyl cellulose in water, from *Standard stock solution*

**Sample stock solution:** 0.25 mg/mL of hydroxypropyl cellulose prepared using the same procedure as the *Standard stock solution*

**Sample solution:** 0.05 mg/mL of hydroxypropyl cellulose in water, from *Sample stock solution*

#### Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV-Vis

**Analytical wavelength:** 620 nm

**Cell length:** 1.0 cm, quartz

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Separately pipet 2 mL of the *Standard solution*, the *Sample solution*, and water, to provide a blank, into individual 50-mL centrifuge tubes. Add to each tube, 6.0 mL of a 0.5 mg/mL solution of anthrone in sulfuric acid, and mix on a vortex mixer. Let the centrifuge tubes cool for approximately 40 min and remix. Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*. [NOTE—Prepare anthrone in sulfuric acid solution just before use in low-actinic glassware, and mix well before adding to the tube. Use it within 12 h of preparation. Avoid contact between the glassware and the paper products during analysis; the cellulose in the paper will react with the sulfuric acid and alter the results.]

Calculate the percentage of hydroxypropyl cellulose in the Ocular System:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of hydroxypropyl cellulose in the *Sample solution* (mg/mL)

**Acceptance criteria:** 85.0%–115.0%

## PERFORMANCE TESTS

### • WEIGHT VARIATION

**Analysis:** Determine the weight of each of a sufficient number of Systems.

**Acceptance criteria:** NMT 1 out of 20 Systems varies more than 25% from the average or, failing that, NMT 6 out of 60 (including the original 20) vary more than 25% (but none more than 35%) from the average weight.

## SPECIFIC TESTS

- **STERILITY TESTS** <71>: Meets the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, at a temperature not exceeding 30°.  
 • **USP REFERENCE STANDARDS** <11>  
 USP Hydroxypropyl Cellulose RS

# Hydroxyurea



CH<sub>4</sub>N<sub>2</sub>O<sub>2</sub> 76.05

Urea, hydroxy-

Hydroxyurea [127-07-1].

» Hydroxyurea contains not less than 97.0 percent and not more than 103.0 percent of CH<sub>4</sub>N<sub>2</sub>O<sub>2</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, in a dry atmosphere.

**USP Reference standards** <11>—

USP Hydroxyurea RS

**Identification, Infrared Absorption** <197K>.

**Loss on drying** <731>—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.50%.

**Heavy metals** <231>: not more than 0.003%.

**Urea and related compounds**—

**Developing solvent**—Shake equal volumes of isobutyl alcohol and water in a separator, and allow the layers to separate. Use the upper layer as the *Mobile phase* and the lower layer as the *Stationary phase*.

**p-Dimethylaminobenzaldehyde solution, 1%**—Dissolve 1.0 g of p-dimethylaminobenzaldehyde in 50 mL of alcohol, add 2 mL of hydrochloric acid, and dilute with alcohol to 100.0 mL.

**pH 6.5 Buffer solution**—Mix 700 mL of 0.2 M dibasic sodium phosphate and 300 mL of 0.1 M citric acid.

**Standard preparation**—Prepare a solution of urea in water, containing 0.1 mg per mL.

**Test preparation**—Dissolve 10.0 mg of Hydroxyurea in 1.0 mL of water.

**Procedure**—Treat a suitable chromatographic paper strip (Whatman No. 1 or equivalent) by dipping it in pH 6.5 Buffer solution. Dry the paper strip, and apply 100 µL of the Test preparation and 50 µL of the Standard preparation. Place

the strip in a chromatographic chamber for descending chromatography containing the *Stationary phase* in the bottom of the chamber and the *Mobile phase* in the trough. Develop for 24 hours, remove the strip from the chamber, air-dry, and develop again for 24 hours. Remove the strip, air-dry, spray with p-Dimethylaminobenzaldehyde solution, 1%, and heat at 90° for 1 to 2 minutes. Not more than two spots, other than the major component, are present in the Test preparation, and their intensities are not greater than the intensity of the spot from the Standard preparation (0.5% of each impurity). The R<sub>f</sub> values relative to hydroxyurea, the principal spot, are 0.65 and 1.26 (urea).

### Assay—

**Solution A**—Dissolve 1.7 g of tetrabutylammonium hydrogensulfate and 1.74 g of dibasic potassium phosphate, anhydrous, in 1000 mL of water, and adjust with 1 N sodium hydroxide or 85% phosphoric acid to a pH of 5.0.

**Solution B:** methanol.

**Mobile phase**—Prepare a solution of filtered, degassed Solution A and Solution B (8.5:1.5). Make adjustments if necessary (see System Suitability under Chromatography <621>).

**Resolution solution**—Dissolve accurately weighed quantities of USP Hydroxyurea RS and hydroxylamine hydrochloride in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.4 mg per mL of each.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydroxyurea RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.4 mg per mL.

**Assay preparation**—Transfer about 200 mg of Hydroxyurea, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

**Chromatographic system** (see Chromatography <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.5 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution, R<sub>s</sub>, between the hydroxylamine and hydroxyurea peaks is not less than 1.5; for the hydroxyurea peak, the column efficiency is not less than 5000; and the tailing factor is not more than 1.5. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of CH<sub>4</sub>N<sub>2</sub>O<sub>2</sub> in the portion of Hydroxyurea taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroxyurea RS in the Standard preparation; and r<sub>U</sub> and r<sub>S</sub> are the peak responses of the hydroxyurea peaks obtained from the Assay preparation and the Standard preparation, respectively.

## Hydroxyurea Capsules

» Hydroxyurea Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyurea (CH<sub>4</sub>N<sub>2</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in tight containers, in a dry atmosphere.

**USP Reference standards** (11)—

USP Hydroxyurea RS

**Identification**—Transfer a portion of the Capsule contents, equivalent to about 30 mg of hydroxyurea, to a suitable centrifuge tube, and add 10 mL of anhydrous methanol. Mix, and centrifuge for 3 minutes. Transfer 1.0 mL of the clear supernatant to a mortar containing 500 mg of potassium bromide, triturate to a homogeneous blend, dry in a vacuum desiccator at 60° for 3 hours, and prepare a suitable disk: the IR absorption spectrum exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroxyurea RS.

**Dissolution** (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

**Procedure**—Determine the amount of  $\text{CH}_4\text{N}_2\text{O}_2$  dissolved, employing the procedure set forth in the Assay, making any necessary modifications.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $\text{CH}_4\text{N}_2\text{O}_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Solution A, Solution B, Mobile phase, Resolution solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under Hydroxyurea.

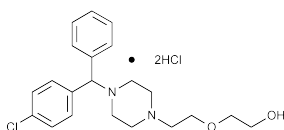
**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Grind to a fine powder, and transfer an accurately weighed portion of the powder, equivalent to about 200 mg of hydroxyurea, to a 500-mL volumetric flask. Add about 300 mL of *Mobile phase*, sonicate for 10 minutes, stir with the aid of a magnetic stirrer for 30 minutes, sonicate for an additional 10 minutes, and dilute as necessary with *Mobile phase* to volume. Filter a portion of the resulting solution, discarding the first 2 mL of the filtrate.

**Procedure**—Proceed as directed for *Procedure* in the Assay under Hydroxyurea. Calculate the quantity, in mg, of hydroxyurea ( $\text{CH}_4\text{N}_2\text{O}_2$ ) in the portion of Capsules taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroxyurea RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Hydrochloride


 $\text{C}_{21}\text{H}_{27}\text{ClN}_2\text{O}_2 \cdot 2\text{HCl}$  447.83

Ethanol, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, dihydrochloride, (±)-  
(±)-2-[2-[4-(p-Chloro-α-phenylbenzyl)-1-piperazinyl]ethoxy]ethanol dihydrochloride  
[2192-20-3].

» Hydroxyzine Hydrochloride, contains not less than 98.0 percent and not more than 102.0 per-

cent of  $\text{C}_{21}\text{H}_{27}\text{ClN}_2\text{O}_2 \cdot 2\text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydroxyzine Hydrochloride RS

USP Hydroxyzine Related Compound A RS

p-Chlorobenzhydrylpiperazine.

**Identification**—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: alcohol.

Absorptivities at 230 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: To 10 mL of a solution (1 in 400) add 2 drops of nitric acid and 1 mL of silver nitrate TS: a curdy, white precipitate, insoluble in 2 N nitric acid, but soluble in 6 N ammonium hydroxide, separates (*presence of chloride*).

**Loss on drying** (731)—Dry it in vacuum at 75° for 3 hours: it loses not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals, Method II** (231): 0.002%.

**Chromatographic purity**—

*Mobile phase, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay.

**Standard solution**—Quantitatively dilute the *Standard preparation* with *Mobile phase* to obtain a solution having a known concentration of about 1.8 µg per mL of Hydroxyzine Hydrochloride.

**Test solution**—Use the Assay stock preparation.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a total time of not less than 1.8 times the retention time of the hydroxyzine peak, and measure the response for each peak, except for the main hydroxyzine peak in the chromatogram obtained from the *Test solution*. Calculate the percentage of each impurity in the specimen taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Hydroxyzine Hydrochloride RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of specimen in the *Test solution*;  $r_U$  is the peak response of a given impurity in the chromatogram obtained from the *Test solution*; and  $r_S$  is the peak response of hydroxyzine in the chromatogram obtained from the *Standard solution*: not more than 0.3% of any impurity is found, and the sum of all impurities found is not greater than 1.5%.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.12 N sulfuric acid (90:10). Make adjustments if necessary (see *System Suitability* under Chromatography (621)).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Hydroxyzine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL.

**Resolution solution**—Dissolve suitable quantities of USP Hydroxyzine Hydrochloride RS and USP Hydroxyzine Related Compound A RS in *Mobile phase* to obtain a solution containing 3.6 µg of each per mL.

**Assay stock preparation**—Transfer an accurately weighed quantity of Hydroxyzine Hydrochloride to a suitable volumetric flask, dissolve in and dilute with *Mobile phase* to volume to obtain a solution containing a known concentration of about 0.6 mg of Hydroxyzine Hydrochloride per mL, and mix.

**Assay preparation**—Quantitatively dilute the *Assay stock preparation* with *Mobile phase* to obtain a final known concentration of about 0.3 mg per mL of Hydroxyzine Hydrochloride.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between hydroxyzine related compound A and hydroxyzine is not less than 1.5; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%. [NOTE—For identification purposes, the relative retention times are about 0.9 for hydroxyzine related compound A and 1.0 for hydroxyzine.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for a total time of not less than 1.8 times the retention time of the hydroxyzine peak, and measure the response for the main hydroxyzine peak in the chromatograms. Calculate the percentage of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ , in the portion of Hydroxyzine Hydrochloride taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which  $C_s$  is the concentration, in mg per mL, of USP Hydroxyzine Hydrochloride RS in the *Standard preparation*;  $C_u$  is the concentration, in mg per mL, of Hydroxyzine Hydrochloride in the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses of hydroxyzine in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Hydrochloride Injection

» Hydroxyzine Hydrochloride Injection is a sterile solution of Hydroxyzine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, protected from light.

### USP Reference standards <11>—

USP Endotoxin RS

USP Hydroxyzine Hydrochloride RS

**Identification**—Dilute a volume of Injection with 0.1 N hydrochloric acid to obtain a solution having a concentration of about 20  $\mu$ g of hydroxyzine hydrochloride per mL: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a 1 in 50,000 solution of USP Hydroxyzine Hydrochloride RS in 0.1 N hydrochloric acid, concomitantly measured.

**Bacterial endotoxins** <85>—It contains not more than 3.6 USP Endotoxin Units per mg of hydroxyzine hydrochloride.

**pH** <791>: between 3.5 and 6.0.

**Other requirements**—It meets the requirements under *Injections* <1>.

### Assay and limit of 4-chlorobenzophenone—

**Mobile phase**—Adjust about 1000 mL of *Buffer No. 1* (see *Phosphate Buffers and Other Solutions* in the section *Media and Diluents*, under *Antibiotics—Microbial Assays* <81>) with 10 N potassium hydroxide to a pH of 6.6. To about 35 volumes of this solution add about 65 volumes of methanol,

mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve accurately weighed quantities of USP Hydroxyzine Hydrochloride RS and 4-chlorobenzophenone in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having known concentrations of about 250  $\mu$ g of USP Hydroxyzine Hydrochloride RS and 0.5  $\mu$ g of 4-chlorobenzophenone per mL. Protect this solution from light.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 125 mg of hydroxyzine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Protect this solution from light.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factors for the 4-chlorobenzophenone and hydroxyzine peaks are not more than 2.5, the resolution,  $R$ , between the 4-chlorobenzophenone and hydroxyzine peaks is not less than 2.0, and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%. The relative retention times are about 0.75 for 4-chlorobenzophenone and 1.0 for hydroxyzine.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$0.5(C / V)(r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Hydroxyzine Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the hydroxyzine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. The ratio of the response of the 4-chlorobenzophenone peak to that of the hydroxyzine peak obtained from the *Assay preparation* does not exceed the corresponding ratio of peak responses obtained from the *Standard preparation* (0.2%).

## Hydroxyzine Hydrochloride Oral Solution

» Hydroxyzine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards <11>—

USP Hydroxyzine Hydrochloride RS

**Identification**—Dilute a volume of Oral Solution, equivalent to about 20 mg of hydroxyzine hydrochloride, with 50 mL of methanol, and mix. Apply 100  $\mu$ L of this solution and 100  $\mu$ L of a solution in the same medium containing about 350  $\mu$ g of USP Hydroxyzine Hydrochloride RS per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>), coated with a 0.25-mm layer of chromatographic silica gel and dried in air for 30 minutes followed by drying in vacuum at 140° for 30 minutes. Allow the spots to dry, and develop the chromatogram in a solvent system

consisting of a mixture of toluene, alcohol, and ammonium hydroxide (150:95:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by lightly spraying with potassium iodoplatinate TS: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay under *Hydroxyzine Hydrochloride Tablets*.

*Standard preparation*—Dissolve a suitable quantity of USP Hydroxyzine Hydrochloride RS, accurately weighed, in water to obtain a solution having a known concentration of about 100 µg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of hydroxyzine hydrochloride, to a 200-mL volumetric flask, dilute with water to volume, mix, and pass a portion through a polytetrafluoroethylene membrane filter having a 5-µm or finer porosity.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Hydroxyzine Hydrochloride Tablets*. Calculate the quantity, in mg, of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ) in each mL of the Oral Solution taken by the formula:

$$0.2(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Hydroxyzine Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Hydrochloride Tablets

» Hydroxyzine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**USP Reference standards** (11)—

USP Hydroxyzine Hydrochloride RS

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of hydroxyzine hydrochloride, with 50 mL of methanol, and filter. Apply 100 µL of this solution and 100 µL of a solution in the same medium containing 2 mg of USP Hydroxyzine Hydrochloride RS per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel and dried in air for 30 minutes followed by drying in vacuum at 140° for 30 minutes. Proceed as directed in the *Identification* test under *Hydroxyzine Hydrochloride Oral Solution*, beginning with "Allow the spots to dry."

**Dissolution** (711)—

TEST 1—

*Medium*: water, 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 230 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroxyzine Hydrochloride RS in the same *Medium*. Calculate the amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  dissolved per Tablet.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  is dissolved in 45 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Medium*: water, 250 mL.

*Apparatus 3*: 30 dips per minute.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 230 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroxyzine Hydrochloride RS in the same *Medium*. Calculate the amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  dissolved per Tablet.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Mobile phase*—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, add 1000 mL of methanol, and mix. Pass the solution through a polytetrafluoroethylene membrane filter having a 5-µm or finer porosity, and degas.

*Standard preparation*—Dissolve a suitable quantity of USP Hydroxyzine Hydrochloride RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 100 µg per mL.

*Assay preparation*—Place 20 Tablets in a high-speed blender jar containing 400.0 mL of methanol, and blend for 5 minutes. The Tablets are completely disintegrated. Allow to settle, and filter a portion of the supernatant through a polytetrafluoroethylene membrane filter having a 1-µm or finer porosity. Dilute an accurately measured volume of the filtrate so obtained quantitatively with methanol to obtain an *Assay preparation* having a concentration of about 100 µg of hydroxyzine hydrochloride per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm × 25-cm column that contains packing L9. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.5%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ) in each Tablet taken by the formula:

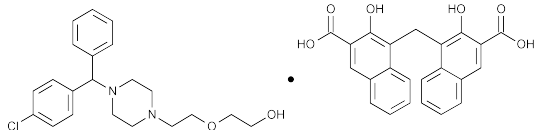
$$(L/D)(C)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of hydroxyzine hydrochloride in each Tablet;  $D$  is the concentration, in µg per mL, of hydroxyzine hydrochloride in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution;  $C$  is the concentration, in µg per mL,



of USP Hydroxyzine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Pamoate



$C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$  763.27

Ethanol, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, ( $\pm$ )-, compd. with 4,4'-methylenedibis[3-hydroxy-2-naphthalenecarboxylic acid] (1:1).

( $\pm$ )-2-[2-[4-(*p*-Chloro- $\alpha$ -phenylbenzyl)-1-piperazinyl]ethoxy]ethanol 4,4'-methylenedibis[3-hydroxy-2-naphthoate] (1:1) [10246-75-0].

» Hydroxyzine Pamoate contains not less than 97.0 percent and not more than 102.0 percent of  $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Hydroxyzine Pamoate RS

USP Hydroxyzine Hydrochloride RS

USP Pamoic Acid RS

$C_{23}H_{16}O_6$  388.38

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water**, *Method I* (921): not more than 5.0%.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals**, *Method II* (231): 20 ppm.

**Assay**—

**Mobile phase**—Dissolve 8.65 g of sodium 1-octanesulfonate in about 1000 mL of water in a 2000-mL volumetric flask, add 4.0 mL of phosphoric acid, dilute with water to volume, mix, and pass through a 0.5- $\mu$ m or finer porosity membrane filter. Prepare a suitable mixture of this solution and acetonitrile (45:55), making any adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydroxyzine Hydrochloride RS in dimethylformamide to obtain a solution having a known concentration of about 1 mg per mL. Transfer 2.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and pass through a 0.5- $\mu$ m or finer porosity membrane filter.

**Assay preparation**—Transfer about 90 mg of Hydroxyzine Pamoate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix. Transfer 2.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and pass through a 0.5- $\mu$ m or finer porosity membrane filter, discarding the first 5 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1.

The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, calculated from the analyte peak, is not less than 2000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2%. Chromatograph the *Assay preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between hydroxyzine and pamoic acid is not less than 1.5.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for hydroxyzine and 1.0 for pamoic acid. Calculate the percentage of  $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$  in the portion of Hydroxyzine Pamoate taken by the formula:

$$100(763.27 / 447.83)(C_S / C_U)(r_U / r_S)$$

in which 763.27 and 447.83 are the molecular weights of hydroxyzine pamoate and hydroxyzine hydrochloride, respectively;  $C_S$  is the concentration, in mg per mL, of USP Hydroxyzine Hydrochloride RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Hydroxyzine Pamoate in the *Assay preparation*; and  $r_U$  and  $r_S$  are the hydroxyzine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Pamoate Capsules

» Hydroxyzine Pamoate Capsules contain hydroxyzine pamoate ( $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Hydroxyzine Pamoate RS

USP Hydroxyzine Hydrochloride RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Mobile phase**—Prepare a suitable degassed solution of methanol and 0.05 M monobasic sodium phosphate (6:4).

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L9. The flow rate is about 1.9 mL per minute. Chromatograph replicate injections of a Standard solution, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%.

**Procedure**—Inject alternately 50  $\mu$ L of a filtered portion of the solution under test and a Standard solution, having a known concentration of USP Hydroxyzine Hydrochloride RS in the same medium, into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  dissolved from the peak response obtained in comparison with the peak response obtained from the Standard solution.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Dissolve 7.0 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.4. Pass the solution through a 5- $\mu$ m porosity polytetrafluoroethylene membrane filter. Mix 900 mL of the filtrate with 900 mL of methanol, and degas by stirring under vacuum prior to use.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydroxyzine Pamoate RS in methanol to obtain a solution having a known concentration of about 0.18 mg per mL (equivalent to about 0.1 mg of hydroxyzine hydrochloride per mL).

**Assay preparation**—Transfer, as completely as possible, the contents of not less than 20 Capsules to a tared beaker, and determine the average weight per capsule. Mix the combined contents, and transfer an accurately weighed portion, equivalent to about 25 mg of hydroxyzine hydrochloride, to a 250-mL volumetric flask. Add 200 mL of methanol to the flask, sonicate for 5 minutes, shake by mechanical means for 30 minutes, and sonicate for 2 minutes. Dilute with methanol to volume, and mix. Filter the solution through a 5- $\mu$ m porosity polytetrafluoroethylene membrane filter equipped with a glass fiber prefilter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L9. The flow rate is about 2.5 mL per minute. Chromatograph four replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 6 minutes for hydroxyzine. Calculate the equivalent quantity, in percentage, of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ) in the portion of Capsules taken by the formula:

$$100(447.83 / 763.27)(C_S / C_U)(r_U / r_S)$$

in which 447.83 and 763.27 are the molecular weights of hydroxyzine hydrochloride and hydroxyzine pamoate, respectively;  $C_S$  is the concentration, in mg per mL, of USP Hydroxyzine Pamoate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of hydroxyzine pamoate in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses of hydroxyzine pamoate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydroxyzine Pamoate Oral Suspension

» Hydroxyzine Pamoate Oral Suspension contains hydroxyzine pamoate ( $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hydroxyzine Hydrochloride RS  
USP Hydroxyzine Pamoate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.5 and 7.0.

**Assay**—

**Mobile phase**—Dissolve 7.0 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.4. Pass the solution through a 5- $\mu$ m porosity polytetrafluoroethylene membrane filter. Mix 900 mL of the filtrate with 900 mL of methanol, and degas by stirring under vacuum prior to use.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Hydroxyzine Pamoate RS in methanol to obtain a solution having a known concentration of about 0.18 mg per mL (equivalent to about 0.1 mg of hydroxyzine hydrochloride per mL).

**Assay preparation**—Transfer an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of hydroxyzine hydrochloride, to a 250-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix. Pass this solution through a polytetrafluoroethylene membrane filter having a 1- $\mu$ m or finer porosity.

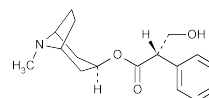
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L9. The flow rate is about 2.5 mL per minute. Chromatograph four replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the equivalent quantity, in percentage, of hydroxyzine hydrochloride ( $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ ) in the portion of the Oral Suspension taken by the formula:

$$100(447.83 / 763.27)(C_S / C_U)(r_U / r_S)$$

in which 447.83 and 763.27 are the molecular weights of hydroxyzine hydrochloride and hydroxyzine pamoate, respectively;  $C_S$  is the concentration, in mg per mL, of USP Hydroxyzine Pamoate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of hydroxyzine pamoate in the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses of the hydroxyzine peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hyoscyamine



$C_{17}H_{23}NO_3$  289.37

Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, [3(*S*)-endo-].

1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol (-)-tropate (ester). [101-31-5].

» Hyoscyamine contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{23}NO_3$ , calculated on the dried basis.

[Caution—Handle Hyoscyamine with exceptional care, since it is highly potent.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hyoscyamine Sulfate RS

**Identification**—

**A:** Transfer 30 mg of Hyoscyamine and 36 mg of USP Hyoscyamine Sulfate RS to individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide and 10 mL of chloroform. Shake for 1 minute, allow the layers to separate, and filter the chloroform extracts through separate filters of about 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide: the IR absorption spectrum, determined in a 1-mm cell, of the solution obtained from the test specimen exhibits maxima only at the same wavelengths as that of the solution obtained from the Reference Standard.

**B:** Dissolve 60 mg in 1 mL of 0.2 N hydrochloric acid, and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Add a small amount of 3 N hydrochloric acid, dissolve the precipitate with the aid of heat, and then allow to cool: lustrous golden yellow scales are formed (*distinction from atropine and scopolamine*).

**Melting range** (741): between 106° and 109°.

**Specific rotation** (781S): between −20° and −23°.

*Test solution:* 10 mg per mL, in dilute alcohol (1 in 2).

**Loss on drying** (731)—Dry it in vacuum over silica gel to constant weight: it loses not more than 0.2% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Limit of foreign alkaloids and other impurities**—Prepare a solution of it in methanol containing 20 mg per mL, and by quantitative dilution of a portion of this solution with methanol, prepare a second solution of Hyoscyamine containing 1 mg per mL. Apply 25  $\mu$ L of the first (20 mg per mL) Hyoscyamine solution, 1  $\mu$ L of the second (1 mg per mL) Hyoscyamine solution, and 5  $\mu$ L of a methanol solution of USP Hyoscyamine Sulfate RS containing 24 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.5-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, acetone, and diethylamine (5:4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with potassium iodoplatinate TS. The  $R_f$  value of the principal spot obtained from each test solution corresponds to that obtained from the Standard solution, and no secondary spot obtained from the first Hyoscyamine solution exhibits intensity equal to or greater than the principal spot obtained from the second Hyoscyamine solution (0.2%).

**Assay**—Dissolve about 500 mg of Hyoscyamine, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of  $C_{17}H_{23}NO_3$ .

## Hyoscyamine Tablets

» Hyoscyamine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hyoscyamine ( $C_{17}H_{23}NO_3$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Hyoscyamine Sulfate RS

**Identification**—Macerate a quantity of powdered Tablets, equivalent to about 5 mg of hyoscyamine, with 20 mL of water, filter, and transfer the filtrate to a separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract the alkaloid with 50 mL of methylene chloride. Divide the methylene chloride layer into two equal portions, and evaporate each to dryness. Perform tests A and B on the residues.

**A:** To one portion of the dry residue add 2 drops of nitric acid, and evaporate on a steam bath to dryness. Cool and add 10 mL of acetone to dissolve the residue. Add a few drops of alcoholic potassium hydroxide TS: a violet color is produced.

**B:** Dissolve the other portion of the residue in 1 mL of 0.1 N hydrochloric acid, and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool: lustrous golden yellow scales are formed.

**Disintegration** (701): 30 minutes, the use of disks being omitted.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*pH 9.0 Buffer*—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.0, determined electrometrically, by the addition of 3 N hydrochloric acid or 1 N sodium hydroxide, as necessary, with mixing.

*Internal standard solution*—Dissolve about 25 mg of homatropine hydrobromide, accurately weighed, in water contained in a 50-mL volumetric flask, add water to volume, and mix. Prepare fresh daily.

*Standard preparation*—Dissolve about 10 mg of USP Hyoscyamine Sulfate RS, accurately weighed, in water contained in a 100-mL volumetric flask, add water to volume, and mix. Prepare fresh daily. Pipet 10.0 mL of this solution into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *pH 9.0 Buffer*, and adjust with 1 N sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under nitrogen to dryness. Dissolve the residue in 2.0 mL of methylene chloride.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.86 mg of hyoscyamine, to a separator containing 5 mL of *pH 9.0 Buffer*, and add, by pipet, 2.0 mL of *Internal standard solution*. Proceed as directed under *Standard preparation*, beginning with “adjust with 1 N sodium hydroxide to a pH of 9.0.”

*Chromatographic system*—Under typical conditions, the instrument contains a 1.8-m  $\times$  2-mm glass column packed with 3% liquid phase G3 on support S1AB, cured as directed (see *Gas Chromatography* (621)). Maintain the column at 225°, and use nitrogen as the carrier gas at a flow rate of 25 mL per minute.

*System suitability*—Chromatograph six to ten injections of the *Standard preparation*, and record peak areas as directed for *Procedure*. The analytical system is suitable for con-

ducting this assay if the relative standard deviation for the ratio of the peak areas does not exceed 2.0%, the resolution factor is not less than 5, and the tailing factor does not exceed 2.0.

**Procedure**—Inject 1- $\mu$ L portions of the *Assay preparation* and the *Standard preparation* successively into the gas chromatograph. Measure the areas under the peaks for hyoscyamine and homatropine in each chromatogram. Calculate the ratio,  $A_U$ , of the area of the hyoscyamine peak to the area of the internal standard peak in the chromatogram from the *Assay preparation*, and similarly calculate the ratio,  $A_S$ , in the chromatogram from the *Standard preparation*. Calculate the quantity, in mg, of hyoscyamine ( $C_{17}H_{23}NO_3$ ) in the portion of Tablets taken by the formula:

$$(289.37 / 676.83)(W / 10)(A_U / A_S)$$

in which 289.37 and 676.83 are the molecular weights of hyoscyamine and anhydrous hyoscyamine sulfate, respectively, and  $W$  is the weight, in mg, of USP Hyoscyamine Sulfate RS taken for the *Standard preparation*.

## Hyoscyamine Hydrobromide

$C_{17}H_{23}NO_3 \cdot HBr$  370.28

Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, hydrobromide [3(S)-endo]-.

1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol (-)-tropate (ester) hydrobromide [306-03-6].

» Hyoscyamine Hydrobromide contains not less than 98.5 percent and not more than 100.5 percent of  $C_{17}H_{23}NO_3 \cdot HBr$ , calculated on the dried basis. [*Caution*—Handle Hyoscyamine Hydrobromide with exceptional care, since it is highly potent.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hyoscyamine Sulfate RS

**Identification**—

**A:** Transfer 30 mg of Hyoscyamine Hydrobromide and 36 mg of USP Hyoscyamine Sulfate RS to individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide and 10 mL of chloroform. Shake for 1 minute, allow the layers to separate, and filter the chloroform extracts through separate filters of about 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide: the IR absorption spectrum, determined in a 1-mm cell, of the solution obtained from the test specimen exhibits maxima only at the same wavelengths as that of the solution obtained from the Reference Standard.

**B:** To about 1 mL of a solution (1 in 20) add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Add a small amount of 3 N hydrochloric acid, dissolve the precipitate with the aid of heat, and allow the solution to cool: lustrous reddish brown scales that may be accompanied by reddish brown needles are formed (*distinction from atropine and scopolamine*).

**C:** To an aqueous solution (1 in 20) add silver nitrate TS: a yellowish-white precipitate is formed, and it is insoluble in nitric acid.

**Melting temperature** (741): not less than 149°.

**Specific rotation** (781S): not less than  $-24^\circ$ .

*Test solution:* 50 mg per mL, in water.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Other alkaloids**—Dissolve 250 mg in 1 mL of 0.1 N hydrochloric acid, and dilute with water to 15 mL. To 5 mL of the solution add a few drops of platinic chloride TS: no precipitate is formed immediately. To another 5-mL portion add 2 mL of 6 N ammonium hydroxide: the mixture may develop a slight opalescence, but no turbidity or precipitate is formed immediately.

**Assay**—Dissolve about 700 mg of Hyoscyamine Hydrobromide, accurately weighed, in a mixture of 50 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.03 mg of  $C_{17}H_{23}NO_3 \cdot HBr$ .

## Hyoscyamine Sulfate

$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O$  712.85

Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-, 8-methyl-8-azabicyclo-

[3.2.1]oct-3-yl ester, [3(S)-endo]-, sulfate (2:1), dihydrate. 1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol (-)-tropate (ester) sulfate (2:1) (salt) dihydrate [6835-16-1].

Anhydrous 676.83 [620-61-1].

» Hyoscyamine Sulfate contains not less than 98.5 percent and not more than 100.5 percent of  $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ , calculated on the anhydrous basis. [*Caution*—Handle Hyoscyamine Sulfate with exceptional care, since it is highly potent.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Hyoscyamine Sulfate RS

USP Hyoscyamine Related Compound A RS

Norhyoscyamine sulfate or (1R,3R,5S)-8-azabicyclo[3.2.1]oct-3-yl(2S)-3-hydroxy-2-phenylpropanoate.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Chromatographic purity*.

**C:** A solution (1 in 20) responds to the tests for *Sulfate* (191).

**Specific rotation** (781S): between  $-24^\circ$  and  $-29^\circ$ , measured at 20°.

*Test solution:* 50 mg per mL, in water.

**Water, Method Ia** (921): between 2.0% and 5.5%.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—

*Buffer solution*—Dissolve 7.0 g of monobasic potassium phosphate in 1000 mL of water, adjust with 0.05 M phosphoric acid to a pH of 3.3, and mix.

*Solution A*—Dissolve 3.5 g of sodium dodecyl sulfate in 606 mL of *Buffer solution*, add 320 mL of acetonitrile, and mix.

*Solution B*—Use acetonitrile.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Hyoscyamine Sulfate RS in *Solution A* to obtain a solution having a known concentration of about 1.2 mg of hyoscyamine sulfate per mL.

*Standard solution*—Dilute a portion of the *Standard stock solution* with *Solution A* to obtain a solution having a known concentration of about 0.24 mg of hyoscyamine sulfate per mL.

*Diluted standard solution*—Dilute the *Standard solution* with *Solution A* to obtain a solution having a known concentration of about 0.24 µg of hyoscyamine sulfate per mL.

*System suitability solution*—Prepare a solution of USP Hyoscyamine Related Compound A RS in *Solution A* having a concentration of about 2.4 µg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 10 mL of the *Standard stock solution*, dilute with *Solution A* to volume and mix.

*Test solution*—Transfer about 60 mg of Hyoscyamine Sulfate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Solution A* to volume, and mix. Transfer 10 mL of this solution to a 50-mL volumetric flask, and dilute with *Solution A* to volume. This solution contains about 0.24 mg of hyoscyamine sulfate per mL.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–2	95	5	isocratic
2–20	95→70	5→30	linear gradient
20–20.1	70→95	30→5	linear gradient
20.1–25	95	5	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between hyoscyamine related compound A and hyoscyamine is greater than 2.0.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Diluted standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the percentage of each impurity in the portion of Hyoscyamine Sulfate taken by the formula:

$$0.1(C_S / C_T) (r_i / r_S)$$

in which  $C_S$  is the concentration, in µg per mL, of hyoscyamine sulfate in the *Diluted standard solution*;  $C_T$  is the concentration, in mg per mL, of hyoscyamine sulfate in the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the hyoscyamine peak response obtained from the *Diluted standard solution*. In addition to not exceeding the limits for each impurity in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 0.5% of total impurities is found.

Table 1

Name	Relative Retention Time	Limit (%)
DL-Tropic acid	0.2	0.2
7-Hydroxyhyoscyamine	0.67	0.2
6-Hydroxyhyoscyamine	0.72	0.2
Scopolamine	0.8	0.2
Norhyoscyamine (Hyoscyamine related compound A)	0.9	0.3
Apoatropine	1.8	0.2
Littorine	1.1	0.2

**Assay**—Dissolve about 0.5 g of Hyoscyamine Sulfate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 67.68 mg of  $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ .

## Hyoscyamine Sulfate Elixir

» Hyoscyamine Sulfate Elixir contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards** <11>—

USP Hyoscyamine Sulfate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH** <791>: between 3.0 and 6.5.

**Alcohol content** <611>: between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Assay**—

*Diluent*—Use 0.01 N hydrochloric acid.

*Buffer solution*—Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, dissolve in about 1800 mL of water, adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , dilute with water to volume, mix, and filter.

*Mobile phase*—Prepare a degassed mixture containing *Buffer solution* and methanol (75:25).

*Standard stock preparation*—Dissolve an accurately weighed quantity of USP Hyoscyamine Sulfate RS in *Diluent* to obtain a solution having a concentration of about 0.16 mg of anhydrous hyoscyamine sulfate per mL. [NOTE—This solution may be stored in a refrigerator for 30 days.]

*Standard preparation*—Transfer 3.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask, dilute with *Diluent*, to volume and mix. Calculate the concentration,  $C$ , in mg per mL, of anhydrous hyoscyamine sulfate in this solution.

*Tropic acid solution*—Dissolve an accurately weighed quantity of tropic acid in *Diluent* to obtain a solution having a concentration of about 4 µg of tropic acid per mL.

*System suitability preparation*—Transfer 3.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask, add 4.0 mL of the *Tropic acid solution*, dilute with *Diluent*, to volume and mix.

*Assay preparation*—Transfer an accurately measured volume of Elixir, equivalent to about 0.25 mg of hyoscyamine

sulfate, to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass an aliquot through a 0.45- $\mu$ m filter, discarding the first 5 mL of the filtrate.

**Chromatographic system**—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm  $\times$  15-cm column that contains 4- $\mu$ m packing L11 and a 3-mm  $\times$  4-mm guard column that contains packing L11. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the elution order is tropic acid peak, followed by hyoscyamine peak; the resolution,  $R$ , between tropic acid and hyoscyamine is not less than 1.5; the tailing factor for the hyoscyamine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$  in each mL of the Elixir taken by the formula:

$$50 \times 1.053 \times (C/V)(r_U / r_S)$$

in which 1.053 is the ratio of the molecular weight of hydrated hyoscyamine sulfate to that of anhydrous hyoscyamine sulfate;  $C$  is as defined in the *Standard preparation*;  $V$  is the volume, in mL, of the Elixir taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hyoscyamine Sulfate Injection

» Hyoscyamine Sulfate Injection is a sterile solution of Hyoscyamine Sulfate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.

### USP Reference standards (11)—

USP Hyoscyamine Sulfate RS  
USP Endotoxin RS

### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** After evaporation to dryness, or appropriate adjustment of concentration, it meets the requirements of the tests for *Sulfate* (191).

**C:** The angular rotation of the Injection is levorotatory.

**Bacterial endotoxins** (85)—It contains not more than 714.3 USP Endotoxin Units per mg of hyoscyamine sulfate.

**pH** (791): between 3.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

**Diluent**—Use 0.01 N hydrochloric acid.

**Buffer solution**—Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, dissolve in about 1800 mL of water, adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , dilute with water to volume, mix, and filter.

**Mobile phase**—With continuous stirring, add 0.3 mL of triethylamine to 1800 mL of the *Buffer solution*. Add 200 mL of acetonitrile, mix well, and degas.

**Standard stock preparation**—Dissolve an accurately weighed quantity of USP Hyoscyamine Sulfate RS in *Diluent* to obtain a solution having a concentration of about 0.16 mg of anhydrous hyoscyamine sulfate per mL. [NOTE—This solution may be stored in a refrigerator for 30 days.]

**Standard preparation**—Transfer 3.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask, dilute with *Diluent*, to volume and mix. Calculate the concentration,  $C$ , in mg per mL, of anhydrous hyoscyamine sulfate in this solution.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 1.0 mg of hyoscyamine sulfate, to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass an aliquot through a 0.45- $\mu$ m filter, discarding the first 5 mL of the filtrate.

**Chromatographic system**—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm  $\times$  15-cm column that contains 4- $\mu$ m packing L11 and a 3-mm  $\times$  4-mm guard column that contains packing L11. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.8, and the relative standard deviation for six replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$  in each mL of the Injection taken by the formula:

$$100 \times 1.053 \times (C/V)(r_U / r_S)$$

in which 1.053 is the ratio of the molecular weight of hydrated hyoscyamine sulfate to that of anhydrous hyoscyamine sulfate;  $C$  is as defined under *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hyoscyamine Sulfate Oral Solution

» Hyoscyamine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Hyoscyamine Sulfate RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.0 and 6.5.

#### Assay—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Hyoscyamine Sulfate Injection*.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 0.5 mg of hyoscyamine sulfate, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass an aliquot through a 0.45- $\mu$ m filter, discarding the first 5 mL of the filtrate.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$  in each mL of the Oral Solution taken by the formula:

$$100 \times 1.053 \times (C/V)(r_U / r_S)$$

in which 1.053 is the ratio of the molecular weight of hydrated hyoscyamine sulfate to that of anhydrous hyoscyamine sulfate; C is defined under *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hyoscyamine Sulfate Tablets

» Hyoscyamine Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Hyoscyamine Sulfate RS

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**B:** A filtered solution of Tablets meets the requirements of the tests for *Sulfate* (191).

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

*Diluent, Buffer solution, Mobile phase, Standard stock preparation, and Standard preparation*—Proceed as directed in the Assay under *Hyoscyamine Sulfate Injection*.

*Tropic acid solution*—Dissolve an accurately weighed quantity of tropic acid in *Diluent* to obtain a solution having a concentration of about 3  $\mu$ g of tropic acid per mL.

*System suitability preparation*—Transfer 3.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask, add 4.0 mL of the *Tropic acid solution*, dilute with *Diluent*, to volume and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 0.125 mg of hyoscyamine sulfate, to a 25-mL volumetric flask. Add about 20 mL of the *Diluent*, and sonicate for 15 minutes with occasional swirling. Allow to cool to room temperature, dilute with *Diluent* to volume, and mix. Pass an aliquot through a 0.45- $\mu$ m filter, discarding the first 5 mL of the filtrate.

*Chromatographic system*—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm  $\times$  15-cm column that contains 4- $\mu$ m packing L11 and a 3-mm  $\times$  4-mm guard column that contains packing L11. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the elution order is the tropic acid peak, followed by the hyoscyamine peak; the resolution,  $R_s$ , between tropic acid and hyoscyamine is not less than 1.5; the tailing factor for the hyoscyamine peak is not more than 1.8; and the relative standard deviation for six replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of hyoscyamine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$  in the portion of Tablets taken by the formula:

$$25 \times 1.053 \times C(r_U / r_S)$$

in which 1.053 is the ratio of the molecular weight of hydrated hyoscyamine sulfate to that of anhydrous hyoscyamine sulfate; C is as defined under *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Hypromellose

Attribute	JP	EP	USP
Definition	+	+	+
Labeling	+	+	+
Identification (A)	+	+	+
Identification (B)	+	+	+
Identification (C)	+	+	+
Identification (D)	+	+	+
Identification (E)	+	+	+
Viscosity, Method 1	+	+	+
Viscosity, Method 2	+	+	+
pH	+	+	+
Heavy Metals	+	+	+
Loss on Drying	+	+	+
Residue on Ignition	+	+	+
Assay	+	+	+

**Legend:** + will adopt and implement; – will not stipulate  
**Nonharmonized attributes:** Packaging and Storage  
**Specific local attributes:** Appearance of solution (EP), Description (JP), Limit of glyoxal (EP)

Cellulose, 2-hydroxypropyl methyl ether;  
 Cellulose hydroxypropyl methyl ether [9004-65-3].

#### DEFINITION

Hypromellose is a methyl and hydroxypropyl mixed ether of cellulose. It contains, calculated on the dried basis, methoxy ( $-OCH_3$ : 31.03) and hydroxypropoxy ( $-OC_3H_6OH$ : 75.09) groups conforming to the limits for the types of Hypromellose (hydroxypropyl methylcellulose) set forth in the table below.

Substitution Type	Methoxy (%)		Hydroxypropoxy (%)	
	Min.	Max.	Min.	Max.
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0

Substitution Type	Methoxy (%)		Hydroxypropoxy (%)	
	Min.	Max.	Min.	Max.
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

**IDENTIFICATION****A. PROCEDURE****Sample:** 1 g**Analysis:** Gently add the *Sample* to the top of 100 mL of water in a beaker, and allow to disperse over the surface, tapping the top of the container to ensure an even dispersion of the substance. Allow the beaker to stand for 1–2 min.**Acceptance criteria:** The powdered material aggregates on the surface.**B. PROCEDURE****Sample:** 1 g**Analysis:** Add the *Sample* to 100 mL of boiling water, and stir the mixture using a magnetic stirrer with a bar 25 mm long.**Acceptance criteria:** A slurry is formed, but the powdered material does not dissolve. Cool the slurry to 10°, and stir using a magnetic stirrer: the resulting liquid is a clear or slightly turbid solution with thickness dependent on the viscosity grade.**C. PROCEDURE****Solution A:** Sulfuric acid and water (9:1). [NOTE—Carefully add sulfuric acid to water.]**Sample solution:** 0.1 mL of the solution prepared for Identification test B**Analysis:** To the *Sample solution*, add 9 mL of *Solution A*, and shake. Heat in a water bath for exactly 3 min, immediately cool in an ice bath, and add carefully 0.6 mL of ninhydrin TS. Shake, and allow to stand at 25°.**Acceptance criteria:** A red color develops at first that changes to purple within 100 min.**D. PROCEDURE****Sample solution:** 2–3 mL of the solution prepared for Identification test B**Analysis:** Pour the *Sample solution* onto a glass slide as a thin film, and allow the water to evaporate.**Acceptance criteria:** A coherent, clear film forms on the glass slide.**E. PROCEDURE****Sample solution:** 50 mL of the solution prepared in Identification test B**Analysis:** Add the *Sample solution* to exactly 50 mL of water in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate, and begin heating at a rate of 2° to 5°/min. Determine the temperature at which a turbidity increase begins to occur, and designate this temperature as the flocculation temperature.**Acceptance criteria:** The flocculation temperature is higher than 50°.**ASSAY****PROCEDURE****[CAUTION—** Hydriodic acid and its reaction byproducts are highly toxic. Perform all steps in the preparation of the *Standard solution* and the *Sample solution* in a properly functioning hood. Specific safety practices to be followed are to be identified to the analyst performing this test.]**Apparatus:** For the reaction vial, use a 5-mL pressure-tight serum vial, 50 mm in height, 20 mm in outside diameter, and 13 mm in inside diameter at the mouth. The vial is equipped with a pressure-tight septum having a polytetrafluoroethylene-faced butyl rubber and an airtight seal using an aluminum crimp or any sealing system that provides sufficient airtightness. Use a heater having a heating module that has a square-shape alu-

minum block with holes 20 mm in diameter and 32 mm in depth, into which the reaction vial fits. The heating module is also equipped with a magnetic stirrer capable of mixing the contents of the vial, or use a reciprocal shaker that performs a reciprocating motion of about 100 times/min.

**Hydriodic acid:** Use a reagent having a typical concentration of HI of about 57%.**Internal standard solution:** 30 mg/mL of *n*-octane in *o*-xylene**Standard solution:** Into a suitable serum vial, weigh between 60 and 100 mg of adipic acid, and add 2.0 mL of *Hydriodic acid* and 2.0 mL of *Internal standard solution*. Close the vial securely with a suitable septum stopper. Weigh the vial and contents, add between 15 µL and 22 µL of isopropyl iodide through the septum with a syringe, weigh again, and calculate the weight of isopropyl iodide added, by difference. Add 45 µL of methyl iodide similarly, weigh again, and calculate the weight of methyl iodide added, by difference. Shake the reaction vial well, and allow the layers to separate. Use the upper layer as the *Standard solution*.**Sample solution:** Transfer 0.065 g of dried Hypromellose to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum-type closure, add between 60 and 100 mg of adipic acid, and pipet 2.0 mL of *Internal standard solution* into the vial. Cautiously pipet 2.0 mL of *Hydriodic acid* into the mixture, immediately cap the vial tightly, and weigh. Using the magnetic stirrer equipped in the heating module, or using a reciprocal shaker, mix the contents of the vial continuously, heating and maintaining the temperature of the contents at 130 ± 2° for 60 min. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-min intervals during the initial 30 min of the heating time. Allow the vial to cool, and weigh. If the weight loss is ≥ 0.50% of the contents or there is evidence of a leak, discard the mixture, and prepare another *Sample solution*.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** GC**Detector:** Thermal conductivity or hydrogen flame-ionization**Column:** 3- to 4-mm × 1.8- to 3-m glass column packed with 20% liquid phase G28 on 100- to 120-mesh support S1C that is not silanized

[NOTE—Use a column giving well-resolved peaks of methyl iodide, isopropyl iodide, and the internal standard, in that order.]

**Column temperature:** 100°**Carrier gas:** Use helium with the thermal conductivity detector; helium or nitrogen can be used for the hydrogen flame-ionization detector.**Flow rate:** With the *Standard solution*, adjust the flow rate so that the retention time of the internal standard is about 10 min.**Injection size:** 1–2 µL**Analysis****Samples:** Upper layer of the *Standard solution* and the *Sample solution*Calculate the percentage of –OCH<sub>3</sub> in the portion of Hypromellose taken:

$$\text{Result} = 21.864 \times (R_{\text{Ua}}/R_{\text{Sa}}) \times (W_{\text{Sa}}/W_{\text{U}})$$

 $R_{\text{Ua}}$  = peak area ratio of methyl iodide to *n*-octane from the *Sample solution* $R_{\text{Sa}}$  = peak area ratio of methyl iodide to *n*-octane from the *Standard solution* $W_{\text{Sa}}$  = weight of methyl iodide in the *Standard solution* (mg) $W_{\text{U}}$  = weight of Hypromellose, calculated on the dried basis, taken for the *Sample solution* (mg)



Calculate the percentage of  $-\text{OC}_3\text{H}_6\text{OH}$  in the portion of Hypromellose taken:

$$\text{Result} = 44.17 \times (R_{\text{Ub}}/R_{\text{Sb}}) \times (W_{\text{Sb}}/W_{\text{U}})$$

- $R_{\text{Ub}}$  = peak area ratio of isopropyl iodide to *n*-octane from the *Sample solution*  
 $R_{\text{Sb}}$  = peak area ratio of isopropyl iodide to *n*-octane from the *Standard solution*  
 $W_{\text{Sb}}$  = weight of isopropyl iodide in the *Standard solution* (mg)  
 $W_{\text{U}}$  = weight of Hypromellose, calculated on the dried basis, taken for the *Sample solution* (mg)

**Acceptance criteria:** See the limits, calculated on the dried basis, in the table in the *Definition*.

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 1.5% on a 1.0-g sample
- **HEAVY METALS**, *Method III* (231): NMT 20 ppm

### SPECIFIC TESTS

- **pH** (791): 5.0–8.0, measured on the solution prepared in the tests for Viscosity at a temperature of  $20 \pm 2^\circ$ . Read the indicated pH value after the probe has been immersed for  $5 \pm 0.5$  min.
- **LOSS ON DRYING** (731): Dry 1.0 g at  $105^\circ$  for 1 h: it loses NMT 5.0% of its weight.
- **VISCOSITY—CAPILLARY VISCOMETER METHODS** (911) and **ROTATIONAL RHEOMETER METHODS** (912)

For hypromellose samples having a viscosity type of less than 600 mPa · s

**Sample solution:** Transfer a quantity of Hypromellose equivalent to 4 g of solids, calculated on the dried basis, to a tared, wide-mouth centrifuge bottle. Add hot water to obtain a total weight of the sample and water of 200.0 g. Capping the bottle, stir by mechanical means at  $400 \pm 50$  rpm for 10–20 min until the particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below  $10^\circ$  for another 20–40 min. Adjust the solution weight, if necessary, to 200.0 g, using cold water. Centrifuge the solution, if necessary, to expel any entrapped air. If any foam is present, remove with a spatula.

**Analysis:** Determine the viscosity in a suitable viscometer of the Ubbelohde type as directed under *Viscosity—Capillary Viscometer Methods* (911).

**Acceptance criteria:** 80%–120% of the viscosity stated on the label

For hypromellose samples having a viscosity type of 600 mPa · s or higher

**Sample solution:** Transfer a quantity of Hypromellose equivalent to 10 g of solids, calculated on the dried basis, to a tared, wide-mouth centrifuge bottle, and add hot water to obtain a total weight of the sample and water of 500.0 g. Capping the bottle, stir by mechanical means at  $400 \pm 50$  rpm for 10–20 min until the particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below  $10^\circ$  for another 20–40 min. Adjust the solution weight if necessary to 500.0 g, using cold water. Centrifuge the solution, if necessary, to expel any entrapped air. If any foam is present, remove with a spatula.

**Analysis:** Equip a suitable single-cylinder type rotational viscometer (Brookfield type LV Model, or equivalent), and determine the viscosity of this solution at  $20 \pm$

$0.1^\circ$  under the operating conditions specified in the table below.

Labeled Viscosity <sup>a</sup> (mPa · s)	Rotor No.	Revolution (rpm)	Calculation Multiplier
600 or more and less than 1400	3	60	20
1400 or more and less than 3500	3	12	100
3500 or more and less than 9500	4	60	100
9500 or more and less than 99,500	4	6	1000
99,500 or more	4	3	2000

<sup>a</sup> The Labeled Viscosity is based on the manufacturer's specifications.

Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of 2 min between subsequent measurements. Repeat the operation twice to rotate the spindle as specified above, and average the three readings.

**Acceptance criteria:** 75%–140% of the viscosity stated on the label

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements are specified.
- **LABELING:** Label it to indicate its substitution type and its nominal viscosity value in millipascals per second (mPa · s).

## Hypromellose Ophthalmic Solution

» Hypromellose Ophthalmic Solution is a sterile solution of Hypromellose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of Hypromellose (hydroxypropyl methylcellulose). It may contain suitable antimicrobial, buffering, and stabilizing agents.

**Packaging and storage—**Preserve in tight containers.

**USP Reference standards** (11)—

USP Hypromellose RS

**Identification—**

**A:** Pour a few mL of Ophthalmic Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

**B:** Heat 5 mL of Ophthalmic Solution in a test tube over a low flame: the warm solution turns cloudy but clears upon chilling.

**Sterility** (71): meets the requirements.

**pH** (791): between 6.0 and 7.8.

**Assay—**

**Standard preparation—**Dissolve a suitable quantity of USP Hypromellose RS, accurately weighed, in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 100 µg per mL.

**Assay preparation—**Dilute an accurately measured volume of Ophthalmic Solution quantitatively with water to obtain a solution having an equivalent concentration of about 100 µg of hypromellose per mL.

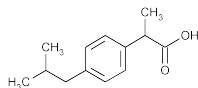
**Procedure—**Pipet 2 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide a blank, into separate, glass-stoppered test tubes. To each tube add 5.0 mL of diphenylamine solution (prepared by dissolving

3.75 g of colorless diphenylamine crystals in 150 mL of glacial acetic acid and diluting the solution with 90 mL of hydrochloric acid), mix, and immediately insert the tubes into an oil bath at 105° to 110° for 30 minutes, the temperature being kept uniform within 0.1° during heating. Remove the tubes, and place them in an ice-water bath for 10 minutes or until thoroughly cool. At room temperature and using a suitable spectrophotometer, concomitantly determine the absorbances of the solutions from the *Standard preparation* and the *Assay preparation* at 635 nm, using the water solution as the blank. Calculate the quantity, in mg, of hypromellose in each mL of the Ophthalmic Solution taken by the formula:

$$0.001Cd(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Hypromellose RS in the *Standard preparation*; *d* is the dilution factor used to obtain the *Assay preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ibuprofen



$C_{13}H_{18}O_2$  206.28

Benzeneacetic acid,  $\alpha$ -methyl-4-(2-methylpropyl), ( $\pm$ )-.

( $\pm$ )-*p*-Isobutylhydratropic acid.

( $\pm$ )-2-(*p*-Isobutylphenyl)propionic acid [15687-27-1].

( $\pm$ ) Mixture [58560-75-1].

» Ibuprofen contains not less than 97.0 percent and not more than 103.0 percent of  $C_{13}H_{18}O_2$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ibuprofen RS

USP Ibuprofen Related Compound C RS

**Identification**—

**A:** *Infrared Absorption* (197M)—Do not dry specimens.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 250  $\mu$ g per mL.

*Medium:* 0.1 N sodium hydroxide.

Respective absorptivities at 264 nm and 273 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**C:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ibuprofen, the retention time of which, relative to that of the internal standard, corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**Water**, *Method I* (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.5%.

**Heavy metals**, *Method II* (231): 0.002%.

**Chromatographic purity**—

*Mobile phase*—Prepare a suitable filtered mixture of water, previously adjusted with phosphoric acid to a pH of 2.5 and acetonitrile (1340:680). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Test preparation*—Prepare a solution of Ibuprofen in acetonitrile containing about 5 mg per mL.

*Resolution solution*—Prepare a solution in acetonitrile containing in each mL about 5 mg of Ibuprofen and 5 mg of valerophenone.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1 and is maintained at 30  $\pm$  0.5°. The flow rate is about 2 mL per minute. Chromatograph a series of 5- $\mu$ L injections of the *Test preparation* to condition the column. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for valerophenone and 1.0 for ibuprofen, and the resolution, *R*, between the valerophenone peak and the ibuprofen peak is not less than 2.0.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Inject about 5  $\mu$ L of the *Test preparation* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity taken by the formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of an individual peak, other than the solvent peak and the main ibuprofen peak, and  $r_t$  is the

sum of the responses of all the peaks, excluding that of the solvent peak: not more than 0.3% of any individual impurity is found, and the sum of all the individual impurities found does not exceed 1.0%.

**Limit of ibuprofen related compound C**—Using the chromatograms of the *Assay preparation* and the *Ibuprofen related compound C standard solution*, obtained as directed in the *Assay*, calculate the percentage of ibuprofen related compound C ( $C_{12}H_{16}O$ ) in the portion of Ibuprofen taken by the formula:

$$10,000(C / W)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ibuprofen Related Compound C RS in the *Ibuprofen related compound C standard solution*; *W* is the weight, in mg, of Ibuprofen taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of ibuprofen related compound C to valerophenone obtained from the *Assay preparation* and the *Ibuprofen related compound C standard solution*, respectively: not more than 0.1% is found.

**Assay**—

*Mobile phase*—Dissolve 4.0 g of chloroacetic acid in 400 mL of water, and adjust with ammonium hydroxide to a pH of 3.0. Add 600 mL of acetonitrile, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of valerophenone in *Mobile phase* having a concentration of about 0.35 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ibuprofen RS in *Internal standard solution* to obtain a solution having a known concentration of about 12 mg per mL.

*Ibuprofen related compound C standard solution*—Quantitatively dissolve an accurately weighed quantity of USP Ibuprofen Related Compound C RS in acetonitrile to obtain a solution having a known concentration of about 0.6 mg per mL. Add 2.0 mL of this stock solution to 100.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.012 mg of ibuprofen related compound C per mL.

*Assay preparation*—Transfer about 1200 mg of Ibuprofen, accurately weighed, to a 100-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.4 for the internal standard and 1.0 for ibuprofen; the resolution, *R*, between ibuprofen and the internal standard is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Ibuprofen related compound C standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for valerophenone and 1.2 for ibuprofen related compound C; the resolution, *R*, between valerophenone and ibuprofen related compound C is not less than 2.5; the tailing factors for the individual peaks are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation*, the *Assay preparation*, and the *Ibuprofen related compound C standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in

mg, of  $C_{13}H_{18}O_2$  in the portion of Ibuprofen taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ibuprofen RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ibuprofen Oral Suspension

» Ibuprofen Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{13}H_{18}O_2$ .

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Ibuprofen RS

USP Ibuprofen Related Compound C RS

### Identification—

**A:** Transfer a volume of Oral Suspension, equivalent to about 200 mg of ibuprofen, to a separator containing about 10 mL of chloroform, and shake for about 1 minute. Allow the layers to separate, and pass the lower chloroform layer through a filter containing about 2 g of anhydrous sodium sulfate. Use the filtrate as the test solution. [NOTE—Retain a portion of this test solution for use in *Identification* test B.] Separately apply 10- $\mu$ L portions of the test solution and of a Standard solution containing 20 mg per mL of USP Ibuprofen RS in chloroform to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 minutes. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-hexane, butyl acetate, and glacial acetic acid (17:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry in a current of cool air. Examine the chromatograms under short-wave-length UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B: Infrared Absorption** (197K)—Prepare the test specimen and the standard as follows. Evaporate about 20 drops of the test solution and the Standard solution retained from *Identification* test A to dryness in a current of air without heating.

### Dissolution (711)—

**Medium:** pH 7.2 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

Determine the percentage of the labeled amount of  $C_{13}H_{18}O_2$  dissolved by the following procedure:

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay*.

**Internal standard solution**—Prepare a solution of benzophenone in acetonitrile containing about 0.3 mg per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Ibuprofen RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.011/ mg per mL,  $J$  being the labeled quantity, in mg, of ibuprofen in each mL of the Oral Suspension. Mix 10.0 mL of this solution and 10.0 mL of the *Internal standard solution*, pass the mixture through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Standard solution*.

**Test solution**—Filter a portion of the solution under test. Mix 10.0 mL of the filtrate and 10.0 mL of the *Internal standard solution*, pass the mixture through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Test solution*.

**Procedure**—Using an accurately tared syringe, draw about 10 mL of well-mixed Oral Suspension into the syringe, which is connected to tubing, and accurately weigh. [NOTE—The tubing of the syringe is placed into a zone that is between the surface of the *Dissolution Medium* and the top of the rotating blade.] Express the Oral Suspension into the *Dissolution Medium*. Promptly reweigh the syringe, and determine the weight,  $W_U$ , in g, of the Oral Suspension added to the *Dissolution Medium*. Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of the labeled amount of  $C_{13}H_{18}O_2$  dissolved by the formula:

$$90,000(C/L)(D/W_U)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ibuprofen RS in the *Standard solution*;  $L$  is the labeled quantity, in mg per mL, of ibuprofen in the Oral Suspension;  $D$  is the density, in g per mL, of the Oral Suspension, determined as directed for *Density* in the *Assay*;  $W_U$  is the weight, in g, of the Oral Suspension added to the *Dissolution Medium*; and  $R_U$  and  $R_S$  are the ratios of the ibuprofen peak areas to the benzophenone peak areas obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{13}H_{18}O_2$  is dissolved in 60 minutes.

### Uniformity of dosage units (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

### Deliverable volume (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.6 and 4.6.

**Deliverable volume** (698): meets the requirements.

### Limit of ibuprofen related compound C—

**Mobile phase and Diluent**—Proceed as directed in the *Assay*.

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Ibuprofen Related Compound C RS in acetonitrile to obtain a stock solution having a known concentration of about 0.5 mg per mL. Transfer 3.0 mL of this stock solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 2.0 mL of this solution to a second 50-mL volumetric flask, add 18 mL of *Diluent*, dilute with acetonitrile to volume, mix, and pass through a filter having a porosity of 0.22- $\mu$ m. This *Standard solution* contains about 0.0012 mg of ibuprofen related compound C per mL.

**Test solution**—Transfer 20.0 mL of the portion of the stock solution retained from the *Assay preparation* in the *Assay* into a 50-mL volumetric flask, dilute with acetonitrile to volume, mix, and pass through a filter having a porosity of 0.22- $\mu$ m.

**System suitability solution**—Transfer 1.5 mL of the stock solution of USP Ibuprofen Related Compound C RS prepared as directed for *Standard solution* and 9 mL of the stock solution of USP Ibuprofen RS prepared as directed for *Standard preparation* in the *Assay* to a 25-mL volumetric flask, dilute with acetonitrile to volume, mix, and pass through a filter having a porosity of 0.22- $\mu$ m. This solution contains about 0.03 mg of ibuprofen related compound C and about 0.4 mg of ibuprofen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 2 mL per minute. Chromatograph

the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.3 for ibuprofen related compound C and 1.0 for ibuprofen; the resolution,  $R$ , between ibuprofen and ibuprofen related compound C is not less than 1.5; and the tailing factor is not more than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 35  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of ibuprofen related compound C in the Oral Suspension, based on the labeled content of ibuprofen, taken by the formula:

$$(12,500C/DL)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ibuprofen Related Compound C RS in the *Standard solution*;  $D$  is the quantity, in mL, of Oral Suspension taken to prepare the stock solution for the *Assay preparation*;  $L$  is the labeled quantity, in mg, of ibuprofen in each mL of Oral Suspension; and  $r_U$  and  $r_S$  are the ibuprofen related compound C peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 0.25% is found.

#### Assay—

**Mobile phase**—Dilute 0.7 mL of phosphoric acid with water to obtain 1000 mL of 0.01 M phosphoric acid. Prepare a mixture of this solution and acetonitrile (63:37). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of acetonitrile and water (1:1).

**Internal standard solution**—Prepare a solution of benzophenone in acetonitrile containing about 3.2 mg per mL.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Ibuprofen RS in *Diluent* to obtain a stock solution having a known concentration of about 1.2 mg per mL. Transfer 20.0 mL of this stock solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, mix, and filter. This solution contains about 0.48 mg of ibuprofen per mL.

**Density**—Using a tared 50-mL volumetric flask, weigh 50 mL of Oral Suspension that has been previously well shaken to ensure homogeneity, allow to stand until the entrapped air has risen, and finally invert carefully just prior to transferring it to the volumetric flask. From the observed weight of 50 mL of the Oral Suspension, calculate the density, in g per mL, of the Oral Suspension.

**Assay preparation**—Transfer an accurately weighed portion of Oral Suspension, equivalent to about 60 mg of ibuprofen, to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix (stock solution). Transfer 20.0 mL of this stock solution and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with acetonitrile to volume, mix, and filter. [NOTE—Retain a portion of the stock solution for use in the test for *Limit of ibuprofen related compound C*.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for benzophenone and 1.0 for ibuprofen; the resolution,  $R$ , between benzophenone and ibuprofen is not less than 1.5; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{13}H_{18}O_2$  in each mL of the Oral Suspension taken by the formula:

$$125C(D/W_U)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ibuprofen RS in the *Standard preparation*;  $D$  is the density, in g per mL, of Oral Suspension;  $W_U$  is the weight, in g, of the portion of Oral Suspension taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the ibuprofen peak areas to the benzophenone peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ibuprofen Tablets

» Ibuprofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{13}H_{18}O_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Where the Tablets are gelatin-coated, the label so states.

#### USP Reference standards (11)—

USP Ibuprofen RS

USP Ibuprofen Related Compound C RS

#### Identification—

**A:** Grind 1 Tablet to a fine powder in a mortar, add about 5 mL of chloroform, and swirl. Filter the mixture, and evaporate the filtrate with the aid of a stream of nitrogen to dryness: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ibuprofen RS.

**B:** Its retention time, relative to that of the internal standard, determined as directed in the *Assay*, corresponds to that of USP Ibuprofen RS.

#### Dissolution (711)—

**Medium:** pH 7.2 phosphate buffer (see under *Buffers* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_{13}H_{18}O_2$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 221 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ibuprofen RS in the same medium. [NOTE—Where the Tablets are labeled as gelatin-coated, determine the amount of  $C_{13}H_{18}O_2$  dissolved from the UV absorbance at the wavelength of maximum absorbance at about 266 nm from which is subtracted the absorbance at 280 nm, in comparison with the Standard solution similarly measured.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{13}H_{18}O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water, Method I** (921): not more than 5.0%, except that Tablets labeled as gelatin-coated are exempt from this requirement.

**Limit of ibuprofen related compound C**—Using the chromatograms of the *Assay preparation* and the *Ibuprofen related compound C standard solution* obtained as directed in

the Assay, calculate the percentage of ibuprofen related compound C ( $C_{12}H_{16}O$ ) in the Tablets taken by the formula:

$$10,000C(A/W)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ibuprofen Related Compound C RS in the *Ibuprofen related compound C standard solution*; A is the average weight, in mg, of a Tablet; W is the weight of Tablet powder taken to prepare the *Assay preparation*; I is the quantity, in mg, of ibuprofen per Tablet as obtained in the *Assay*; and  $R_U$  and  $R_S$  are the ratios of the ibuprofen related compound C peak response to the valerophenone peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.25% per Tablet is found.

#### Assay—

*Mobile phase, Internal standard solution, and Standard preparation*—Prepare as directed in the *Assay under Ibuprofen*.

*Ibuprofen related compound C standard solution*—Quantitatively dissolve an accurately weighed quantity of USP Ibuprofen Related Compound C RS in acetonitrile to obtain a stock solution having a known concentration of about 0.6 mg per mL. Add 2.0 mL of this stock solution to 100 mL of *Internal standard solution*, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1200 mg of ibuprofen, to a suitable container, add 100.0 mL of *Internal standard solution*, and shake for 10 minutes. [NOTE—Where the Tablets are coated, place an accurately counted number of Tablets, equivalent to not less than 1200 mg of ibuprofen, in a container, add an accurately measured volume of *Internal standard solution*, sufficient to obtain an *Assay preparation* containing about 12 mg of ibuprofen per mL, and about 15 glass beads, and shake until the Tablets are completely disintegrated.] Centrifuge a portion of the suspension so obtained and use the clear supernatant as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.75 for ibuprofen and 1.0 for valerophenone; the resolution,  $R$ , between ibuprofen and valerophenone is not less than 2.5; the tailing factors for the individual peaks are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Ibuprofen related compound C standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for valerophenone and 1.2 for ibuprofen related compound C; the resolution,  $R$ , between valerophenone and ibuprofen related compound C is not less than 2.5; the tailing factors for the individual peaks are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation*, the *Assay preparation*, and the *Ibuprofen related compound C standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ibuprofen ( $C_{13}H_{18}O_2$ ) in each Tablet taken by the formula:

$$100C(A/W)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ibuprofen RS in the *Standard preparation*; A is the average weight, in mg, of a Tablet; W is the weight, in mg, of Tablet powder taken to prepare the *Assay preparation*; and  $R_U$  and  $R_S$  are the ratios of the ibuprofen peak response to the valerophenone peak response obtained from the *Assay prepara-*

*tion* and the *Standard preparation*, respectively; or where intact Tablets were taken, calculate the quantity, in mg, of  $C_{13}H_{18}O_2$  in each Tablet taken by the formula:

$$(CV/N)(R_U/R_S)$$

in which V is the volume, in mL, of *Internal standard solution* used to prepare the *Assay preparation*; N is the number of Tablets taken; and the other terms are as defined above.

## Ibuprofen and Pseudoephedrine Hydrochloride Tablets

» Ibuprofen and Pseudoephedrine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Ibuprofen ( $C_{13}H_{18}O_2$ ) and Pseudoephedrine Hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards <11>—

USP Ibuprofen RS

USP Pseudoephedrine Hydrochloride RS

#### Identification—

**A:** Place a Tablet in a small beaker, crack the Tablet coating, add 10 mL of methanol, and stir by mechanical means for about 10 minutes. Allow to settle, and use the clear supernatant as the *Test solution*. Prepare a Standard solution in methanol containing about 20 mg of USP Ibuprofen RS and 20J mg of USP Pseudoephedrine Hydrochloride RS per mL, J being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of ibuprofen per Tablet. Separately apply 10  $\mu$ L each of the *Test solution* and the *Standard solution* to a thin-layer chromatographic plate (see *Chromatography* <621>) covered with a 0.25-mm layer of chromatographic silica gel mixture and activated by heating the plate at 105° for about 30 minutes. Place the plate in a chromatographic chamber equilibrated with a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (80:15:5). Develop the chromatograms until the solvent has moved about 10 cm from the origin. Remove the plate from the chromatographic chamber, place it in a chamber containing iodine vapors for about 10 minutes, and examine the chromatograms: the principal spots obtained from the *Test solution* correspond in  $R_f$  value and appearance to those obtained from the *Standard solution*.

**B:** The retention times of the pseudoephedrine and ibuprofen peaks, relative to that of the butylparaben internal standard peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution <711>—

*Medium:* pH 7.2 phosphate buffer (see under *Buffers* in the section *Reagents, Indicators, and Solutions*); 900 mL.

*Apparatus 2:* 50 rpm.

*Times:* 30 minutes (ibuprofen); 45 minutes (pseudoephedrine hydrochloride).

*Procedure for ibuprofen*—Determine the amount of ibuprofen ( $C_{13}H_{18}O_2$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 224 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Ibuprofen RS in the same medium.

*Procedure for pseudoephedrine hydrochloride*—

**MOBILE PHASE**—Prepare a solution of monobasic potassium phosphate in water containing 500 mg per 1000 mL. Filter

through a filter having a porosity of 0.5  $\mu\text{m}$  or finer. Prepare a mixture of this solution and acetonitrile (500:500), and adjust with phosphoric acid to a pH of  $3.3 \pm 0.1$ . Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)). Increasing the concentration of monobasic potassium phosphate or increasing the pH increases the retention time of pseudoephedrine.

**STANDARD PREPARATION**—Prepare a solution of USP Pseudoephedrine Hydrochloride RS in *Dissolution Medium* having a known concentration of about  $P/900$  mg per mL,  $P$  being the labeled quantity, in mg, of pseudoephedrine hydrochloride per Tablet.

**CHROMATOGRAPHIC SYSTEM** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector, a guard column containing packing L10, and a 4.6-mm  $\times$  25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**PROCEDURE**—Pass a portion of the solution under test through a filter having a porosity of 0.5  $\mu\text{m}$  or finer. Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) dissolved by the formula:

$$900C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are pseudoephedrine peak responses obtained from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amounts of ibuprofen ( $\text{C}_{13}\text{H}_{18}\text{O}_2$ ) and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) are dissolved in 30 minutes and in 45 minutes, respectively.

#### Uniformity of dosage units (905)—

**Procedure for content uniformity**—Proceed as directed in the *Assay*, preparing the *Assay preparation* as follows. Transfer 1 Tablet to a glass-stoppered conical flask, add 10.0 mL of *Internal standard solution*, and stir with a magnetic stirrer until the Tablet disintegrates. Add 10.0 mL of acetonitrile, stir for about 15 minutes, and filter.

#### Assay—

**Mobile phase**—Dissolve 2.5 g of docusate sodium in a mixture of water and acetonitrile (590:410). Add 1.0 mL of phosphoric acid, and adjust with ammonium hydroxide to a pH of  $3.2 \pm 0.05$ . Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)). Decreasing the proportion of docusate sodium increases the resolution between pseudoephedrine and ibuprofen.

**Internal standard solution**—Prepare a solution of butylparaben in *Mobile phase* containing about 0.15 mg per mL.

**Standard preparation**—Prepare a solution in *Internal standard solution* having known concentrations of about 20 mg of USP Ibuprofen RS and 20/ mg of USP Pseudoephedrine Hydrochloride RS per mL,  $J$  being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of ibuprofen per Tablet. To the resulting solution add an equal volume of acetonitrile, accurately measured, and mix. Pass through a filter having 0.5  $\mu\text{m}$  porosity or finer, and use the filtrate as the *Standard preparation*. This solution contains about 10 mg of USP Ibuprofen RS and 10/ mg of USP Pseudoephedrine Hydrochloride RS per mL.

**Assay preparation**—Transfer an accurately counted number of Tablets, equivalent to about 2000 mg of ibuprofen, to a glass-stoppered conical flask, add 100 mL of *Internal standard solution*, and stir with a magnetic stirrer until the Tablets disintegrate. Add 100 mL of acetonitrile, and mix. Filter through a filter of 0.5  $\mu\text{m}$  porosity or finer, and use the filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector, a guard column that contains packing L1, and a 4.6-mm  $\times$  10-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.55 for butylparaben, 0.7 for pseudoephedrine, and 1.0 for ibuprofen; the resolution,  $R$ , between the butylparaben peak and the pseudoephedrine peak and between the pseudoephedrine peak and the ibuprofen peak is not less than 2.0; the tailing factors for the butylparaben peak, the pseudoephedrine peak, and the ibuprofen peak are not more than 3.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of ibuprofen ( $\text{C}_{13}\text{H}_{18}\text{O}_2$ ) and of pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) in each Tablet taken by the formula:

$$200(C / N)(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ibuprofen RS or USP Pseudoephedrine Hydrochloride RS, as appropriate, in the *Standard preparation*;  $N$  is the number of Tablets taken; and  $R_U$  and  $R_S$  are the ratios of the relevant analyte peak response to the butylparaben peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ichthammol

Ichthammol.

Ichthammol [8029-68-3].

» Ichthammol is obtained by the destructive distillation of certain bituminous schists, sulfonation of the distillate, and neutralization of the product with ammonia. Ichthammol yields not less than 2.5 percent of ammonia ( $\text{NH}_3$ ) and not less than 10.0 percent of total sulfur ( $S$ ).

**Packaging and storage**—Preserve in well-closed containers.

#### Identification—

**A:** Dilute 10 mL with 90 mL of water, and stir for 5 minutes with a magnetic stirrer. Add 25 mL of hydrochloric acid, and mix: a heavy, resinous precipitate is formed. Remove the liquid by decantation, and wash the precipitate with 2 N hydrochloric acid until the last washing is nearly colorless. Transfer the precipitate to absorbent paper, allow it to stand for 10 minutes, and then transfer 10 mg of the precipitate to a 250-mL conical flask. To the flask add 100 mL of ether, attach an air condenser to the flask, and stir for 30 minutes with a magnetic stirrer: the precipitate does not dissolve completely.

**B:** To a solution (1 in 10) add 1 N sodium hydroxide, and heat to the boiling point: ammonia is evolved.

**Loss on drying** (731)—Dry it at 80° for 8 hours, and continue the drying at 100° for constant weight: it loses not more than 50.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Limit for ammonium sulfate**—Accurately weigh about 1 g, transfer to a 100-mL beaker, and add 25 mL of alcohol. Stir, filter, and wash the filter with a mixture of equal volumes of ether and alcohol until the last washing is clear and colorless. Air-dry the filter and residue, and pass 200 mL of warm water, slightly acidified with hydrochloric acid, through the residue on the filter. Heat the filtrate to boiling, add barium chloride TS in excess, and heat for 1 hour on a steam bath. Collect the precipitate of barium sulfate on a filter, wash it well, dry, and ignite to constant weight. Each g of barium sulfate is equivalent to 566.1 mg of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Ichthammol contains not more than 8.0% of ammonium sulfate.

**Assay for ammonia**—Dissolve about 5 g of Ichthammol, accurately weighed, in 100 mL of water, transfer the solution to a distillation flask, add 3 g of paraffin, and add 20 mL of sodium hydroxide solution (4 in 10). Connect the flask to a condenser by means of a spray trap, and immerse the lower outlet tube of the condenser in 30.0 mL of 0.5 N sulfuric acid VS. Distill slowly, collect about 50 mL of distillate, and then titrate the excess acid with 0.5 N sodium hydroxide VS, using methyl red TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sulfuric acid is equivalent to 8.515 mg of NH<sub>3</sub>.

**Assay for total sulfur**—Transfer from 500 mg to 800 mg of Ichthammol, accurately weighed, to a Kjeldahl flask with the aid of 20 mL of water. Add 3 g of potassium chlorate, then add slowly 30 mL of nitric acid, and evaporate the mixture on a hot plate to about 5 mL. Cool, repeat the oxidation with 3 g of potassium chlorate and 30 mL of nitric acid, and evaporate to about 5 mL. Add 25 mL of hydrochloric acid, and again evaporate to about 5 mL. Add 100 mL of water, heat to boiling, filter, and wash well. To the hot filtrate add 25 mL of barium chloride TS, and heat on a steam bath for 1 hour. Collect the barium sulfate on a previously ignited and tared filtering crucible, wash, dry, and ignite, then cool, and weigh. Each g of barium sulfate is equivalent to 137.4 mg of S.

## Ichthammol Ointment

» Ichthammol Ointment contains an amount of Ichthammol equivalent to not less than 0.25 percent of ammonia (NH<sub>3</sub>).

Ichthammol . . . . .	100 g
Lanolin . . . . .	100 g
Petrolatum . . . . .	800 g
to make . . . . .	1000 g

Thoroughly incorporate the Ichthammol with the Lanolin, and combine this mixture with the Petrolatum.

**Packaging and storage**—Preserve in collapsible tubes or in tight containers, and avoid prolonged exposure to temperatures exceeding 30°.

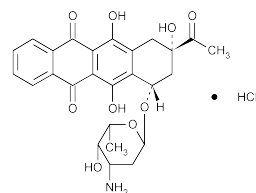
### Assay—

**Assay preparation**—Transfer an accurately weighed portion of Ointment, equivalent to about 2 g of ichthammol, to a 250-mL beaker, and add about 70 mL of boiling water.

Mix with a glass rod, heat on a steam bath, with frequent agitation, for 10 minutes, cover with a watch glass without removing the stirring rod, and allow to stand at room temperature for 15 to 20 minutes. Place in a refrigerator to cause the upper layer to congeal, form a second opening through the congealed layer with the glass rod, and transfer the dark-colored aqueous extract to a funnel containing a pledget of cotton, collecting the filtrate in a 500-mL volumetric flask. Repeat the extraction of the portion of the Ointment several times in the same manner until the aqueous extract is practically colorless, passing each extract through the same cotton filter into the flask containing the main extract. Dilute with water to volume, and mix.

**Procedure for ammonia**—Transfer 100.0 mL of the *Assay preparation* to a suitable distillation flask, add 3 g of paraffin, and add 20 mL of sodium hydroxide solution (4 in 10). Connect the flask to a condenser by means of a spray trap, and immerse the lower outlet tube of the condenser in 30.0 mL of 0.05 N sulfuric acid VS. Distill slowly, collect about 50 mL of distillate, and then titrate the excess acid with 0.05 N sodium hydroxide VS, using methyl red TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N sulfuric acid is equivalent to 0.8515 mg of NH<sub>3</sub>.

## Idarubicin Hydrochloride



C<sub>26</sub>H<sub>27</sub>NO<sub>9</sub> · HCl 533.95

5,12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7*S*-*cis*)-(1*S*,3*S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside, hydrochloride [57852-57-0].

» Idarubicin Hydrochloride contains not less than 960 μg and not more than 1030 μg of C<sub>26</sub>H<sub>27</sub>NO<sub>9</sub> · HCl per mg, calculated on the anhydrous basis.

**Caution**—Great care should be taken to prevent inhaling particles of Idarubicin Hydrochloride and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The amorphous form is so labeled.

**USP Reference standards** (11)—

USP Idarubicin Hydrochloride RS

### Identification—

**A: Infrared Absorption** (197K).

**B:** The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for idarubicin, the retention time of which corresponds to that in the chromatogram of the *Standard preparation* obtained in the *Assay*.

**Crystallinity** (695): meets the requirements, except where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions.

**pH** (791): between 5.0 and 6.5, in a solution containing 5 mg per mL.



**Water, Method I** (921): not more than 5.0%.

**Chromatographic purity**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, and disregarding the solvent peak, calculate the percentage of each impurity taken by the formula:

$$100r_i / r_s$$

in which  $r_i$  is the response of each impurity peak; and  $r_s$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and the sum of all impurities is not more than 3.0%.

**Assay**—

**Mobile phase**—Prepare a mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 g of sodium lauryl sulfate in 1000 mL of this solution, adjust with 2 N sodium hydroxide to a pH of  $3.6 \pm 0.1$ , pass through a filter having a porosity of 0.5  $\mu\text{m}$  or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare as directed for *Mobile phase*, except to omit the sodium lauryl sulfate.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Idarubicin Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 500  $\mu\text{g}$  of idarubicin hydrochloride per mL.

**Assay preparation**—Transfer about 50 mg of Idarubicin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in *Diluent*, dilute with *Diluent* to volume, and mix.

**Resolution solution**—Prepare an aqueous solution containing 1 mg of Idarubicin Hydrochloride per mL. To 2 mL of this solution in a test tube, add 20  $\mu\text{L}$  of hydrochloric acid, and heat in an oil bath at  $95^\circ$  for about 8 minutes. Mix 1 mL of this solution and 9 mL of *Diluent*. This *Resolution solution* contains a mixture of 4-demethoxydaunorubicinone and idarubicin.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L13. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for 4-demethoxydaunorubicinone and 1.0 for idarubicin; and the resolution,  $R$ , between the 4-demethoxydaunorubicinone peak and the idarubicin peak is not less than 9.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the idarubicin peak is not less than 10 and not more than 20; the tailing factor for the idarubicin peak is not less than 0.85 and not more than 1.2; the column efficiency calculated from the idarubicin peak is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of  $\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$  in each mg of the Idarubicin Hydrochloride taken by the formula:

$$100(C/M)(r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of idarubicin hydrochloride ( $\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$ ) in the *Standard preparation*;  $M$  is the quantity, in mg, of Idarubicin Hydrochloride taken to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the responses of the idarubicin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Idarubicin Hydrochloride for Injection

» Idarubicin Hydrochloride for Injection is a sterile mixture of Idarubicin Hydrochloride and Lactose. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$ .

**Caution**—Great care should be taken to prevent inhaling particles of Idarubicin Hydrochloride and exposing the skin to it.

**Packaging and storage**—Preserve in Containers for Sterile Solids as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Idarubicin Hydrochloride RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for idarubicin, the retention time of which corresponds to that in the chromatogram of the *Standard preparation* obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 8.9 USP Endotoxin Units per mg of idarubicin hydrochloride, a solution of Idarubicin Hydrochloride for Injection containing 0.07 mg of idarubicin hydrochloride per mL being used in the *Test Procedure*.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.0, in a solution constituted as directed in the labeling, water being used as the diluent.

**Water, Method I** (921): not more than 4.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* under *Injections* (1).

**Assay**—

**Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the *Assay* under *Idarubicin Hydrochloride*.

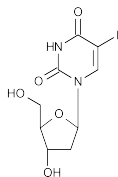
**Assay preparation**—Dilute the contents of 1 container of Idarubicin Hydrochloride for Injection quantitatively with *Diluent* to obtain a solution containing about 0.5 mg of idarubicin hydrochloride per mL.

**Procedure**—Proceed as directed for *Procedure* under *Idarubicin Hydrochloride*. Calculate the quantity, in mg, of  $\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$  in the container of Idarubicin Hydrochloride for Injection taken by the formula:

$$(C / 1000)(L / D)(r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of idarubicin hydrochloride ( $\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$ ) in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of idarubicin hydrochloride in the container;  $D$  is the concentration, in mg per mL, of idarubicin hydrochloride in the *Assay preparation* on the basis of the labeled quantity in the container and the extent of dilution; and  $r_u$  and  $r_s$  are the responses of the idarubicin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Idoxuridine



$C_9H_{11}IN_2O_5$  354.10  
Uridine, 2'-deoxy-5-iodo-  
2'-Deoxy-5-iodouridine [54-42-2].

» Idoxuridine contains not less than 98.0 percent and not more than 101.0 percent of  $C_9H_{11}IN_2O_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Idoxuridine RS

### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

Solution: 35  $\mu$ g per mL.

**Medium:** pH 12.0 buffer (prepared from 7.46 g of potassium chloride and 24 mL of 1 N sodium hydroxide dissolved in 2000 mL of water).

Absorptivities at 279 nm, calculated on the dried basis for the test sample only, do not differ by more than 2.0%.

**Loss on drying** (731)—Dry about 500 mg, accurately weighed, in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Assay**—Dissolve about 250 mg of Idoxuridine, accurately weighed, in 20 mL of dimethylformamide that previously has been neutralized with 0.1 N sodium methoxide in toluene VS, a solution of 300 mg of thymol blue in 100 mL of methanol being used as the indicator. Titrate with 0.1 N sodium methoxide in toluene VS to a blue endpoint, taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 35.41 mg of  $C_9H_{11}IN_2O_5$ .

## Idoxuridine Ophthalmic Ointment

» Idoxuridine Ophthalmic Ointment is Idoxuridine in a Petrolatum base. It contains not less than 0.45 percent and not more than 0.55 percent of  $C_9H_{11}IN_2O_5$ . It is sterile.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes in a cool place.

**USP Reference standards** (11)—  
USP Idoxuridine RS

**Identification**—The UV absorption spectrum of the solution from the Ophthalmic Ointment employed for measurement of absorbance in the Assay exhibits maxima and minima at the same wavelengths as that of the *Standard preparation* prepared for the Assay.

**Sterility** (71): meets the requirements.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

### Assay—

**Chromatographic column**—Mix 4 g of chromatographic siliceous earth with 4 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Transfer to a 19- × 250-mm chromatographic tube (see *Chromatography* (621)) that contains a pledget of glass wool and is fitted with a stopcock at the bottom. Tamp gently to compress to a uniform mass.

**Standard preparation**—Transfer about 25 mg of USP Idoxuridine RS, accurately weighed, to a 50-mL volumetric flask, add methanol to volume, and mix. Dilute 5.0 mL of this solution with a mixture of 1 volume of butyl alcohol and 5 volumes of chloroform to 100.0 mL, and mix.

**Assay preparation**—Mix 4 g of chromatographic siliceous earth with 2 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Add a quantity of Ophthalmic Ointment, equivalent to about 5 mg of idoxuridine and accurately weighed, to the mixture, and mix.

**Procedure**—Transfer the Assay preparation to the prepared *Chromatographic column*. Transfer 2 g of chromatographic siliceous earth and 2 mL of 0.1 N hydrochloric acid to the glass mortar, and mix until fluffy, using this material to rinse the mortar and pick up any remaining Ophthalmic Ointment. Transfer about half of this mixture to the tube, and tamp gently until the column appears uniform. Transfer the remaining portion to the *Chromatographic column*, and tamp as before. Wipe the walls of the mortar with a small pledget of glass wool, and insert the pledget in the top of the column. Pass 50 mL of chloroform through the column at a flow rate of approximately 1 mL per minute, and discard the chloroform. Elute with about 200 mL of a mixture of 1 volume of butyl alcohol and 5 volumes of chloroform at the same flow rate, discarding the first 20 mL of the eluate. Collect the remainder of the eluate in a 200-mL volumetric flask, dilute with the eluting solvent to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* in 1-cm cells at 320 nm and at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer, using a mixture of butyl alcohol and chloroform as the blank. Calculate the quantity, in mg, of  $C_9H_{11}IN_2O_5$  in the Ophthalmic Ointment taken by the formula:

$$0.2C(A_{283} - A_{320})_U / (A_{283} - A_{320})_S$$

in which C is the concentration, in  $\mu$ g per mL, of USP Idoxuridine RS in the *Standard preparation*; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Ophthalmic Ointment (U) and the *Standard preparation* (S), respectively.

## Idoxuridine Ophthalmic Solution

» Idoxuridine Ophthalmic Solution is a sterile, aqueous solution of Idoxuridine. It contains not less than 0.09 percent and not more than 0.11 percent of  $C_9H_{11}IN_2O_5$ . It may contain suitable buffers, stabilizers, and antimicrobial agents.

**Packaging and storage**—Preserve in tight, light-resistant containers in a cold place.

**USP Reference standards** (11)—  
USP Idoxuridine RS

**Identification**—The UV absorption spectrum of the solution employed for measurement of absorbance in the Assay exhibits maxima and minima at the same wavelengths as that of the *Standard preparation* prepared for the Assay.

**Sterility** (71): meets the requirements.

**pH** (791): between 4.5 and 7.0.

**Assay—**

**Chromatographic column and Standard preparation—**Prepare as directed in the Assay under *Idoxuridine Ophthalmic Ointment*.

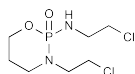
**Assay preparation—**Mix an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of idoxuridine, with 3 g of chromatographic siliceous earth in a glass mortar until the mixture is fluffy.

**Procedure—**Proceed as directed for *Procedure* in the Assay under *Idoxuridine Ophthalmic Ointment*, omitting the treatment of the column with 50 mL of chloroform. Calculate the quantity, in mg, of  $C_7H_{15}Cl_2N_2O_2P$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.2C(A_{283} - A_{320})_U / V(A_{283} - A_{320})_S$$

in which *C* is the concentration, in  $\mu\text{g}$  per mL, of USP Idoxuridine RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the Solution (*U*) and the *Standard preparation* (*S*), respectively.

## Ifosfamide



$C_7H_{15}Cl_2N_2O_2P$  261.09

2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,  
3-bis(2-chloroethyl)tetrahydro-, 2-oxide.

3-(2-Chloroethyl)-[(2-chloroethyl)amino]tetrahydro-2*H*-1,3,  
2-oxazaphosphorine 2-oxide [3778-73-2].

» Ifosfamide contains not less than 98.0 percent and not more than 102.0 percent of  $C_7H_{15}Cl_2N_2O_2P$ .

**Caution—***Great care should be taken in handling Ifosfamide, as it is a potent cytotoxic agent and suspected carcinogen.*

**Packaging and storage—**Preserve in tight containers at a temperature not exceeding 25°.

**Labeling—**Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Ifosfamide RS

**Identification—**

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the Assay.

**pH** (791): between 4.0 and 7.0 in a solution (1 in 10).

**Water, Method I** (921): not more than 0.3%.

**Heavy metals, Method I** (231): not more than 0.002%.

**Ionic chloride—**

**Standard sodium chloride solution—**Transfer about 118.7 mg of sodium chloride, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with water to

volume, and mix. This solution contains 360 ppm of ionic chloride.

**Procedure—**Pipet 10 mL of *Standard sodium chloride solution* into a beaker, and add 90 mL of water and 10 mL of acetic acid. Titrate with 0.01 N silver nitrate VS (prepared fresh daily), determining the endpoint potentiometrically using silver and silver-silver chloride electrodes. Record the volume,  $V_1$ , of 0.01 N silver nitrate VS consumed. Transfer about 2.0 g of Ifosfamide, accurately weighed, into a beaker, and add 90 mL of water and 10 mL of acetic acid. Pipet 10 mL of *Standard sodium chloride solution* into the beaker, and stir, if necessary, until solution is complete. Titrate with 0.01 N silver nitrate VS as directed above, and record the volume,  $V_2$ , of 0.01 N silver nitrate VS consumed. Calculate the difference in volume, *V*, of 0.01 N silver nitrate VS consumed between the two determinations by subtracting  $V_1$  from  $V_2$ : a difference of not more than 1.0 mL corresponding to not more than 0.018% of ionic chloride is found.

**Chloroform-insoluble phosphorus—**

**Ammonium molybdate solution—**[NOTE—Prepare fresh on the day of use.] Dissolve 25 g of ammonium molybdate in 300 mL of water (*Solution A*). Cautiously add 75 mL of sulfuric acid to 100 mL of water, cool to room temperature, and dilute with water to 200.0 mL (*Solution B*). Mix *Solution A* and *Solution B* to obtain *Ammonium molybdate solution*.

**Hydroquinone solution—**Dissolve 0.5 g of hydroquinone in 100 mL of water, and add one drop of concentrated sulfuric acid. [NOTE—When this solution darkens, discard it and prepare fresh.]

**Sodium sulfite solution—**Prepare a solution of sodium sulfite in water having a concentration of 200 mg per mL. [NOTE—Prepare fresh at the time of use.]

**Phosphorus stock solution—**Transfer 0.1824 g of monobasic potassium phosphate, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Phosphorus intermediate solution—**Transfer 10.0 mL of *Phosphorus stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Prepare this solution fresh on the day of use.

**Phosphorus standard solution—**Transfer 10.0 mL of *Phosphorus intermediate solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Test preparation—**Transfer 1 g of Ifosfamide, accurately weighed, to a 100-mL volumetric flask, dissolve in 50 mL of water, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a separatory funnel, and add 5 mL of water. Add 15 mL of chloroform, shake vigorously for 30 seconds, allow the layers to separate and drain, and discard the lower chloroform layer. Repeat this extraction four times, each time with 15 mL of chloroform, discarding the chloroform layer after each extraction. Transfer the aqueous portion to a conical flask, wash the separatory funnel with two 5-mL portions of water, and collect all the aqueous washings in the same flask. Add 3 mL of sulfuric acid, and heat under a hood until white fumes appear. Remove the flask from the heat, and with swirling, add 0.6 mL of hydrogen peroxide. Heat until white fumes reappear. If the solution is not colorless, repeat additions of hydrogen peroxide followed by heating until all color is gone. Cool to room temperature, add 25 mL of water, and cautiously add 10 mL of ammonium hydroxide solution. Cool to room temperature, add 2 drops of phenolphthalein TS, and then add hydrochloric acid dropwise until all pink color has disappeared. Transfer the contents of the flask to a 100-mL flask, dilute with water to volume, and mix.

**Blank solution—**To 3 mL of sulfuric acid in a second conical flask, adding 0.6 mL of hydrogen peroxide, proceed as directed for the *Test preparation*, beginning with "Heat until white fumes reappear."

**Procedure**—Transfer 15.0 mL each of the *Test preparation*, the *Blank solution*, and the *Phosphorus standard solution* to three separate 25-mL volumetric flasks. Add 2.5 mL of *Ammonium molybdate solution* to each of the flasks, swirl, and allow to stand for about 30 seconds. To each of the three flasks in order, rapidly add 2.5 mL each of *Hydroquinone solution* and *Sodium sulfite solution*. Dilute the contents of each flask with water to volume, mix, and allow the flasks to stand for 30 minutes. Concomitantly determine the absorbances of the solutions obtained from the *Test preparation* and the *Phosphorus standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 730 nm, with a suitable spectrophotometer, using the solution obtained from the *Blank solution* as the blank. Calculate the percentage of chloroform-insoluble phosphorus in the portion of Ifosfamide taken by the formula:

$$100(C / W)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of phosphorus in the *Phosphorus standard solution*; *W* is the weight, in mg, of Ifosfamide taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances from the solutions obtained from the *Test preparation* and the *Phosphorus standard solution*, respectively: not more than 0.0415% is found.

#### Limit of 2-chloroethylamine hydrochloride—

**Standard solution**—Dissolve an accurately weighed quantity of 2-chloroethylamine hydrochloride in *N,N*-dimethylacetamide, and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution having a known concentration of about 0.025 mg per mL.

**Test solution**—Transfer about 100 mg of Ifosfamide, accurately weighed, to a flask, add 10.0 mL of *N,N*-dimethylacetamide, and shake until dissolved.

**Chromatographic system**—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm × 1.8-m column packed with 10% liquid phase G16 containing 2% potassium hydroxide on 80- to 100-mesh support S1A. The injection port is maintained at a temperature of about 200°, the detector is maintained at a temperature of about 300°, the oven is maintained at a temperature of about 140°, and nitrogen is used as the carrier gas at a flow rate of about 25 mL per minute.

**Procedure**—Separately inject equal volumes (about 1.0 µL) of the *Test solution* and the *Standard solution* into the gas chromatograph, record the chromatograms, and measure the areas of the peaks due to 2-chloroethylamine hydrochloride. Calculate the percentage of 2-chloroethylamine hydrochloride in the portion of Ifosfamide taken by the formula:

$$1000(C / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of 2-chloroethylamine hydrochloride in the *Standard solution*; *W* is the weight, in mg, of Ifosfamide taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the areas of the 2-chloroethylamine peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% of 2-chloroethylamine hydrochloride is found.

**Other requirements**—Where the label states that Ifosfamide is sterile, it meets the requirements for *Sterility Tests* (71) and for *Bacterial endotoxins* under *Ifosfamide for Injection*. Where the label states that Ifosfamide must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ifosfamide for Injection*.

**Assay**—[NOTE—Ifosfamide degrades in solution. Prepare solutions of Ifosfamide fresh daily and do not store for more than 24 hours. Prepare the *Standard preparation* and the *Assay preparation* simultaneously.]

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 50 mg of ethylparaben, accurately weighed, to a 100-mL volumetric flask, and add 25 mL of alcohol to dissolve. Dilute with water to volume, and mix.

**Standard preparation**—Transfer about 15 mg of USP Ifosfamide RS, accurately weighed, to a 25-mL volumetric flask, add 1.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Assay preparation**—Transfer about 150 mg of Ifosfamide, accurately weighed, to a 250-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 195-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between ifosfamide and ethylparaben is not less than 6.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P in the portion of Ifosfamide taken by the formula:

$$250C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ifosfamide RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the responses of the ifosfamide peak to the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ifosfamide for Injection

» Ifosfamide for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P.

**Caution**—Great care should be taken in handling Ifosfamide, as it is a potent cytotoxic agent and suspected carcinogen.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), at controlled room temperature.

#### USP Reference standards (11)—

USP Endotoxin RS  
USP Ifosfamide RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

#### Identification—

**A:** (See *Thin-layer Chromatographic Identification Tests* (201).)

**Developing solvent**—Prepare a mixture of isopropyl alcohol and toluene (1:1).

**Standard solution**—Dissolve 20.0 mg of USP Ifosfamide RS in 1.0 mL of alcohol.

**Test solution**—Dissolve 20 mg of Ifosfamide for Injection in 1.0 mL of alcohol.

**Procedure**—Apply separately 10 µL each of the *Standard solution* and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, allow the spots to dry, and develop the plate in a paper-lined

chromatographic chamber equilibrated with *Developing solvent* for about 15 minutes prior to use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, mark the solvent front, and air-dry for 5 minutes. Place the plates into a chromatographic chamber containing iodine crystals, and view the spots that develop. [NOTE—For better detection, overspray the iodine stain with a mixture of alcohol and water (1:1).] The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.125 USP Endotoxin Unit per mg.

**pH** (791): between 4.0 and 7.0 in a solution prepared as directed for *Constituted Solutions* under *Injections* (1), determined 30 minutes after its preparation.

**Water, Method I** (921): not more than 0.3%.

**Other requirements**—It meets the requirements for *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and *Labeling* under *Injections* (1).

#### Assay—

*Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Ifosfamide*.

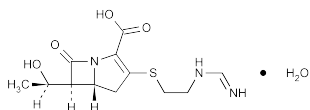
*Assay preparation*—Select an accurately counted number of containers of Ifosfamide for Injection, the combined contents of which are equivalent to about 6 g of Ifosfamide. Dissolve the contents of each container in water and combine all of the solutions in a 1000-mL volumetric flask. Rinse each container with water, and add the rinsings to the volumetric flask. Dilute with water to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Ifosfamide*. Calculate the quantity, in g, of  $C_7H_{15}Cl_2N_2O_2P$  in each container of Ifosfamide for Injection taken by the formula:

$$10(C / N)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Ifosfamide RS in the *Standard preparation*; N is the number of containers selected for the *Assay preparation*, and  $R_U$  and  $R_S$  are the ratios of the responses of the ifosfamide peak to the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Imipenem



$C_{12}H_{17}N_3O_4S \cdot H_2O$  317.36

1-Azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, 6-(1-hydroxyethyl)-3-[[2-(iminomethyl)amino]ethyl]thio]-7-oxo-, monohydrate, [5*R*]-[5 $\alpha$ ,6 $\alpha$ ( $R^*$ )]-.

(5*R*,6*S*)-3-[[2-(Formimidoylamino)ethyl]thio]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate [74431-23-5].

Anhydrous 299.35 [64221-86-9].

» Imipenem contains the equivalent of not less than 98.0 percent and not more than 101.0 per-

cent of imipenem monohydrate ( $C_{12}H_{17}N_3O_4S \cdot H_2O$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), and store in a cold place.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile.

#### USP Reference standards (11)—

USP Endotoxin RS

USP Imipenem Monohydrate RS

**Identification, Infrared Absorption** (197M).

**Specific rotation** (781S): between +84° and +89°.

*Test solution:* 5 mg per mL, in a pH 7 buffer. Prepare the pH 7 buffer solution as follows. Dissolve 5 g of monobasic potassium phosphate and 11 g of dibasic potassium phosphate in 900 mL of water, adjust with phosphoric acid or 5 N sodium hydroxide to a pH of 7, dilute with water to 1000 mL, and mix.

**Crystallinity** (695): meets the requirements.

**Bacterial endotoxins** (85) (where the label states that Imipenem is sterile)—It contains not more than 0.17 USP Endotoxin Unit per mg.

**Sterility** (71) (where the label states that Imipenem is sterile)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen dissolved in 200 mL of *Fluid A* being used.

**Loss on drying** (see *Thermal Analysis* (891))—[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on drying*.] Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument, using 5 to 10 mg of Imipenem, accurately weighed. Heat the specimen under test at a rate of 20° per minute under vacuum. Record the thermogram to 200°, and calculate the weight loss at the plateau or inflection point at about 150°: it loses not less than 5.0% and not more than 8.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): not more than 0.002%.

#### Solvents—

*Internal standard solution*—Add 1 mL of *n*-propyl alcohol to 2000 mL of water, and mix.

*Standard preparation*—Transfer 1.0 mL of acetone and 2.0 mL of isopropyl alcohol to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with water to volume, and mix. Each mL of this *Standard preparation* contains 31.6 µg of acetone and 63.2 µg of isopropyl alcohol.

*Test preparation*—Transfer about 250 mg of Imipenem, accurately weighed, to a 10-mL volumetric flask, add 4.0 mL of 1 N ammonium hydroxide, and dissolve by swirling. Add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 1.8-m column containing 10% phase G16 on support S5. The column temperature is programmed to operate at 70° for 8 minutes, then to increase at a rate of 32° per minute to 170°, and to maintain the temperature at 170° for 8 minutes. The injection port is maintained at 200°, the detector is maintained at 250°, and helium is used as the carrier gas at a flow rate of about 19 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.3 for acetone, 0.5 for isopropyl alcohol, and 1.0 for *n*-propyl alcohol, and the relative standard deviation of each of the ratios of the

response of the respective analyte peak to the response of the *n*-propyl alcohol peak for replicate injections is not more than 5%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, using the solvent (water) flush technique, record the chromatograms, and measure the responses for the acetone, isopropyl alcohol, and *n*-propyl alcohol peaks. Calculate the percentages of acetone and isopropyl alcohol in the portion of Imipenem taken by the same formula:

$$(C/W)(R_U/R_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of the appropriate analyte in the *Standard preparation*; *W* is the quantity, in mg, of Imipenem taken to prepare the *Test preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the ratios of the peak response of each of the corresponding analytes to the peak responses of *n*-propyl alcohol obtained from the *Test preparation* and the *Standard preparation*, respectively. Add the percentages of acetone and isopropyl alcohol found: the total is not more than 0.25%.

#### Assay—

**Mobile phase**—Dissolve 0.54 g of monobasic potassium phosphate in 3600 mL of water, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of  $6.8 \pm 0.1$ , dilute with water to make 4000 mL of solution, and mix. Filter this solution through a filter of 0.5- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Imipenem Monohydrate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL. Store this solution in an ice bath, and discard after 8 hours.

**Assay preparation**—Transfer about 100 mg of Imipenem, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Store this solution in an ice bath, and discard the unused portion after 8 hours.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L1, and is maintained at a temperature of  $30 \pm 1.0^\circ$ . The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of imipenem monohydrate ( $C_{12}H_{17}N_3O_4S \cdot H_2O$ ) in the portion of Imipenem taken by the formula:

$$(317.36/299.35)(0.25CP)(r_U/r_S)$$

in which 317.36 and 299.35 are the molecular weights of imipenem monohydrate and anhydrous imipenem, respectively; *C* is the concentration, in mg per mL, of USP Imipenem Monohydrate RS in the *Standard preparation*; *P* is the content, in  $\mu$ g per mg, of anhydrous imipenem ( $C_{12}H_{17}N_3O_4S$ ) in USP Imipenem Monohydrate RS; and *r<sub>U</sub>* and *r<sub>S</sub>* are the imipenem peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Imipenem and Cilastatin for Injection

» Imipenem and Cilastatin for Injection is a sterile mixture of Imipenem, Cilastatin Sodium, and Sodium Bicarbonate. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of imipenem ( $C_{12}H_{17}N_3O_4S$ ) and cilastatin ( $C_{16}H_{26}N_2O_5S$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1), and store at controlled room temperature.

**Labeling**—Label it to indicate that after constitution it is to be solubilized in a suitable parenteral fluid prior to intravenous infusion.

#### USP Reference standards (11)—

USP Cilastatin Ammonium Salt RS

USP Endotoxin RS

USP Imipenem Monohydrate RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—The retention times of the peaks for imipenem and cilastatin in the chromatogram of the *Assay preparation* correspond to those in the chromatograms of the *Imipenem standard preparation* and the *Cilastatin standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.17 USP Endotoxin Unit per mg of imipenem and not more than 0.17 USP Endotoxin Unit per mg of cilastatin.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the specimen being dissolved in *Fluid A*.

**pH** (791): between 6.5 and 8.5, when constituted as directed in the labeling.

**Uniformity of dosage units** (905): meets the requirements.

**Loss on drying** (731)—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 hours: it loses not more than 3.5% of its weight.

**Particulate matter** (788): meets the requirements for small-volume injections.

#### Assay—

**pH 6.8 Buffer**—Dissolve 0.54 g of monobasic potassium phosphate in 3600 mL of water, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of  $6.8 \pm 0.1$ , dilute with water to make 4000 mL of solution, and mix. Pass this solution through a filter of 0.5- $\mu$ m or finer porosity.

**Mobile phase**—Dissolve 2.0 g of sodium 1-hexanesulfonate in 800 mL of *pH 6.8 Buffer*, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of  $6.8 \pm 0.1$ , and dilute with *pH 6.8 Buffer* to make 1000 mL of solution. Pass this solution through a filter of 0.5- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Imipenem standard preparation**—Transfer about 13 mg of USP Imipenem Monohydrate RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of saline TS, 0.5 mL of a 0.1% solution of sodium bicarbonate, and about 15 mL of *pH 6.8 Buffer*, and dissolve by shaking and sonicating.

[NOTE—The duration of sonication should not exceed 1 minute.] Dilute with *pH 6.8 Buffer* to volume, and mix. This solution contains the equivalent of about 500  $\mu$ g of anhydrous imipenem per mL. Use this solution immediately.

**Cilastatin standard preparation**—Transfer about 12.5 mg of USP Cilastatin Ammonium Salt RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of saline TS, 0.5 mL of a 0.1% solution of sodium bicarbonate, and about 15 mL of

*pH 6.8 Buffer*, and dissolve by shaking and sonicating. [NOTE—The duration of sonication should not exceed 1 minute.] Dilute with *pH 6.8 Buffer* to volume, and mix. This solution contains the equivalent of about 500 µg of cilastatin per mL. Use this solution immediately.

**Assay preparation**—Constitute Imipenem and Cilastatin for Injection in a volume of saline TS, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively transfer this suspension to a 100-mL volumetric flask with the aid of *pH 6.8 Buffer*, dilute with *pH 6.8 Buffer* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *pH 6.8 Buffer* to obtain an *Assay preparation* having a concentration of about 500 µg of imipenem per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L1, and is maintained at a temperature of 50 ± 1.0°. The flow rate is about 2 mL per minute. Chromatograph the *Imipenem standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the imipenem peak is not less than 600 theoretical plates when calculated by the formula:

$$5.545(t / W_{h/2})^2$$

the tailing factor for the imipenem peak is not more than 1.5 when calculated by the formula:

$$W_{0.1} / 2f$$

where  $W_{0.1}$  is the width of the peak at 10% height, and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Cilastatin standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the cilastatin peak is not less than 600 theoretical plates when calculated by the formula:

$$5.545(t / W_{h/2})^2$$

the tailing factor for the cilastatin peak is not more than 1.5 when calculated by the formula:

$$W_{0.1} / 2f$$

and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Imipenem standard preparation*, the *Cilastatin standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of anhydrous imipenem ( $C_{12}H_{17}N_3O_4S$ ) and cilastatin ( $C_{16}H_{26}N_2O_5S$ ) in the container, taken by the same formula:

$$(CPL / D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Imipenem Monohydrate RS or USP Cilastatin Ammonium Salt RS in the appropriate *Standard preparation*;  $P$  is the content, in µg per mg, of anhydrous imipenem ( $C_{12}H_{17}N_3O_4S$ ) or cilastatin ( $C_{16}H_{26}N_2O_5S$ ) in the relevant Reference Standard;  $L$  is the labeled quantity, in mg, of imipenem or cilastatin in the container;  $D$  is the concentration, in µg per mL, of imipenem or cilastatin in the *Assay preparation* based on the labeled quantity in the container and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the appropriate *Standard preparation*, respectively.

## Imipenem and Cilastatin for Injectable Suspension

» Imipenem and Cilastatin for Injectable Suspension is a sterile mixture of Imipenem and Cilastatin Sodium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of imipenem ( $C_{12}H_{17}N_3O_4S$ ) and cilastatin ( $C_{16}H_{26}N_2O_5S$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* <1>, and store at controlled room temperature.

**Labeling**—Label it to indicate that the suspension obtained when constituted as directed in the labeling is for intramuscular injection only.

**USP Reference standards** <11>—

USP Cilastatin Ammonium Salt RS

USP Endotoxin RS

USP Imipenem Monohydrate RS

**Identification**—The retention times of the peaks for imipenem and cilastatin in the chromatogram of *Assay preparation 1* correspond to those of the *Imipenem standard preparation* and the *Cilastatin standard preparation* as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 0.23 USP Endotoxin Unit per mg of imipenem and not more than 0.23 USP Endotoxin Unit per mg of cilastatin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the specimen being dissolved in *Fluid A*.

**pH** <791>: between 6.0 and 7.5, when constituted as directed in the labeling.

**Uniformity of dosage units** <905>: meets the requirements.

**Loss on drying** <731>—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.5% of its weight.

**Assay**—

*pH 6.8 Buffer*, *Mobile phase*, *Imipenem standard preparation*, *Cilastatin standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Imipenem and Cilastatin for Injection*.

**Assay preparation**—Constitute Imipenem and Cilastatin for Injectable Suspension in a volume of saline TS, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with saline TS to obtain a stock solution containing about 2500 µg of imipenem per mL. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *pH 6.8 Buffer* to volume, and mix.

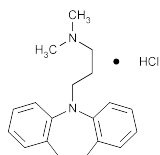
**Procedure**—Separately inject equal volumes (about 10 µL) of the *Imipenem standard preparation*, the *Cilastatin standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of anhydrous imipenem ( $C_{12}H_{17}N_3O_4S$ ) and cilastatin ( $C_{16}H_{26}N_2O_5S$ ) withdrawn from the container taken by the formula:

$$(CPL / D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Imipenem Monohydrate RS or USP Cilastatin Ammonium Salt RS in the appropriate *Standard preparation*;  $P$  is the content, in µg per mg, of anhydrous imipenem ( $C_{12}H_{17}N_3O_4S$ ) or cilastatin ( $C_{16}H_{26}N_2O_5S$ ) in the relevant Reference Stan-

dard;  $L$  is the labeled quantity, in mg, of imipenem or cilastatin in the container;  $D$  is the concentration, in  $\mu\text{g}$  per mL, of imipenem or cilastatin in the *Assay preparation* based on the labeled quantity in the container and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the appropriate *Standard preparation*, respectively.

## Imipramine Hydrochloride



$\text{C}_{19}\text{H}_{24}\text{N}_2 \cdot \text{HCl}$  316.87

5*H*-Dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N,N*-dimethyl-, monohydrochloride.

5-3-(Dimethylamino)propyl-10,11-dihydro-5*H*-dibenz[*b,f*]azepine monohydrochloride [113-52-0].

» Imipramine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{19}\text{H}_{24}\text{N}_2 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Desipramine Hydrochloride RS

USP Imipramine Hydrochloride RS

USP Iminodibenzyl RS

$\text{C}_{14}\text{H}_{13}\text{N}$  195.28

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**C:** Dissolve 0.10 g in 2 mL of alcohol, and add 1 mL of 2 N nitric acid and 3 drops of silver nitrate TS: a white precipitate is formed, and it dissolves on the dropwise addition of ammonium hydroxide.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.001%.

**Related compounds**—[NOTE—Use low-actinic glassware throughout the following procedure.]

*Mobile phase*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve accurately weighed quantities of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in acetonitrile, and dilute with a mixture of water and acetonitrile (5:3) to obtain a solution having known concentrations of about 2.5  $\mu\text{g}$  of each component per mL.

*Test solution*—Transfer about 63 mg of Imipramine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3), and mix.

*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.8 for *N*-(dimethylaminopropyl) iminostilbene and 1.0 for

imipramine. Calculate the percentage of iminodibenzyl in the portion of Imipramine Hydrochloride taken by the formula:

$$5(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Iminodibenzyl RS in the *Standard solution*;  $W$  is the weight, in mg, of Imipramine Hydrochloride taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the iminodibenzyl peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% of iminodibenzyl is found. Calculate the percentage of each other impurity in the portion of Imipramine Hydrochloride taken by the formula:

$$5(C / W)(r_i / r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Imipramine Hydrochloride RS in the *Standard solution*;  $W$  is the weight, in mg, of Imipramine Hydrochloride taken to prepare the *Test solution*;  $r_i$  is the peak response of each individual impurity, excluding iminodibenzyl, obtained from the *Test solution*, and  $r_S$  is the peak response of imipramine obtained from the *Standard solution*: not more than 0.1% of *N*-(dimethylaminopropyl)iminostilbene is found; not more than 0.2% of any other impurity is found; and the total of all impurities found is not more than 1.0%.

**Assay**—[NOTE—Use low-actinic glassware throughout the following procedure.]

*Mobile phase*—Prepare a filtered and degassed mixture of 0.06 M sodium perchlorate, acetonitrile, and triethylamine (625:375:1), and adjust with perchloric acid to a pH of 2.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Transfer about 15 mg of USP Imipramine Hydrochloride RS and about 15 mg of USP Desipramine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3) to volume, and mix.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Imipramine Hydrochloride RS in a mixture of water and acetonitrile (5:3) to obtain a solution having a known concentration of about 0.3 mg per mL.

*Assay preparation*—Transfer about 30 mg of Imipramine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3) to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 269-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the imipramine and desipramine peaks is not less than 1.3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0% for imipramine.

*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $\text{C}_{19}\text{H}_{24}\text{N}_2 \cdot \text{HCl}$  in the portion of Imipramine Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Imipramine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the imipramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Imipramine Hydrochloride Injection

» Imipramine Hydrochloride Injection is a sterile solution of Imipramine Hydrochloride in Water for Injection. It contains, in each mL, not less than 11.5 mg and not more than 13.5 mg of  $C_{19}H_{24}N_2 \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Imipramine Hydrochloride RS

**Identification**—Transfer 10 mL of Injection to a separator, add 2 mL of 2 N hydrochloric acid, extract with 10 mL of chloroform, filter, and evaporate the chloroform solution to about 2 mL. Carefully add ether until the liquid becomes turbid, heat on a steam bath to produce a clear solution, then cool, and allow to stand. Filter the crystalline precipitate, wash with ether, and dry in vacuum at 105° for 30 minutes: the precipitate so obtained responds to *Identification test A* under *Imipramine Hydrochloride*.

**Bacterial endotoxins** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of imipramine hydrochloride.

**pH** (791): between 4.0 and 5.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of imipramine hydrochloride, to a 100-mL volumetric flask, add 0.5 N hydrochloric acid to volume, and mix. Pipet 10 mL of this solution into a separator, add 10 mL of 1 N sodium hydroxide, and extract with four 20-mL portions of ether, shaking each portion for 2 minutes and collecting the extracts in a second separator. Extract the combined ether extracts with four 20-mL portions of 0.5 N hydrochloric acid, and combine the extracts in a 250-mL beaker. Aerate this solution with nitrogen to remove residual ether, then transfer to a 100-mL volumetric flask, and rinse the beaker with 0.5 N hydrochloric acid, collecting the rinsings in the flask. Add the 0.5 N acid to volume, and mix. Dissolve an accurately weighed quantity of USP Imipramine Hydrochloride RS in 0.5 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 25 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 250 nm, with a suitable spectrophotometer, using 0.5 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{19}H_{24}N_2 \cdot HCl$  in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which *C* is the concentration, in µg per mL, of USP Imipramine Hydrochloride RS in the Standard solution; *V* is the volume, in mL, of Injection taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Injection and the Standard solution, respectively.

## Imipramine Hydrochloride Tablets

» Imipramine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of imipramine hydrochloride ( $C_{19}H_{24}N_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Imipramine Hydrochloride RS

**Identification**—Powder a suitable number of Tablets, equivalent to about 100 mg of imipramine hydrochloride, and macerate the powder with 10 mL of chloroform. Filter the chloroform extract through paper into a wide-mouth test tube, and evaporate the filtrate to about 3 mL. Carefully add ether until the liquid becomes turbid, heat on a steam bath to produce a clear solution, then cool, and allow to stand. The precipitate that is formed may be recrystallized from acetone. Filter the crystalline precipitate, wash with ether, and dry in vacuum at 105° for 30 minutes: the precipitate so obtained meets the requirements for *Identification test A* under *Imipramine Hydrochloride*.

**Dissolution** (711)—

*Medium*: 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

**Procedure**—Determine the amount of  $C_{19}H_{24}N_2 \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Imipramine Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 75% (*Q*) of the labeled amount of  $C_{19}H_{24}N_2 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of 70 mL of dilute hydrochloric acid (1 in 100), and shake by mechanical means for 30 minutes. Add dilute hydrochloric acid (1 in 100) to volume, mix, and filter, if necessary, discarding the first 20 mL of the filtrate. Transfer an aliquot of the filtrate, equivalent to about 2.5 mg of imipramine hydrochloride, to a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 100) to volume, and mix. Dissolve an accurately weighed quantity of USP Imipramine Hydrochloride RS in dilute hydrochloric acid (1 in 100), and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 25 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 250 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of  $C_{19}H_{24}N_2 \cdot HCl$  in the Tablet by the formula:

$$10(C/V)(A_U/A_S)$$

in which *C* is the concentration, in µg per mL, of USP Imipramine Hydrochloride RS in the Standard solution; *V* is the volume, in mL, of the aliquot taken of the solution from the Tablet; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

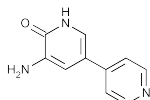
**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of imipramine hydrochloride, to a 200-mL volumetric flask, add about 100 mL of dilute hydrochloric acid (1 in 25), and shake vigorously by mechanical means for 1 hour. Add the dilute acid to volume, mix, and filter, discarding the first 20 mL of the filtrate. Pipet 5 mL of the filtrate into a separator, and proceed as directed in the Assay under *Imipramine Hydrochloride Injection*, beginning with "add 10 mL of 1 N sodium hydroxide." Calculate

the quantity, in mg, of imipramine hydrochloride ( $C_{19}H_{24}N_2 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$4C(A_U / A_S)$$

in which C is the concentration, in  $\mu g$  per mL, of USP Imipramine Hydrochloride RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablets and the Standard solution, respectively.

## Inamrinone



$C_{10}H_9N_3O$  187.20  
[3,4'-Bipyridin]-6(1H)-one, 5-amino-  
5-Amino[3,4'-bipyridin]-6(1H)-one [60719-84-8].

» Inamrinone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{10}H_9N_3O$ , calculated on the anhydrous basis.

*Caution—Inamrinone is a cardiotonic agent.*

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at  $25^\circ$ , excursions permitted between  $15^\circ$  and  $30^\circ$ .

### USP Reference standards (11)—

USP Inamrinone RS

USP Inamrinone Related Compound A RS

5-Carboxamide[3,4'-bipyridin]-6(1H)-one.

$C_{11}H_9N_3O_2$  215.21

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*pH 8.9 Buffer*—Dissolve 107 g of dibasic sodium phosphate in water, adjust, if necessary, with 0.1 M sodium hydroxide or 0.1 M phosphoric acid to a pH of  $8.9 \pm 0.1$ , dilute with water to 1000 mL, and mix.

*Solution:* 6  $\mu g$  per mL, prepared as follows. Dissolve 100 mg in 20 mL of water and 1.0 mL of 1 N hydrochloric acid in a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute 5.0 mL of this solution to 50.0 mL with 0.01 N hydrochloric acid, mix, and transfer 3.0 mL to a 50-mL volumetric flask. Dilute with *pH 8.9 Buffer* to volume, and mix.

*Ratio:*  $A_{237} / A_{318}$  do not differ by more than 3.0%.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

### Chromatographic purity—

*Solution A*—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, add 2 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Solution B*—Prepare a mixture of *Solution A* and acetonitrile (85:15).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

*Diluting solution*—Dissolve 0.25 g of sodium metabisulfite in 1000 mL of *Solution A*.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Inamrinone RS in *Diluting solution* to obtain

a solution having a known concentration of about 2 mg per mL.

*Standard solution*—Dilute a suitable volume of *Standard stock solution* quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of 4  $\mu g$  of USP Inamrinone RS per mL.

*System suitability solution*—Prepare a solution of USP Inamrinone Related Compound A RS in *Diluting solution* having a concentration of 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

*Test solution*—Transfer about 100 mg of Inamrinone, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

[NOTE—Use this solution within 1 hour after preparation.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 315-nm detector and a 4.0-mm  $\times$  25-cm analytical column that contains packing L1 and is fitted with a guard column that contains packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	87	13	equilibration
0–1	87	13	isocratic
1–29	87→0	13→100	linear gradient
29–30	0	100	isocratic

Allow the system to equilibrate at the original conditions before making subsequent injections. Chromatograph 15  $\mu L$  of the *System suitability solution*, record the chromatograms, and measure the peak responses as described for *Procedure*: the relative retention times are 0.6 for inamrinone and 1.0 for inamrinone related compound A; and the resolution,  $R$ , between inamrinone and inamrinone related compound A is not less than 4.0. Chromatograph about 15  $\mu L$  of the *Standard solution*, and record the peak responses for inamrinone as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

*Procedure*—Separately inject equal volumes (about 15  $\mu L$ ) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms allowing the *Test solution* to elute for not less than five times the retention times of inamrinone, and measure the areas of all the peaks observed in the chromatogram of the *Test solution*. Calculate the percentage of each impurity in the portion of Inamrinone taken by the formula:

$$5000(C/W)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Inamrinone RS in the *Standard solution*; W is the weight, in mg, of inamrinone taken for the *Test solution*;  $r_i$  is the response of each impurity peak; and  $r_s$  is the mean response for the *Standard solution*: not more than 0.2% of any individual impurity is found; and the sum of all impurities is not more than 1.0%.

**Assay**—Weigh accurately about 500 mg of Inamrinone, and proceed as directed under *Nitrite Titration* (451). Each mL of 0.1 M sodium nitrite is equivalent to 18.72 mg of  $C_{10}H_9N_3O$ .

## Inamrinone Injection

» Inamrinone Injection is a sterile solution of Inamrinone in Water for Injection, prepared with the aid of Lactic Acid. It contains not less than

90.0 percent and not more than 110.0 percent of the labeled amount of inamrinone ( $C_{10}H_9N_3O$ ).

**Caution**—Inamrinone is a cardiotonic agent.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at room temperature.

**USP Reference standards** (11)—

USP Inamrinone RS

USP Inamrinone Related Compound B RS

*N*-(1,6-Dihydro-6-oxo-(3,4'-bipyridine)-5-yl)-2-hydroxypropanamide.

$C_{13}H_{13}N_3O_3$  259.3

USP Inamrinone Related Compound C RS

1,6-Dihydro-6-oxo-(3,4'-bipyridine)-5-carbonitrile.

$C_{11}H_7N_3O$  197.20

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer a volume of Injection, equivalent to about 50 mg of inamrinone, to a glass-stoppered container. Add about 2 g of 50- to 100-mesh sulfonic acid cation-exchange resin, and shake for about 2 minutes or until the supernatant becomes essentially colorless. Filter, and collect the filtrate in an arsine generator flask (see *Apparatus* under *Arsenic* (211)). Add 5 mL of diluted sulfuric acid, and boil gently on a hot plate for 5 to 10 minutes. Cool to room temperature. Add 10 mL of potassium permanganate TS, attach the scrubber unit and absorber tube, and place the apparatus on a warm hot plate. Add 1 mL of indicator solution (freshly prepared by dissolving 250 mg of sodium nitroferriyanide in sufficient water to make 9 mL and mixing with 1 mL of morpholine) to the absorber tube. Heat gently, allowing the vapors to bubble through the indicator: the indicator turns blue within 5 minutes (*presence of lactate*).

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of inamrinone.

**pH** (791): between 3.2 and 4.0.

**Lactic acid content**—

**Ion-exchange column**—Place a small pledget of glass wool at the bottom of a 100- × 6-mm glass column, equipped with a stopcock and a 25-mL reservoir. Soak a suitable quantity of 50- to 100-mesh sulfonic acid cation-exchange resin in 6 N hydrochloric acid for several minutes. Wash with water until the wash is neutral to wide-range pH indicator paper. Fill the column with the prepared resin to the base of the reservoir. Wash the column with about 50 mL of water in several portions, draining each wash to the top of the resin before adding the next portion. Discard the washes.

**Procedure**—Place a 125-mL conical flask below the *ion-exchange column*. Pipet a volume of Injection, equivalent to about 50 mg of inamrinone, onto the column. Allow the specimen to pass through the column at the rate of about 0.5 mL to 1 mL per minute, draining the specimen to the top of the column and collecting the eluate in the flask. Wash the column with five 5-mL portions of water, collecting the washings in the flask. Add several small glass beads to the solution in the flask, and boil on a hot plate for about 10 minutes. Add 10.0 mL of 0.1 N sodium hydroxide, and boil for 20 minutes. Add phenolphthalein TS, and titrate the warm solution with 0.1 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N hydrochloric acid is equivalent to 9.008 mg of  $C_3H_5O_3$ : the lactic acid content is between 5.0 and 7.5 mg per mL of Injection.

**Chromatographic purity**—

**Mobile phase**—Dilute 11.4 mL of phosphoric acid with water to 990 mL. Prepare a filtered and degassed mixture of the dilute phosphoric acid and acetonitrile (99:1). Make ad-

justments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer accurately weighed quantities of about 10 mg of USP Inamrinone RS and about 25 mg of USP Inamrinone Related Compound B RS to a 100-mL volumetric flask, add about 60 mL of lactic acid solution (1 in 85), and sonicate for about 2 minutes to effect solution. Cool, dilute with lactic acid solution (1 in 85) to volume, and mix. Pipet 10.0 mL of this solution into a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution**—Immediately before use, pipet a volume of Injection, equivalent to about 100 mg of inamrinone, into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 313-nm detector and a 4-mm × 15-cm column that contains base-deactivated packing L7. The column temperature is maintained at a temperature between 30° and 35°, and the flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between inamrinone and inamrinone related compound B is not less than 10; and the relative standard deviation for replicate injections is not more than 10%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Calculate the percentage of inamrinone related compound B relative to inamrinone in the volume of Injection taken by the formula:

$$5(C/W)(r_U/r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Inamrinone Related Compound B RS in the *Standard solution*; *W* is the weight, in mg, of inamrinone in the volume of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the inamrinone related compound B peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Separately calculate the percentage of any other impurity present in the volume of Injection taken by the formula:

$$5(C/W)(r_i/r_S)$$

in which *r<sub>i</sub>* is the peak response for each impurity, and the other terms are as previously defined. Not more than 2.0% of inamrinone related compound B and not more than 0.5% of any other individual impurity is found, and the sum of all impurities is not more than 3.0%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—[NOTE—Prepare all inamrinone-containing solutions immediately before injection into the chromatograph.]

**pH 7 sodium borate buffer, 0.5 M**—Transfer 31 g of boric acid to a beaker containing approximately 800 mL of water. Slowly add sodium hydroxide solution (1 in 5) in small quantities, stirring well after each addition, until all of the boric acid is dissolved and the pH is constant at  $7.0 \pm 0.3$ . Transfer this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and *pH 7 sodium borate buffer, 0.5 M* (500:480:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve accurately weighed quantities of USP Inamrinone RS and USP Inamrinone Related Compound C RS in *Mobile phase* to obtain a solution containing about 50  $\mu$ g of each per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Inamrinone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase*

to obtain a solution having a known concentration of about 50 µg per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of inamrinone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

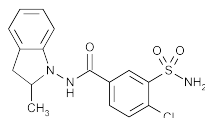
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are 0.6 for inamrinone related compound C and 1.0 for inamrinone; the resolution, *R*, between the inamrinone related compound C and inamrinone peaks is not less than 3; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amrinone (C<sub>10</sub>H<sub>9</sub>N<sub>3</sub>O) in each mL of the Injection taken by the formula:

$$(0.1 C / V)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Inamrinone RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Indapamide



C<sub>16</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>S 365.83

Benzamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-.

4-Chloro-*N*-(2-methyl-1-indolyl)-3-sulfamoylbenzamide [26807-65-8].

» Indapamide contains not less than 98.0 percent and not more than 101.0 percent of C<sub>16</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>S, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—

USP Indapamide RS

**Identification**—

**A:** Infrared Absorption <197K>.

**B:** Ultraviolet Absorption <197U>—

*Solution:* 5 µg per mL.

*Medium:* methanol.

**Loss on drying** <731>—Dry it at 105° for 4 hours: it loses not more than 3.0% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Chromatographic purity**—[Caution—Minimize exposure to light while weighing the samples and spotting on the thin-layer chromatographic plate. Use low-actinic glassware or wrap the

glassware with aluminum foil and protect all the chromatographic solutions from light. Place the chromatographic tanks in a dark room or cover them with aluminum foil during the development. The paperlined chamber should be saturated with solvent vapor for 1 hour before development of the plates.]

**Standard preparations**—Dissolve USP Indapamide RS in methanol, and mix to obtain *Standard preparation A* having a known concentration of 0.30 mg per mL. Dilute quantitatively with methanol to obtain *Standard preparation B* and *Standard preparation C* containing 0.15 mg and 0.075 mg of USP Indapamide RS per mL, respectively.

**Test preparation**—Dissolve an accurately weighed quantity of Indapamide in methanol, and dilute quantitatively with methanol to obtain a solution containing 30 mg per mL.

**Procedure**—Apply separately 10 µL of the *Test preparation* and 10 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of toluene, ethyl acetate, and glacial acetic acid (70:30:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a current of air. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: no secondary spot from the chromatograms of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation B* (0.5%), and the sum of the intensities of the secondary spots obtained from the *Test preparation* corresponds to not more than 2.0%.

**Assay**—[NOTE—Where peak responses are indicated, use peak areas.]

**Mobile phase**—Prepare a filtered and degassed mixture consisting of water, acetonitrile, methanol, and glacial acetic acid (650:175:175:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Internal standard solution**—Dissolve a suitable quantity of *p*-chloroacetanilide in methanol to obtain a solution having a concentration of about 5.0 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Indapamide RS in *Internal standard solution*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL of the Reference Standard and about 0.25 mg per mL of the internal standard.

**Assay preparation**—Transfer about 100 mg of Indapamide, accurately weighed, to a 100-mL volumetric flask, dissolve in 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution, *R*, between any peak of interest and any adjacent peak is not less than 2.0, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time, relative to indapamide, is about 0.65 for *p*-chloroacetanilide.

Calculate the quantity, in mg, of  $C_{16}H_{16}ClN_3O_3S$  in the portion of Indapamide taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Indapamide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak area of indapamide to the peak area of internal standard in the *Assay preparation* and the *Standard preparation*, respectively.

## Indapamide Tablets

» Indapamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{16}ClN_3O_3S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Indapamide RS

**Identification**—

**A:** Crush a quantity of Tablets, equivalent to about 15 mg of indapamide, remove and discard any coating material, and finely powder the remaining tablet cores. Agitate the powdered tablets with two 30-mL portions of 0.2 N sodium hydroxide in a centrifuge tube for 10 minutes. Centrifuge each mixture, and combine the supernatants in a 250-mL separator. Acidify the liquid with about 12 mL of dilute hydrochloric acid (1 in 10). Extract the acidic solution with two 4.0-mL portions of ether, filter the extracts through anhydrous sodium sulfate contained in a filter paper, and evaporate the ether, with the aid of a current of dry air, on a water bath. Dry the crystals at 105° for 1 hour: the crystals so obtained respond to *Identification test A* under *Indapamide*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**Dissolution** (711)—

**Medium:** 0.05 M pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

Determine the amount of  $C_{16}H_{16}ClN_3O_3S$  dissolved by employing the following method.

**Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Indapamide RS in methanol, and dilute quantitatively, and stepwise if necessary, with a mixture of *Medium* and methanol (99:1) to obtain a solution having a known concentration equivalent to the solution under test.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 242-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of a filtered portion of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Determine the amount of  $C_{16}H_{16}ClN_3O_3S$  dissolved.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{16}ClN_3O_3S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Dissolve 1.08 g of sodium 1-octanesulfonate in 700 mL of water, add 10 mL of glacial acetic acid, and mix. Add 300 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of 2'-chloroacetophenone in acetonitrile having a concentration of about 0.25 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Indapamide RS in acetonitrile to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 5.0 mL of this solution and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with a mixture of water and acetonitrile (50:10) to volume, and mix.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of powder, equivalent to about 2.5 mg of indapamide, to a 50-mL volumetric flask, add about 25 mL of acetonitrile, and sonicate for about 20 minutes. Cool, dilute with acetonitrile to volume, and mix. Transfer this solution to a 50-mL centrifuge tube, and centrifuge at 2000 rpm for about 10 minutes. Transfer 10.0 mL of the supernatant to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution*, dilute with a mixture of water and acetonitrile (70:4) to volume, and mix.

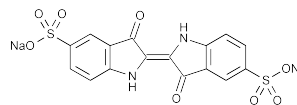
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 242-nm detector and a 4.5-mm × 10-cm column that contains a 3-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the analyte peak and the internal standard peak is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time, relative to indapamide, is about 1.18 for the internal standard. Calculate the quantity, in mg, of  $C_{16}H_{16}ClN_3O_3S$  in the portion of Tablets taken by the formula:

$$250C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Indapamide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of indapamide to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Indigotindisulfonate Sodium



$C_{16}H_8N_2Na_2O_8S_2$  466.35

1*H*-Indole-5-sulfonic acid, 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-, disodium salt.

Sodium 3,3'-dioxo[Δ<sup>2,2'</sup>-biindoline]-5,5'-disulfonate [860-22-0].

» Indigotindisulfonate Sodium contains not less than 96.0 percent and not more than 102.0 per-

cent of sodium indigotinsulfonates, calculated on the dried basis as  $C_{16}H_8N_2Na_2O_8S_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Indigotindisulfonate Sodium RS

**Identification**—

**A:** Incinerate a portion of it: the residue responds to the tests for *Sodium* (191) and for *Sulfate* (191).

**B:** The addition of hydrochloric acid to a solution of it changes the color to bluish violet, and further dilution with water restores the original color.

**C:** The addition of 1 N sodium hydroxide to a solution of it changes the color to yellow or olive-brown.

**D:** The addition of sodium chloride to a solution of it produces a blue precipitate.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 5.0% of its weight.

**Water-insoluble substances**—Dissolve 1.0 g in 100 mL of water, pass through a tared filtering crucible, wash with water until the filtrate is practically colorless, and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 5 mg.

**Arsenic, Method II** (211): 8 ppm.

**Lead**—Place 4.0 g in a Kjeldahl flask, moisten with water, and add 10 mL of sulfuric acid and 5 mL of nitric acid. As soon as the first violent reaction subsides, heat until most of the brown fumes are expelled. Repeat the addition of nitric acid, 1 to 3 mL at a time, and heat until the Indigotindisulfonate Sodium is practically decomposed and most of the organic matter is in solution. Then add, *cautiously and in small portions*, 5 mL of perchloric acid. When the violent reaction subsides, continue the addition of small amounts of nitric acid, and heat as before until a colorless solution is obtained. (If the solution fails to become clear in 10 to 20 minutes after the addition of the perchloric acid, add 1 to 3 mL more of this acid, and continue the nitric acid treatment until the solution is colorless.) Boil for 10 to 15 minutes, cool, and neutralize with 1 N sodium hydroxide. Transfer to a 100-mL volumetric flask, and dilute with water to volume. Five mL of this solution contains not more than 2 µg of lead (corresponding to not more than 0.001%) when tested according to the limit test for *Lead* (251), 3 mL of *Ammonium Citrate Solution*, 1 mL of *Potassium Cyanide Solution*, and 0.5 mL of *Hydroxylamine Hydrochloride Solution* being used.

**Sulfur content**—Place about 25 mg, accurately weighed, in halide-free filter paper measuring about 4 cm square, and fold the paper to enclose it. Proceed as directed under *Oxygen Flask Combustion* (471), using a 1-L flask and using a mixture of 25 mL of water and 5 mL of hydrogen peroxide TS as the absorbing liquid. When the combustion is complete, place a few mL of water in the cup, loosen the stopper, then rinse the stopper, the specimen holder, and the sides of the flask with about 20 mL of water. Add 2 mL of hydrochloric acid, dilute with water to 250 mL, heat to boiling, and slowly add 10 mL of barium chloride TS. Heat the mixture on a steam bath for 1 hour, collect the precipitate of barium sulfate on a filter, wash it until free from chloride, dry, ignite, and weigh. Each g of residue is equivalent to 137.4 mg of sulfur (S). Between 13.0% and 14.0%, calculated on the dried basis, of S is found.

**Assay**—Dissolve about 500 mg of Indigotindisulfonate Sodium, accurately weighed, in dilute hydrochloric acid (1 in 100), and dilute quantitatively and stepwise with the dilute acid to obtain a solution containing about 10 µg per mL. Concomitantly determine the absorbances of this solution and a Standard solution of USP Indigotindisulfonate Sodium RS in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of

maximum absorbance at about 610 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of  $C_{16}H_8N_2Na_2O_8S_2$  in the portion of Indigotindisulfonate Sodium taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Indigotindisulfonate Sodium RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Indigotindisulfonate Sodium and the Standard solution, respectively.

## Indigotindisulfonate Sodium Injection

» Indigotindisulfonate Sodium Injection is a sterile solution of Indigotindisulfonate Sodium in Water for Injection. It contains not less than 90.0 percent and not more than 105.0 percent of the labeled amount of  $C_{16}H_8N_2Na_2O_8S_2$ .

**Packaging and storage**—Preserve in single-dose, light-resistant containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Indigotindisulfonate Sodium RS

**Identification**—It responds to *Identification* tests B, C, and D under *Indigotindisulfonate Sodium*.

**Bacterial endotoxins** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of indigotindisulfonate sodium.

**pH** (791): between 3.0 and 6.5.

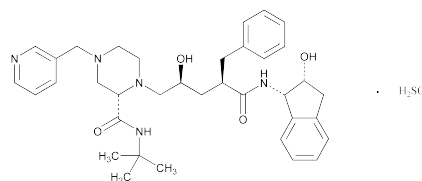
**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Quantitatively dilute a portion of Injection, equivalent to about 40 mg of indigotindisulfonate sodium, with dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 10 µg of indigotindisulfonate sodium per mL. Proceed as directed in the *Assay* under *Indigotindisulfonate Sodium*, beginning with "Concomitantly determine the absorbances." Calculate the quantity, in mg, of  $C_{16}H_8N_2Na_2O_8S_2$  in each mL of the Injection taken by the formula:

$$4(C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Indigotindisulfonate Sodium RS in the Standard solution; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Injection and the Standard solution, respectively.

## Indinavir Sulfate



$C_{36}H_{47}N_5O_4 \cdot H_2SO_4$

711.87

D-erythro-Pentonamide, 2,3,5-trideoxy-N-(2,3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[[1,1-dimethylethyl]amino]]

carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-, [1(1*S*,2*R*),5(*S*)]-, sulfate (1:1) (salt); ( $\alpha$ *R*, $\gamma$ *S*,2*S*)- $\alpha$ -Benzyl-2-(*tert*-butylcarbamoyl)- $\gamma$ -hydroxy-*N*-[(1*S*,2*R*)-2-hydroxy-1-indanyl]-4-(3-pyridinylmethyl)-1-piperazinevaleramide sulfate (1:1) (salt) [157810-81-6].

## DEFINITION

Indinavir Sulfate contains NLT 98.5% and NMT 101.5% of  $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ , calculated on the anhydrous, solvent-free basis.

## IDENTIFICATION

- A. INFRARED ABSORPTION** (197M): Maxima at about 3.0–3.1, 5.9, 6.2, and 13.6  $\mu$ m
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Sulfate** (191): Meets the requirements  
*Sample solution*: A solution of 10 mg/mL in water

## ASSAY

### PROCEDURE

**Solution A**: Dibutyl ammonium phosphate and water (1:50). Adjust with sodium hydroxide TS to a pH of 6.5  $\pm$  0.5.

**Mobile phase**: Acetonitrile and *Solution A* (9:11)

**Standard solution**: 0.5 mg/mL of USP Indinavir RS in *Mobile phase*

**Sample solution**: 0.6 mg/mL of Indinavir Sulfate in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 260 nm

**Column**: 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature**: 40°

**Flow rate**: 1 mL/min

**Injection size**: 10  $\mu$ L

### System suitability

**Sample**: *Standard solution*

### Suitability requirements

**Column efficiency**: NLT 4000 theoretical plates

**Tailing factor**: Less than 2.0

**Relative standard deviation**: NMT 1.0%

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$  in the portion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Indinavir RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Indinavir Sulfate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of indinavir sulfate, 711.87

$M_{r2}$  = molecular weight of indinavir, 613.79

**Acceptance criteria**: 98.5%–101.5% on the anhydrous, solvent-free basis

## OTHER COMPONENTS

### PROCEDURE 1: CONTENT OF SULFATE

**Solution A**: Methanol and formaldehyde (1000:0.3)

**Diluent**: *Solution A* and water (1:1)

**Sample solution**: 6.25 mg/mL of Indinavir Sulfate in *Diluent*

**Analysis**: Titrate with 0.1 M lead perchlorate VS, using a lead-specific electrode in conjunction with a suitable reference electrode. Each mL of 0.1 M lead perchlorate VS is equivalent to 9.604 mg of sulfate.

**Acceptance criteria**: 13.2%–14.4%, calculated on the anhydrous and solvent-free basis

### PROCEDURE 2: CONTENT OF ALCOHOL

**Standard solution**: 0.001 mL/mL of dehydrated alcohol, in water. [NOTE—Dehydrated alcohol is at 20°.]

**Sample solution**: 4 mg/mL of Indinavir Sulfate in water

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: GC

**Detector**: Flame ionization

**Column**: 0.53-mm  $\times$  30-m capillary column with a

1.0- $\mu$ m film of phase G14

### Temperature

**Column**: 35°

**Injector**: 140°

**Detector**: 220°

[NOTE—At the end of each 5-min isothermal run, increase the oven temperature to 200° before adjusting the column temperature to 35° for the next injection.]

**Flow rate**: 10 mL/min

**Carrier gas**: Helium

**Injection size**: 0.1  $\mu$ L

### System suitability

**Sample**: *Standard solution*

### Suitability requirements

**Relative standard deviation**: NMT 2.0%

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of alcohol in the portion of Indinavir Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of dehydrated alcohol in the *Standard solution* (mL/mL)

$C_U$  = concentration of Indinavir Sulfate in the *Sample solution* (mg/mL)

$D$  = density of alcohol at 20°, 790 mg/mL

**Acceptance criteria**: 5.0%–8.0%

## IMPURITIES

### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.1%

### HEAVY METALS (231)

**Standard solution**: Transfer 2 mL of *Standard Lead Solution* (10  $\mu$ g/mL) to a 50-mL color-comparison tube, and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as an external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Dilute with water to 40 mL.

**Sample solution**: Transfer 2 g of Indinavir Sulfate to a 50-mL color-comparison tube, and dissolve in 25 mL of water. Using a pH meter or a suitable short-range pH indicator paper as an external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Dilute with water to 40 mL.

**Blank solution**: To a 50-mL color-comparison tube, add 25 mL of water. Using a pH meter or a suitable short-range pH indicator paper as an external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Dilute with water to 40 mL.

**Analysis**: To each tube, add 10 mL of hydrogen sulfide TS, allow to stand for 5 min, and view downward over a white surface.

**Acceptance criteria**: The color of the *Sample solution* is not darker than that of the *Standard solution*, and the intensity of the color of the *Blank solution* is less than or equal to the intensity of that of the *Sample solution*.

### Organic Impurities

#### PROCEDURE

**Solution A**: 0.27 g/L of monobasic potassium phosphate and 1.395 g/L of dibasic potassium phosphate, in water

**Solution B:** Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	30	70
45	30	70
47	80	20
52	80	20

Diluent: *Solution A* and *Solution B* (1:1)System suitability solution: 0.4 mg/mL of USP Indinavir System Suitability RS in *Diluent*Sample solution: 0.5 mg/mL of Indinavir Sulfate in *Diluent***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

**System suitability**Sample: *System suitability solution***Suitability requirements**

Resolution: NLT 1.8 between indinavir and indinavir related compound C

Tailing factor: More than 0.95 and less than 2.0, determined from the indinavir peak

**Analysis**Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Indinavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak area response for each impurity $r_T$  = sum of the responses of all the peaks**Acceptance criteria**Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
<i>cis</i> -Aminoindanol <sup>a</sup>	0.18	0.1
Desnicotinyl indinavir <sup>b</sup>	0.80	0.1
<i>threo</i> -Indinavir <sup>c</sup>	0.98	0.1
Indinavir lactone <sup>d</sup>	1.14	0.1
Diindanyl indinavir <sup>e</sup>	1.30	0.1

<sup>a</sup> (1*S*,2*R*)-1-Aminoindanol-2-ol.<sup>b</sup> (5*S*)-1-[(2*S*,4*R*)-4-Benzyl-2-hydroxy-5-[(1*S*,2*R*)-2-hydroxyindan-1-ylamino]-5-oxopentyl]-*N*-*tert*-butylpiperazine-2-carboxamide.<sup>c</sup> (5*S*)-1-[(2*R*,4*R*)-4-Benzyl-2-hydroxy-5-[(1*S*,2*R*)-2-hydroxyindan-1-ylamino]-5-oxopentyl]-*N*-*tert*-butyl-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide.<sup>d</sup> (5*S*)-1-[(2*S*,4*R*)-4-Benzyl-5-oxotetrahydrofuran-2-yl]methyl]-*N*-*tert*-butyl-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide.<sup>e</sup> (2*R*,2'*R*,4*S*,4'*S*)-5,5'-[(5*S*)-2-(*tert*-Butylcarbamoyl)piperazine-1,4-diyl]bis[2-benzyl-4-hydroxy-*N*-[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]pentanamide].**SPECIFIC TESTS**

- OPTICAL ROTATION, Specific Rotation <781S>:** +122° to +129°, at 365 nm, determined on the anhydrous, solvent-free basis

Sample solution: 10 mg/mL in water

- WATER DETERMINATION, Method I <921>:** NMT 1.5%, using 0.25 g

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from moisture. Store at 25°, excursions permitted between 15° and 30°.
- USP REFERENCE STANDARDS <11>**  
USP Indinavir RS  
USP Indinavir System Suitability RS

**Indium In 111 Capromab Pendetide Injection**

» Indium In 111 Capromab Pendetide Injection is a sterile, nonpyrogenic, murine monoclonal antibody, 7E11-C 5.3, (CYT-351), an immunoconjugate prepared by specific modification of the carbohydrate groups and covalent binding to the tripeptide linker chelator, glycytyrosyl-(*N*, *E*-diethylenetriaminepentaacetic acid)-lysine hydrochloride that is complexed with <sup>111</sup>In. It contains not less than 90.0 percent and not more than 110.0 percent of the specified amount of <sup>111</sup>In capromab pendetide, expressed in megabecquerels (or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity. Immediately prior to use, the radiolabeling is performed with Indium In 111 Chloride Solution in the presence of a sodium acetate buffer. It contains sodium chloride and buffering agents as stabilizers. The immuno-reactive fraction, determined by a validated method, is not less than 70 percent. The monomer content is not less than 95 percent determined by a validated electrophoretic mobility method.

**Packaging and storage**—Preserve in adequately shielded single-dose containers at controlled room temperature for not more than 8 hours.

**Labeling**—Label it to include the following in addition to the information specified for *Labeling* under *Injections* <1>: the time and date of calibration; the amount of <sup>111</sup>In capromab pendetide as total MBq (or mCi) and concentration of MBq (or mCi) per mL, at the time of calibration; the expiration date and time; and the storage temperature and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>111</sup>In is 67.2 hours.

**USP Reference standards <11>—**

USP Endotoxin RS

**Bacterial endotoxins <85>—**The limit of endotoxin content is not more than 175/V USP Endotoxin Units per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH <791>:** between 5.0 and 7.0.**Radiochemical purity—**

Absorbent: 1-cm × 8-cm instant silica gel strip.

Test solution: a mixture of the Injection and 0.05 M pentetic acid (1:1).

Application volume: 10 μL.

Developing solvent system: 0.9% sodium chloride solution.



**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621) by ascending chromatography. Determine the distribution of radioactivity on the chromatogram by scanning with a suitable collimated radiochromatogram strip scanner, and determine the percentage of radiochemical purity of the test specimen. Not less than 90% of the In 111 activity is present as a band between the  $R_f$  values of 0 and 0.1.

**Other requirements**—It meets the requirements for *Radionuclide identification* and *Radionuclidic purity* under *Indium In 111 Chloride Solution*. It meets also the requirements under *Injections* (1), except that the radioactive component may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of manufacture.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the total radioactivity, in MBq (or  $\mu$ Ci), of the unshielded Injection by use of a calibrated system.

## Indium In 111 Chloride Solution

Indium Chloride ( $^{111}\text{InCl}_3$ ).

Indium ( $^{111}\text{In}$ ) trichloride [10025-82-8].

» Indium In 111 Chloride Solution is a sterile, nonpyrogenic solution of radioactive indium ( $^{111}\text{In}$ ) in dilute hydrochloric acid suitable for the radiolabeling of proteins such as monoclonal antibodies, peptides, or small biologically active organic molecules. The concentration of acid and  $^{111}\text{In}$  per mL of Indium In 111 Chloride Solution may require adjustment for the specific antibody or peptide being labeled. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{111}\text{In}$  expressed as megabecquerels (or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity. [NOTE—Indium In 111 Chloride Solution is generally recommended for use with specific antibodies or peptides. Consult the product labeling for recommendations and applications for radiolabeling.]

**Specific activity:** not less than 1.85 gigabecquerels (50 millicuries) per  $\mu\text{g}$  of indium at the date and time of calibration.

**Packaging and storage**—Preserve in single-unit containers at controlled room temperature.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{111}\text{In}$  as labeled chloride expressed as total megabecquerels (or millicuries) and the concentration as megabecquerels per mL (or as millicuries per mL) on the date and time of calibration; the expiration date; the statement, "Not for direct administration. Use only as an ingredient for radiolabeling;" and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{111}\text{In}$  is 67.3 hours.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Identification**—Add 1 drop of it to 2 drops of 0.1 M silver nitrate in a glass test tube: a white precipitate is formed (presence of chloride).

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**Acidity**—Pipet 20  $\mu\text{L}$  of Solution into a plastic tube containing 1 drop of bromocresol green, and titrate with 0.0025 N sodium carbonate to a blue endpoint. Calculate the acidity of the Solution by the formula:

$$0.0025 V_T / 20$$

in which  $V_T$  is the volume of titrant consumed: the molarity of the Solution is between 0.035 and 0.045.

**Radionuclide identification** (821)—Its gamma-ray spectrum is identical to that of a specimen of  $^{111}\text{In}$  that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.

**Radionuclidic purity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity of each radionuclidic impurity, in kBq per MBq ( $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ , in the Solution by use of a calibrated system as directed under *Radioactivity* (821).

**INDIUM 110M**—The limit of  $^{110\text{m}}\text{In}$  is 3 kBq per MBq (or 3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ . The presence of  $^{110\text{m}}\text{In}$  in the Solution is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.66 and 0.91 MeV.  $^{110\text{m}}\text{In}$  decays with a half-life of 4.9 hours.

**INDIUM 114M**—The limit of  $^{114\text{m}}\text{In}$  is 3 kBq per MBq (or 3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .  $^{114\text{m}}\text{In}$  is quantified by counting the beta emissions of ground state  $^{114}\text{In}$  with a beta-liquid scintillation counter having a high-energy channel set to discriminate against all counts arising from  $^{111}\text{In}$ .

**ZINC 65**—The limit of  $^{65}\text{Zn}$  is 3 kBq per MBq (or 3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ . The presence of  $^{65}\text{Zn}$  in the Solution is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.116 MeV.  $^{65}\text{Zn}$  decays with a radioactive half-life of 243.9 days.

**Radiochemical purity**—

**Adsorbent:** instant thin-layer chromatography (ITLC) strips (2.5 cm  $\times$  10 cm).<sup>1</sup>

**Test solution**—Dispense about 50  $\mu\text{L}$  of Solution into 1 mL of 0.05 M hydrochloric acid, taking care to use polypropylene tips prewashed in 0.05 M hydrochloric acid for all dispensings.

**Application volume:** 2  $\mu\text{L}$ . The amount of  $^{111}\text{In}$  spotted should be between 0.5  $\mu\text{Ci}$  and 30  $\mu\text{Ci}$  as of the day of the test.

**Developing solvent system:** a mixture of a 1 in 10 solution of ammonium acetate and methanol (1:1).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate with an appropriate scanner, and determine the percentage of radiochemical purity of the *Test solution*. The indium chloride will remain at the origin. Not less than 95% of indium is present as ionic indium.

**Chemical purity**—

**Copper**—Determine the copper, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the copper, as directed by the manufacturer of the instrument used, and measuring the absorbance at 324.8 nm against a standard.

**Nickel**—Determine the nickel, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the nickel, as directed by the manufacturer of the instrument used, and measuring the absorbance at 232.0 nm against a standard.

<sup>1</sup>Type SG impregnated glass microfiber sheet (Gelman Sciences, Ann Arbor, MI).

**Cadmium**—Determine the cadmium, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the cadmium, as directed by the manufacturer of the instrument used, and measuring the absorbance at 228.8 nm against a standard.

**Lead**—Determine the lead, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the lead, as directed by the manufacturer of the instrument used, and measuring the absorbance at 217.0 nm against a standard.

**Mercury**—Determine the mercury, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the mercury, as directed by the manufacturer of the instrument used, and measuring the absorbance at 253.7 nm against a standard.

**Iron**—Determine the iron, in  $\mu\text{g}$  per mL, in the Solution by atomic absorption spectrometry (see *Spectrophotometry and Light-scattering* (851)), using a graphite furnace to volatilize the iron, as directed by the manufacturer of the instrument used, and measuring the absorbance at 248.3 nm against a standard.

**Zinc**—Prepare a zinc stock solution in dilute hydrochloric acid (1 in 100) having a concentration of 1  $\mu\text{g}$  of zinc per mL. Pipet 10 mL of the zinc stock solution into a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of 0.1  $\mu\text{g}$  of zinc per mL (*Standard solution A*). Pipet 20 mL of the zinc stock solution into a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of 0.2  $\mu\text{g}$  of zinc per mL (*Standard solution B*). Pipet 0.1 mL of Indium Chloride In 111 Solution into a 10-mL volumetric flask, dilute with water to volume, and mix to obtain the test solution. Determine the absorbances of the *Standard solutions* and the test solution at the zinc emission line at 213.9 nm with an atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a zinc hollow-cathode lamp and an air-acetylene flame, using water as the blank. Determine the quantity of zinc, in  $\mu\text{g}$  per mL, in the Solution.

The composite total metal ion content is not greater than 1.0  $\mu\text{g}$  per mL.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Solution may be distributed or dispensed prior to completion of the test for *Sterility* (71), the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity, in MBq (or in microcuries or millicuries) per mL, of the Solution, by the use of a calibrated system.

## Indium In 111 Ibritumomab Tiuxetan Injection

» Ibritumomab Tiuxetan is the immunoconjugate resulting from a stable thiourea covalent bond between the monoclonal antibody Ibritumomab and the linker-chelator tiuxetan [*N*-[2-bis(carboxymethyl)amino]-3-(*p*-isothiocyanato phenyl)propyl]-[*N*-[2-bis(carboxymethyl)amino]-2-(methyl)ethyl]glycine. This chelate provides a high-affinity, conformationally restricted chelation site for Yttrium-90 and Indium-111. The approxi-

mate molecular weight of Ibritumomab Tiuxetan is 148 kD.

Ibritumomab is a murine IgG<sub>1</sub> kappa monoclonal antibody directed against the CD20 antigen, which is found on the surface of normal and malignant B lymphocytes. Ibritumomab is produced in Chinese hamster ovary cells and is composed of two murine gamma 1 heavy chains of 445 amino acids each and two kappa light chains of 213 amino acids each.

Indium In 111 Ibritumomab Tiuxetan Injection is a sterile, nonpyrogenic preparation of the immunoconjugate of ibritumomab and tiuxetan that is labeled with <sup>111</sup>In and is suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>111</sup>In as the ibritumomab complex, expressed in megabecquerels (or millicuries) per mL at the time indicated in the labeling. It may contain buffers and stabilizers. It contains no antimicrobial agents. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The immunoreactive fraction, as determined by a validated method, is not less than 90 percent.

**Packaging and storage**—Preserve in single-dose containers, and store in a refrigerator for not more than 12 hours. [NOTE—Translucent protein particles may develop, which are removed by filtration prior to administration using a 0.22 micron low-protein-binding filter.]

**Labeling**—Label it to include the following in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of <sup>111</sup>In Ibritumomab Tiuxetan as total MBq (or mCi) and concentration of MBq (or mCi) per mL at the time of calibration; the expiration date and time; the storage temperature; and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>111</sup>In is 67.3 hours.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Bacterial endotoxins** (85)—The limit of endotoxin content is not more than 175/V USP Endotoxin Units per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 5.5 and 7.5.

**Radiochemical purity**—

*Absorbent*: 1- × 8-cm instant silica gel strip.

*Test solution*: the Injection.

*Application volume*: 10  $\mu\text{L}$ .

*Developing solvent system*: 0.9 % sodium chloride solution.

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621) by ascending chromatography. Determine the distribution of radioactivity on the chromatogram by scanning with a suitable collimated radiochromatogram strip scanner, and determine the percentage of radiochemical purity of the test specimen. Not less than 95% of the In 111 activity is present as a band between the *R<sub>f</sub>* values of 0 and 0.1.

**Other requirements**—It meets the requirements for *Radioisotope identification* and *Radionuclidic purity* under *Indium In 111 Chloride Solution*. It meets also the requirements under *Injections* (1), except that the radioactive component may

be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of manufacture.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the total radioactivity, in MBq (or  $\mu\text{Ci}$ ) of the unshielded Injection by use of a calibrated system.

### Indium In 111 Oxyquinoline Solution

» Indium In 111 Oxyquinoline Solution is a sterile, nonpyrogenic, isotonic aqueous solution suitable for the radiolabeling of blood cells, especially leukocytes and platelets, containing radioactive indium ( $^{111}\text{In}$ ) in the form of a complex with 8-hydroxyquinoline, the latter being present in excess. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{111}\text{In}$  as the 8-hydroxyquinoline complex expressed as megabecquerels (millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride, surfactants, and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Specific activity:** not less than 1.85 GBq (50 millicuries) per  $\mu\text{g}$  of indium.

**Packaging and storage**—Preserve in single-unit containers at a temperature between  $15^\circ$  and  $25^\circ$ .

**Labeling**—Label it to contain the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{111}\text{In}$  as the 8-hydroxyquinoline complex expressed as total megabecquerels (millicuries) and concentration as megabecquerels (millicuries) per mL on the date and time of calibration; the expiration date; the statement "Not for direct administration. Use only for radiolabeling of leukocytes in vitro. Administer radiolabeled cells subsequently by intravenous injection," and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{111}\text{In}$  is 67.9 hours.

**Pyrogen**—It meets the requirements of the *Pyrogen Test* (151).

**pH** (791): between 6.5 and 7.5.

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{111}\text{In}$  that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.

**Radiochemical purity**—Place a suitable volume, about 100  $\mu\text{L}$ , of Solution, dilute with 3 mL of 0.9 percent sodium chloride solution in a separator, and extract with 6 mL of *n*-octanol by vigorous shaking. Allow the phases to separate and then drain the lower, aqueous layer into a suitable stoppered counting tube. Drain the residual, organic layer into a similar counting tube. Rinse the separator with 1 mL of *n*-octanol, and drain this rinse into the counting tube containing the organic layer. Rinse the separator with 5 mL of 2 N hydrochloric acid, and drain this rinse into a third counting tube. Insert the stopper and measure the radioactivity in each of the three tubes in a suitable gamma counter or

ionization chamber calibrated for  $^{111}\text{In}$ . The radiochemical purity is calculated by the formula:

$$(A / B)$$

where *A* is the radioactivity measured in the organic layer and *B* is the sum of the radioactivity measured in the organic, aqueous, and acid solutions. The radioactivity of the 8-hydroxyquinoline complex is not less than 90.0% of the total radioactivity and is found in the organic layer.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity of each radionuclidic impurity, in kBq per MBq ( $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ , in the Solution by use of a calibrated system as directed under *Radioactivity* (821).

**INDIUM 114m**—The limit of  $^{114m}\text{In}$  is 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .  $^{114m}\text{In}$  is quantified by counting the beta emissions of ground state  $^{114}\text{In}$  using a beta-liquid scintillation counter with a high-energy channel set to discriminate against all counts arising from  $^{111}\text{In}$ .

**ZINC 65**—The limit of  $^{65}\text{Zn}$  is 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ . The presence of  $^{65}\text{Zn}$  in the Solution is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.116 MeV.  $^{65}\text{Zn}$  decays with a radioactive half-life of 243.9 days.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq (mCi) per mL, of Solution by the use of a calibrated system as directed under *Radioactivity* (821).

### Indium In 111 Pentetate Injection

» Indium In 111 Pentetate Injection is a sterile, isotonic solution suitable for intrathecal administration, containing radioactive indium ( $^{111}\text{In}$ ) in the form of a chelate of pentetic acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{111}\text{In}$  as the pentetic acid complex expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{111}\text{In}$  as labeled pentetic acid complex expressed as total megabecquerels (millicuries or microcuries) and concentration as megabecquerels (microcuries or millicuries) per mL on the date and time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{111}\text{In}$  is 2.83 days.

**USP Reference standards** (11)—

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 14/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.0 and 8.0.

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{111}\text{In}$  that exhibits major photopeaks having energies of 0.173 and 0.247 MeV.

**Radiochemical purity**—Place 2  $\mu\text{L}$  to 5  $\mu\text{L}$  of Injection about 17 mm from one end of a 65-  $\times$  97-mm piece of silica gel-impregnated glass microfiber sheet (see under *Reagents* in the section *Reagents, Indicators, and Solutions*) (see also *Chromatography* (621)), and allow to dry. Repeat applications may be made to obtain a suitable count rate. Develop the chromatogram over a suitable period of time by ascending chromatography, using dilute methanol (8.5 in 10), and dry in an oven at  $105 \pm 5^\circ$  for 5 minutes. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the indium pentetic acid complex band is not less than 90.0% of the total radioactivity, and the  $R_f$  value is between 0.8 and 1.0.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity of each radionuclidic impurity, in kBq per MBq ( $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ , in the Injection by use of a calibrated system as directed under *Radioactivity* (821).

**INDIUM 114m**—The presence of  $^{114\text{m}}\text{In}$  in the Injection is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.192, 0.558, and 0.724 MeV.  $^{114\text{m}}\text{In}$  decays with a radioactive half-life of 49.5 days. The amount of  $^{114\text{m}}\text{In}$  is not greater than 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .

**ZINC 65**—The presence of  $^{65}\text{Zn}$  in the Injection is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.115 MeV.  $^{65}\text{Zn}$  decays with a radioactive half-life of 243.9 days. The amount of  $^{65}\text{Zn}$  is not greater than 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Indium In 111 Pentetreotide Injection

» Indium In 111 Pentetreotide Injection is a sterile solution, suitable for intravenous administration, containing radioactive indium ( $^{111}\text{In}$ ) in the form of a chelate of pentetreotide. It contains not less than 90.0 percent and not more than 110 percent of the labeled amount of  $^{111}\text{In}$  as the pentetreotide complex expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride, stabilizers, and buffers. Other forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1):

the time and date of calibration; the amount of  $^{111}\text{In}$  as labeled pentetreotide complex expressed as total megabecquerels (or millicuries) and the concentration expressed as megabecquerels (or millicuries) per mL on the date and time of calibration; the expiration date; and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and states that the radioactive half-life of  $^{111}\text{In}$  is 67.3 hours.

**USP Reference standards** (11)—

USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{111}\text{In}$  that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 3.8 and 4.3.

**Radiochemical purity**—

**Solution A**—Dissolve 6.8 g of sodium acetate in 500 mL of water. Adjust with glacial acetic acid to a pH of 5.5, dilute with water to 1000 mL, and mix. Filter through a filter having a porosity of 0.5  $\mu\text{m}$  or less, and degas.

**Solution B**—Use methanol.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 3.9-mm  $\times$  30-cm stainless steel column that contains 10- $\mu\text{m}$  packing L1. It is also equipped with a flow-through gamma-ray detector having a cell volume of about 50  $\mu\text{L}$  and calibrated to provide a linear response within the range of 0.5 to 15 MBq (14 to 400  $\mu\text{Ci}$ ). The column temperature is maintained at  $35^\circ$ . The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B*, and the initial flow rate is about 1 mL per minute. The column is equilibrated for at least 15 minutes with a mobile phase consisting of 60% *Solution A* and 40% *Solution B*. After injection, the composition of the mobile phase is changed linearly to 20% *Solution A* and 80% *Solution B* at 20 minutes, then changed to 100% *Solution B* over the next 0.1 minute, and is held at that percentage while the flow rate is increased linearly from 1 to 2 mL over the next 5 minutes, which is the end of the run. Counts are recorded and charted for 25 minutes at about 2-second intervals.

**Procedure**—Constitute the Injection, and allow to stand for 30 minutes. Inject a volume of Injection having an activity of 0.5 to 15 MBq (14 to 400  $\mu\text{Ci}$ ) into the chromatograph, and record the chromatogram. The retention time of the  $^{111}\text{In}$  pentetreotide peak (which should elute as a double peak) is between 4 and 5 relative to that of unbound  $^{111}\text{In}$ . Record the counts for the  $^{111}\text{In}$  pentetreotide, unbound  $^{111}\text{In}$ , other impurity peaks, and a representative baseline segment, and calculate the percentage of radioactivity from  $^{111}\text{In}$  pentetreotide by the formula:

$$100P / (P + O)$$

in which P is the count of the  $^{111}\text{In}$  pentetreotide peak, and O is the count for all other peaks, each being corrected for its corresponding baseline count. The radioactivity of  $^{111}\text{In}$  pentetreotide is not less than 90% of the total radioactivity.

**Radionuclidic purity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly*), determine the radioactivity of each radionuclidic impurity, in kBq per MBq (or  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ , in the Injection by use of a calibrated system.

**INDIUM 114m**—The presence of  $^{114\text{m}}\text{In}$  in the Injection is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.192, 0.558, and

0.724 MeV.  $^{114m}\text{In}$  decays with a radioactive half-life of 49.5 days. The amount of  $^{114m}\text{In}$  is not greater than 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .

**ZINC 65**—The presence of  $^{65}\text{Zn}$  in the Injection is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.115 MeV.  $^{65}\text{Zn}$  decays with a radioactive half-life of 243.9 days. The amount of  $^{65}\text{Zn}$  is not greater than 3 kBq per MBq (3  $\mu\text{Ci}$  per mCi) of  $^{111}\text{In}$ .

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility* (71), the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity** (821)—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq per mL of Injection, by use of a calibrated system.

## Indium In 111 Satumomab Pendetide Injection

» Indium In 111 Satumomab Pendetide Injection is a sterile, nonpyrogenic, virus-free preparation of monoclonal antibody B72.3 that is labeled with  $^{111}\text{In}$ . Satumomab pendetide is prepared by site-specific conjugation of the linker-chelator, glycyl-tyrosyl-(*N*,*E*-diethylene triamine pentaacetic acid)-lysine hydrochloride to the oxidized oligosaccharide component of the monoclonal antibody B72.3. Satumomab pendetide is radio-labeled by the addition of a sterile, nonpyrogenic solution of a buffered Indium In 111 Chloride solution. [NOTE—Other chemical forms of indium are not to be used in the radiolabeling.] It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{111}\text{In}$  as labeled satumomab pendetide expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity. It may contain buffers and stabilizers. The immunoreactive fraction, as determined by a validated method, is not less than 60 percent.

**Packaging and storage**—Preserve in adequately shielded single-dose containers, at controlled room temperature.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{111}\text{In}$  as labeled satumomab pendetide expressed as total megabecquerels (or millicuries) and concentration as megabecquerels (or millicuries) per mL at the time of calibration; the expiration date; and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay and also indicates that the radioactive half-life of  $^{111}\text{In}$  is 67.3 hours.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Units per mL of the Injection, when compared with USP Endotoxin RS, in which V is the maxi-

mum recommended total dose, in mL, at the expiration date or time.

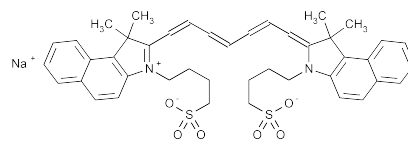
**pH** (791): between 5.5 and 6.5.

**Radiochemical purity**—Mix equal parts of Injection with 0.05 M diethylenetriamine pentaacetic acid in a clean glass vial. Apply a drop of this solution 1 cm from the bottom of a 1-cm  $\times$  8-cm instant thin-layer chromatographic silica gel strip. Allow the spot to air-dry, and develop the strip by ascending chromatography, using 0.9% sodium chloride solution as the solvent. Allow the solvent front to migrate 6 cm from the origin. Remove the strip from the solvent, and air-dry. Determine the distribution of radioactivity on the chromatogram by scanning with a suitable collimated radiochromatogram strip scanner, or cut the strip 1.6 cm from the bottom and determine the radioactivity of each piece in a suitable detector. Not less than 90% of the In-111 activity must be present as a band between  $R_f = 0$  and 0.1.

**Other requirements**—It meets the requirements of the tests for *Radionuclide identification* and *Radionuclidic purity* under *Indium In 111 Chloride Solution*. It meets also the requirements under *Injections* (1), except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ) per mL of the Injection by use of a calibrated system, as directed under *Radioactivity* (821).

## Indocyanine Green



$\text{C}_{43}\text{H}_{47}\text{N}_2\text{NaO}_6\text{S}_2$  774.96

1-*H*-Benz[e]indolium, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[e]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-, hydroxide, inner salt, sodium salt.

2-[7-[1,1-Dimethyl-3-(4-sulfobutyl)benz[e]indolin-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-1*H*-benz[e]indolium hydroxide, inner salt, sodium salt [3599-32-4].

» Indocyanine Green contains not less than 89.0 percent and not more than 100.0 percent of  $\text{C}_{43}\text{H}_{47}\text{N}_2\text{NaO}_6\text{S}_2$ , calculated on the dried basis. It contains not more than 5.0 percent of sodium iodide, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—  
USP Endotoxin RS  
USP Indocyanine Green RS

**Identification—**

**A:** Incinerate a portion of it: the residue responds to the tests for *Sodium* (191) and for *Sulfate* (191).

**B:** To a solution (1 in 20,000) add 10 drops of 1 N sodium hydroxide, and heat to about 60°. Add 10 drops of hydrogen peroxide TS, and mix: a red color develops within about 4 minutes and, on standing, fades to a pale orange.

**Loss on drying** (731)—Dry it in vacuum at 50° for 3 hours: it loses not more than 6.0% of its weight.

**Arsenic, Method II** (211): 8 ppm.

**Lead**—A 5-mL portion of the solution prepared for the test for *Arsenic* (211) contains not more than 2 µg of lead (corresponding to not more than 0.001%) when tested by the limit test for *Lead* (251), 3 mL of *Ammonium Citrate Solution*, 1 mL of *Potassium Cyanide Solution*, and 0.5 mL of *Hydroxylamine Hydrochloride Solution* being used.

**Sodium iodide**—Dissolve about 200 mg, accurately weighed, in 100 mL of water, add 1 mL of nitric acid, mix, and titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically, using silver and glass electrodes. Each mL of 0.01 N silver nitrate is equivalent to 1.499 mg of sodium iodide. Not more than 5.0% of sodium iodide, calculated on the dried basis, is found.

**Other requirements**—Where the label states that Indocyanine Green is sterile, it meets the requirements for *Sterility Tests* (71) and for *Bacterial endotoxins* under *Indocyanine Green for Injection*. Where the label states that Indocyanine Green must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Indocyanine Green for Injection*.

**Assay**—Dissolve a quantity of Indocyanine Green, equivalent to about 100 mg of dried indocyanine green and accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution containing about 2 µg per mL. Dissolve a quantity of USP Indocyanine Green RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a Standard solution having a known concentration of about 2 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 785 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of  $C_{43}H_{47}N_2NaO_6S_2$  in the Indocyanine Green taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Indocyanine Green RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution of Indocyanine Green and the Standard solution, respectively.

## Indocyanine Green for Injection

» Indocyanine Green for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{43}H_{47}N_2NaO_6S_2$ .

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Indocyanine Green RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

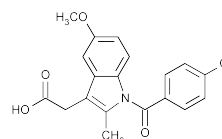
**Bacterial endotoxins** (85)—It contains not more than 7.1 USP Endotoxin Units per mg of indocyanine green.

**pH** (791): between 5.5 and 7.5, in a solution (1 in 200).

**Content variation**—Transfer the contents of each of 5 containers individually to separate 100-mL volumetric flasks with the aid of methanol. To each flask add methanol to volume. Dilute the solutions quantitatively and stepwise with methanol, to obtain a concentration of about 2.5 µg per mL. Proceed as directed in the *Assay* under *Indocyanine Green*, beginning with "Concomitantly determine the absorbances." The requirements are met if the content of each of not less than 4 of the containers tested is within the limits specified under *Uniformity of Dosage Units* (905).

**Other requirements**—It responds to the *Identification* tests, and meets the requirements for *Arsenic*, *Lead*, *Sodium iodide*, and *Assay* under *Indocyanine Green*. It meets also the requirements for *Sterility Tests* (71) and for *Labeling* under *Injections* (1).

## Indomethacin



$C_{19}H_{16}ClNO_4$  357.79

1*H*-Indole-3-acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-

1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid [53-86-1].

» Indomethacin contains not less than 98.0 percent and not more than 101.0 percent of  $C_{19}H_{16}ClNO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Indomethacin RS

**Identification—**

**A:** *Infrared Absorption* (197M).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 25 µg per mL.

*Medium:* hydrochloric acid in methanol (1 in 120).

Absorptivities at 318 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Its X-ray diffraction pattern (see *X-ray Diffraction* (941)) conforms to that of USP Indomethacin RS.

**Loss on drying** (731)—Dry it at a pressure below 5 mm of mercury at 100° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

**Assay—**

*Mobile phase*—Prepare a suitable solution of 0.01 M monobasic sodium phosphate and 0.01 M dibasic sodium phosphate in acetonitrile and water (approximately 1:1).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Indomethacin RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Weigh accurately about 100 mg of Indomethacin, and transfer to a 100-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix.

Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 10-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub> in the portion of Indomethacin taken by the formula:

$$1000C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Indomethacin RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained at equivalent retention times from the *Assay preparation* and the *Standard preparation*, respectively.

## Indomethacin Capsules

» Indomethacin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub>.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards <11>—

USP Indomethacin RS

### Identification—

**A:** Shake a portion of the contents of Capsules, equivalent to about 50 mg of indomethacin, with 10 mL of acetone for about 2 minutes, and filter. Transfer 5 mL of the filtrate to a stoppered flask, add 20 mL of water, and shake for about 2 minutes until a precipitate forms and crystallizes. Filter, and collect the crystals. Dry the crystals in air, then dry at a pressure below 5 mm of mercury at 100° for 2 hours: the IR absorption spectrum of a potassium bromide dispersion of the dried residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Indomethacin RS that has been similarly recrystallized from a solution of 25 mg in 5 mL of acetone.

**B:** Shake a portion of the contents of Capsules, equivalent to about 25 mg of indomethacin, with 25 mL of methanol, and filter. Separately apply 2 μL of the filtrate so obtained (test solution) and 2 μL of a Standard solution in methanol containing 1 mg of USP Indomethacin RS per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture, and dry the spots with the aid of a current of air. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow it to dry, and locate the spots under short-wavelength UV light: the intensity and *R<sub>F</sub>* value of the principal spot obtained from the test solution correspond to that obtained from the Standard solution.

### Dissolution <711>—

**Medium:** 1 volume of pH 7.2 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) mixed with 4 volumes of water; 750 mL.

**Apparatus 1:** 100 rpm.

**Time:** 20 minutes.

**Procedure**—Determine the amount of C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub> dissolved from UV absorbances at the wavelength of maximum absorbance at about 318 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Indomethacin RS in the same medium.

**Tolerances**—Not less than 80% (*Q*) of the labeled amount of C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub> is dissolved in 20 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—Transfer the contents of 1 Capsule to a 100-mL volumetric flask, add 10 mL of water, and allow to stand for 10 minutes, swirling occasionally. Add 60 mL of methanol, shake for 10 minutes, dilute with methanol to volume, mix, and centrifuge. Dilute a portion of the clear solution quantitatively and stepwise, if necessary, with a mixture of equal volumes of methanol and pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to obtain a solution containing about 25 μg of indomethacin per mL. Concomitantly determine the absorbances of this solution and a Standard solution of USP Indomethacin RS, in the methanol and pH 7.0 phosphate buffer mixture (1:1) having a known concentration of about 25 μg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 318 nm, with a suitable spectrophotometer, using the methanol and pH 7.0 phosphate buffer mixture as the blank. Calculate the quantity, in mg, of C<sub>19</sub>H<sub>16</sub>ClNO<sub>4</sub> in the Capsule taken by the formula:

$$(TC / D)(A_u / A_s)$$

in which *T* is the labeled quantity, in mg, of indomethacin in the Capsule, *C* is the concentration, in μg per mL, of USP Indomethacin RS in the Standard solution, *D* is the concentration, in μg per mL, of indomethacin in the test solution, based upon the labeled quantity per Capsule and the extent of dilution, and *A<sub>u</sub>* and *A<sub>s</sub>* are the absorbances of the solution from the Capsule and the Standard solution, respectively.

### Assay—

**Standard preparation**—Transfer about 25 mg of USP Indomethacin RS, accurately weighed, to a 200-mL volumetric flask, dissolve in 2 mL of methanol, dilute with pH 7.2 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to volume, and mix. Transfer 25.0 mL of this solution to a separator, and extract with three 25-mL portions of methylene chloride. Filter the extracts through a pledget of cotton into a 100-mL volumetric flask, rinse the filter with methylene chloride, dilute with methylene chloride to volume, and mix to obtain a *Standard preparation* having a known concentration of about 31 μg per mL.

**Assay preparation**—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared container, and determine the average content weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion, equivalent to about 25 mg of indomethacin, to a 200-mL volumetric flask, add 2 mL of methanol, shake for 10 minutes, dilute with pH 7.2 phosphate buffer to volume, and mix. Transfer about 50 mL to a centrifuge tube, and centrifuge for 15 minutes. Transfer 25.0 mL of the supernatant to a 125-mL separator, and extract with three 25-mL portions of methylene chloride. Filter the extracts through a pledget of cotton into a 100-mL vol-

umetric flask, rinse the filter with methylene chloride, dilute with methylene chloride to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 318 nm, with a suitable spectrophotometer, using methylene chloride as the blank. Calculate the quantity, in mg, of  $C_{19}H_{16}ClNO_4$  in the portion of Capsules taken by the formula:

$$0.8C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Indomethacin RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Indomethacin Extended-Release Capsules

### DEFINITION

Indomethacin Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ).

### IDENTIFICATION

- A.** **Standard solution:** 5 mg/mL of USP Indomethacin RS in acetone

**Sample solution:** Shake a portion of Capsule contents, nominally equivalent to 50 mg of indomethacin, with 10 mL of acetone for about 2 min, and filter.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Transfer 5 mL of each of the *Samples* to individual stoppered flasks, add 20 mL of water to each flask, and shake for 2 min until a precipitate forms and crystallizes. Filter and collect the crystals. Dry the crystals in air, then dry at a pressure below 5 mm of mercury at 100° for 2 h.

**Acceptance criteria:** The IR absorption spectrum of a potassium bromide dispersion of the dried crystals from the *Sample solution* so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the *Standard solution*.

- B.** **Standard solution:** 1 mg/mL of USP Indomethacin RS in methanol

**Sample solution:** Shake a portion of Capsule contents, nominally equivalent to 25 mg of indomethacin, with 25 mL of methanol, and filter.

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 2  $\mu\text{L}$

**Developing solvent system:** Chloroform and methanol (4:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Dry the spots with the aid of a current of air. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow it to dry, and locate the spots under short-wavelength UV light.

**Acceptance criteria:** The intensity and  $R_f$  value of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

### C.

**Sample solution:** Equivalent to 1 mg/mL of indomethacin in sodium hydroxide solution (0.4 mg/mL) from powdered Capsule contents

**Analysis:** Shake the *Sample solution* for 5 min, and filter. To 1 mL of the clear filtrate add 1 mL of 1 mg/mL sodium nitrite solution, mix, and allow to stand for 5 min. Add 0.5 mL of sulfuric acid.

**Acceptance criteria:** A golden yellow color develops.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol, water, and phosphoric acid (600: 400: 0.8)

**Diluent:** Phosphoric acid and water (1:99)

**Standard solution A:** 0.8 mg/mL of USP Indomethacin RS, prepared by dissolving 60% of the flask volume in acetonitrile and diluting with *Diluent* to volume

**Standard stock solution B:** 0.18 mg/mL of 4-chlorobenzoic acid in acetonitrile

**Standard solution B:** 0.0036 mg/mL of 4-chlorobenzoic acid in *Diluent*, from *Standard stock solution B*

**Sample solution:** Weigh and finely powder the contents of NLT 20 Capsules. Transfer a portion of the powder, nominally equivalent to 75 mg of indomethacin, to a 100-mL volumetric flask, add 40 mL of *Diluent*, and shake for 1 h. Sonicate for 15 min, add 40 mL of acetonitrile, sonicate for 15 min, and dilute with acetonitrile to volume. Centrifuge a portion of this solution, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution A* and *Standard solution B*

#### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates from the indomethacin peak, *Standard solution A*

**Tailing factor:** NMT 2.0 for the indomethacin peak, *Standard solution A*

**Capacity factor,  $k'$ :** NLT 4.0 for the indomethacin peak, *Standard solution A*; and NLT 0.9 for the 4-chlorobenzoic acid peak, *Standard solution B*

**Relative standard deviation:** NMT 2.0%, *Standard solution A*

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of indomethacin in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from *Standard solution A*

$C_S$  = concentration of USP Indomethacin RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

**Test 1:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** pH 6.2 phosphate buffer (see *Reagents, Indicators, and Solutions*); 750 mL

**Apparatus 1:** 75 rpm

**Times:** 1, 2, 4, 6, 12, and 24 h

**Sample solution:** Sample per *Dissolution* <711>. Dilute with *Medium* as necessary, filtered.



**Standard solution:** USP Indomethacin RS at a known concentration in *Medium*

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** 318 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved
1	10%–25%
2	20%–40%
4	35%–55%
6	45%–65%
12	60%–80%
24	NLT 80%

The percentages of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ) dissolved at the times specified conform to *Acceptance Table 2*.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Apparatus, Sample solution, Standard solution, and**

**Analysis:** Proceed as directed in *Test 1*.

**Medium:** pH 6.2 phosphate buffer (see *Reagents, Indicators, and Solutions*); 900 mL

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Amount Dissolved
1	12%–32%
2	27%–52%
4	50%–80%
12	NLT 80%

The percentages of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ) dissolved at the times specified conform to *Acceptance Table 2*.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions*); 750 mL

**Apparatus, Sample solution, Standard solution, and**

**Analysis:** Proceed as directed in *Test 1*.

**Tolerances:** See *Table 3*.

**Table 3**

Time (h)	Amount Dissolved
1	15%–40%
2	35%–55%
4	55%–75%
6	65%–85%
12	NLT 75%
24	NLT 85%

The percentages of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ) dissolved at the times specified conform to *Acceptance Table 2*.

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** pH 6.2 phosphate buffer (see *Reagents, Indicators, and Solutions*); 900 mL

**Apparatus 1:** 75 rpm

**Times:** 1, 2, 4, 12, and 24 h

**Mobile phase:** Acetonitrile and 0.1% phosphoric acid (60:40)

**Standard stock solution:** 0.4 mg/mL of USP Indomethacin RS in solution prepared as follows. Transfer a suitable amount of USP Indomethacin RS into a suitable volumetric flask. Add 10% of the flask volume of acetonitrile, and sonicate to promote dissolution, if necessary. Dilute with *Medium* to volume.

**Standard solution:** (L/900) mg/mL of USP Indomethacin RS in *Medium* from the *Standard stock solution*, where L is the label claim, in mg

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 100-mm; 3.5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 3%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result} = (r_U/r_S) \times C_S$$

$r_U$  = peak response of indomethacin from the *Sample solution*

$r_S$  = peak response of indomethacin from the *Standard solution*

$C_S$  = concentration of USP Indomethacin RS in the *Standard solution*

Calculate the percentages of the labeled amount ( $Q_i$ ) of indomethacin ( $C_{19}H_{16}ClNO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_i = \{[C_i \times (V - ([i - 1] \times V_S))] + [(C_{[i-1]} + C_{[i-2]} + \dots + C_1) \times V_S]\} \times (1/L) \times 100$$

$C_i$  = concentration of indomethacin in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of indomethacin (mg/Capsule)

$V_S$  = volume of the *Sample solution* withdrawn from the *Medium* (mL)

**Tolerances:** See *Table 4*.

**Table 4**

Time (h)	Time Point (i)	Amount Dissolved
1	1	10%–30%
2	2	20%–40%
4	3	35%–55%
12	4	60%–80%
24	5	NLT 75%

The percentages of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ) dissolved at the times specified conform to *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** (905)

**Analysis for content uniformity**

**Solution A:** Dissolve 17.42 g of dibasic potassium phosphate in 800 mL of water, adjusting with phosphoric acid to a pH of 7.5, and diluting with water to 1000 mL (pH 7.5 phosphate buffer).

**Standard solution:** 25 µg/mL of USP Indomethacin RS in a mixture of methanol and *Solution A* (1:1)

**Sample solution:** 25 µg/mL of indomethacin in a mixture of methanol and *Solution A* (1:1). Prepare as follows. Transfer the contents of 1 Capsule to a 200-mL volumetric flask, and add 100 mL of a mixture of methanol and *Solution A* (1:1). Sonicate until the contents are dispersed, dilute with the methanol and *Solution A* mixture (1:1) to volume, and centrifuge. Dilute a portion of the clear solution with the methanol and *Solution A* mixture (1:1) to obtain the above concentration.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** 318 nm

**Cell:** 1 cm

**Blank:** Methanol and *Solution A* (1:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Indomethacin RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of indomethacin in the *Sample solution* (µg/mL)

**Acceptance criteria:** Meet the requirements

**IMPURITIES**

• **LIMIT OF 4-CHLOROBENZOIC ACID**

**Mobile phase, Diluent, Standard solution A, Standard solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution B* and *Sample solution*

Using the peak responses measured and recorded in the *Assay*, calculate the percentage of 4-chlorobenzoic acid ( $C_7H_5ClO_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of 4-chlorobenzoic acid in *Standard solution B* (mg/mL)

$C_U$  = measured concentration of indomethacin in the *Sample solution* as determined from the *Assay* (mg/mL)

**Acceptance criteria:** NMT 0.44%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.

• **USP REFERENCE STANDARDS** (11)

USP Indomethacin RS

## Indomethacin Topical Gel

» Indomethacin Topical Gel contains not less than 0.90 g and not more than 1.10 g of Indomethacin in 100 mL of gel. Prepare Indomethacin Topical Gel as follows:

Indomethacin . . . . .	1.0 g
Carbomer 941 . . . . .	2.0 g
Purified Water . . . . .	10 mL
Alcohol (95% ethyl alcohol), a sufficient quantity to make . . . . .	100 mL

Transfer the Indomethacin to a suitable beaker, and dissolve it in 55 mL of Alcohol. Transfer this solution to a glass mortar, and slowly add the Carbomer 941 so that it is thoroughly distributed. Press out any white lumps until a smooth gel is formed. Slowly add the Purified Water with mixing. Add a sufficient quantity of Alcohol to obtain a final volume of 100 mL, and mix. Transfer the Gel to a wide-mouth container or ointment jar.

**Packaging and storage**—Preserve in tight, light-resistant, wide-mouth containers or ointment jars. Store at controlled room temperature.

**Labeling**—Label it to state that it is for topical, external use only, that it should be used only as directed, and that the container should be kept tightly closed.

**Beyond-use date**—Thirty days after the day on which it was compounded.

## Indomethacin Suppositories

» Indomethacin Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{19}H_{16}ClNO_4$ .

**Packaging and storage**—Preserve in well-closed containers, at controlled room temperature.

**USP Reference standards** (11)—

USP Indomethacin RS

**Identification**—

**Standard preparation**—Prepare a solution, containing about 125 µg of USP Indomethacin RS per mL, by first dissolving the Reference Standard in a volume of methanol that is one one-hundredth of the volume of the solution to be prepared, then adding ether to volume, and mixing.

**Test preparation**—Use the ether extract contained in the 200-mL volumetric flask obtained as directed under *Assay preparation* in the *Assay*.

**Procedure**—Separately apply 10 µL each of the *Test preparation* and the *Standard preparation* to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and glacial acetic acid (19:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light: the  $R_f$  value of

the principal spot in the chromatogram of the *Test preparation* corresponds to that obtained from the *Standard preparation*.

#### Dissolution <711>—

**Medium:** 0.1 M, pH 7.2 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_{19}H_{16}ClNO_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 320 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Indomethacin RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{19}H_{16}ClNO_4$  is dissolved in 60 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—Place 1 Suppository into a 100-mL volumetric flask containing 80 mL of a solution of methanol and glacial acetic acid (199:1), shake by mechanical means until the Suppository is dissolved, dilute with the methanol-glacial acetic acid solution to volume, and mix. Filter a portion of the solution, discarding the first 15 mL of the filtrate, and dilute an accurately measured volume of the clear filtrate quantitatively and stepwise, if necessary, with the methanol-glacial acetic acid solution to obtain a solution containing about 25 µg of indomethacin per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Indomethacin RS in the same medium having a known concentration of about 25 µg per mL at the wavelength of maximum absorbance at about 320 nm, with a suitable spectrophotometer, using the methanol-glacial acetic acid solution as the blank. Calculate the quantity, in mg, of  $C_{19}H_{16}ClNO_4$  in the Suppository taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of indomethacin in the Suppository; *C* is the concentration, in µg per mL, of USP Indomethacin RS in the Standard solution; *D* is the concentration, in µg per mL, of indomethacin in the solution from the Suppository, on the basis of the labeled quantity per Suppository and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Suppository and the Standard solution, respectively.

#### Assay—

**Solvent mixture**—Prepare a solution of methanol and glacial acetic acid (199:1).

**Standard preparation**—Prepare a solution, having a known concentration of about 165 µg of USP Indomethacin RS per mL, by first dissolving an accurately weighed quantity of the Reference Standard in a volume of methanol that is one one-hundredth of the nominal volume of the volumetric flask being used, then adding ether to volume, and mixing. Transfer 15.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 25 µg of USP Indomethacin RS per mL.

**Assay preparation**—Weigh, mash, and then mix not less than 10 Suppositories. Transfer an accurately weighed portion of the mass, equivalent to about 25 mg of indomethacin, to a 125-mL separator, add 15 mL of water and 50 mL of ether, and shake until the mass is dissolved. Transfer the ether layer to a 200-mL volumetric flask, extract the aqueous layer with two additional 50-mL portions of ether, and combine the ether extracts in the 200-mL volumetric flask. Discard the aqueous layer. Dilute with *Solvent mixture* to

volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 320 nm, with a suitable spectrophotometer, using *Solvent mixture* as a blank. Calculate the quantity, in mg, of  $C_{19}H_{16}ClNO_4$  in the portion of Suppositories taken by the formula:

$$C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Indomethacin RS in the *Standard preparation*; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Indomethacin Oral Suspension

» Indomethacin Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards <11>—

USP Indomethacin RS

#### Identification—

**A:** Mix a portion of Oral Suspension, equivalent to about 25 mg of indomethacin, with 25 mL of a 1 in 200 solution of glacial acetic acid in methanol, and filter. Separately apply 2 µL of the filtrate so obtained (test solution) and 2 µL of a Standard solution in methanol containing 1 mg of USP Indomethacin RS per mL to a suitable thin-layer chromatographic plate (see *Chromatography* <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture, and dry the spots with the aid of a current of air. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and glacial acetic acid (19:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow it to dry, and locate the spots under short-wavelength UV light: the intensity and *R<sub>F</sub>* value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B:** The retention time of the indomethacin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

#### Dissolution <711>—

**Medium:** 0.01 M pH 7.2 phosphate buffer prepared by dissolving 1.36 g of monobasic potassium phosphate in 1 L of water and adjusting with 0.1 N sodium hydroxide to a pH of 7.2 ± 0.1; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 20 minutes.

**Procedure**—Transfer to the surface of the *Medium* in the dissolution vessel an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of indomethacin. Determine the amount of  $C_{19}H_{16}ClNO_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 320 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Indomethacin RS in the same *Medium*. [NOTE—An amount of methanol not to exceed 1.0% of the volume of the Stan-

dard solution may be used to bring the USP Reference Standard into solution prior to dilution with *Medium*, and the solution may be sonicated to effect complete dissolution of the USP Reference Standard.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{19}H_{16}ClNO_4$  is dissolved in 20 minutes.

**Uniformity of dosage units** (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 2.5 and 5.0.

**Limit of 4-chlorobenzoic acid**—Using the chromatograms obtained as directed for the Assay, calculate the percentage of 4-chlorobenzoic acid ( $C_7H_5ClO_2$ ) in the Oral Suspension taken by the formula:

$$5(C_4 / C_A)(r_A / r_4)$$

in which  $C_4$  is the concentration, in  $\mu\text{g}$  per mL, of 4-chlorobenzoic acid in the *Standard 4-chlorobenzoic acid preparation*;  $C_A$  is the quantity, in mg, of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in the portion of Oral Suspension taken to prepare the *Assay preparation*, determined as directed in the Assay; and  $r_A$  and  $r_4$  are the 4-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard 4-chlorobenzoic acid preparation*, respectively: not more than 0.44% is found.

**Content of sorbic acid (if present)**—Using the chromatograms obtained as directed for the Assay, calculate the quantity, in mg, of sorbic acid ( $C_6H_8O_2$ ) in each mL of the Oral Suspension taken by the formula:

$$50(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of sorbic acid in the *Standard indomethacin preparation*;  $V$  is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the sorbic acid peak responses obtained from the *Assay preparation* and the *Standard indomethacin preparation*, respectively. It contains between 80% and 120% of the labeled amount.

**Assay**—

**Phosphoric acid solution**—Dilute 2 mL of phosphoric acid with water to make 1000 mL of solution.

**Solvent mixture**—Prepare a solution consisting of a mixture of dehydrated alcohol and butyl alcohol (8:5).

**Mobile phase**—Prepare a suitable mixture of *Phosphoric acid solution* and *Solvent mixture* (610:390), pass through a suitable filter having a 0.5- $\mu\text{m}$  or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard indomethacin preparation**—Transfer about 40 mg of USP Indomethacin RS, accurately weighed, to a 50-mL volumetric flask. Where the Oral Suspension is represented as containing a stated amount of sorbic acid, add 40/ $J$  mg of sorbic acid, accurately weighed,  $J$  being the ratio of the labeled amount, in mg, of sorbic acid to the labeled amount, in mg, of indomethacin per mL of the Oral Suspension. Add 10 mL of *Phosphoric acid solution* and 15 mL of *Solvent mixture*, and sonicate for 5 minutes. Dilute with *Phosphoric acid solution* to volume, and mix. This solution contains about 0.8 mg of USP Indomethacin RS and, where added, about 0.8/ $J$  mg of sorbic acid.

**Standard 4-chlorobenzoic acid preparation**—[NOTE—Prepare this *Standard 4-chlorobenzoic acid preparation* and chromatograph it as directed under *Chromatographic system* and *Procedure* only if the test for *Limit of 4-Chlorobenzoic acid* is being performed.] Dissolve a suitable quantity of 4-chlorobenzoic acid, accurately weighed, in *Solvent mixture* to obtain a solution having a known concentration of about

0.09 mg per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask containing 15 mL of *Solvent mixture*, dilute with *Phosphoric acid solution* to volume, and mix. This solution contains about 1.8  $\mu\text{g}$  of 4-chlorobenzoic acid per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 40 mg of indomethacin, to a 50-mL volumetric flask, add 15 mL of *Solvent mixture*, and sonicate for 10 minutes. Dilute with *Phosphoric acid solution* to volume, mix, and pass through a suitable filter having a 0.5- $\mu\text{m}$  or finer porosity.

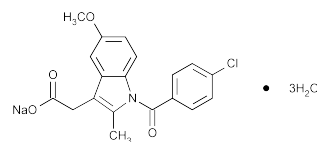
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and an 8-mm  $\times$  10-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph the *Standard indomethacin preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for indomethacin is not less than 2.5; the column efficiency determined from the analyte peak is not less than 500 theoretical plates; the resolution,  $R$ , between sorbic acid (where present) and indomethacin is not less than 4.0; the tailing factor for the analyte peak(s) is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Standard 4-chlorobenzoic acid preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the 4-chlorobenzoic acid peak, is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.5%.

**Procedure**—Separately inject equal volumes (about 15  $\mu\text{L}$ ) of the *Standard indomethacin preparation*, the *Standard 4-chlorobenzoic acid preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in each mL of the Oral Suspension taken by the formula:

$$50(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Indomethacin RS in the *Standard indomethacin preparation*;  $V$  is the volume, in mL, of Oral Suspension taken; and  $r_U$  and  $r_S$  are the peak responses of the analyte obtained from the *Assay preparation* and the *Standard indomethacin preparation*, respectively.

## Indomethacin Sodium



$C_{19}H_{15}ClNNaO_4 \cdot 3H_2O$  433.82  
1*H*-Indole-3-acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-, sodium salt, trihydrate.  
Sodium 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate, trihydrate [74252-25-8].  
Anhydrous 379.78

» Indomethacin Sodium contains not less than 98.0 percent and not more than 101.0 percent of  $C_{19}H_{15}ClNNaO_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must

be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Indomethacin RS

**Identification—**

**A:** It responds to *Identification test B* under *Indomethacin for Injection*.

**B:** Ignite a small amount of it on a platinum wire in a non-luminous flame: an intense yellow flame is produced.

**C:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**Loss on drying** (731)—Dry it at 100° for 2 hours at a pressure not exceeding 5 mm of mercury: it loses between 11.5% and 13.5% of its weight.

**Heavy metals, Method II** (231): 0.002%.

**Limit of acetone—**

**Standard solution**—Transfer 1.0 mL of acetone to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix. Insert the stopper, and cool in an ice bath.

**Test solution**—Transfer about 100 mg of Indomethacin Sodium, accurately weighed, to a 15-mL centrifuge tube, and dissolve in 1.0 mL of cool water. While vortexing this solution, add 1.0 mL of 0.24 N hydrochloric acid, centrifuge promptly, and filter the supernatant. Collect the filtrate in a suitable tube, cap, and cool in an ice bath.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 1.8-m column that contains support S3. The column temperature is maintained at 165°. The carrier gas is nitrogen. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *K'*, for acetone is between 4 and 7; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Using the solvent flush technique, with water as the flushing agent, separately inject equal volumes (about 3 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for 6 minutes, and measure the areas for the acetone peaks. Calculate the percentage of acetone in the portion of Indomethacin Sodium taken by the formula:

$$0.79(10 / W_U)(r_U / r_S)$$

in which 0.79 is the specific gravity of acetone; *W<sub>U</sub>* is the quantity, in mg, of Indomethacin Sodium taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetone peak areas obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

**Chromatographic purity—**

**Mobile phase, Diluent, and Chromatographic system**—Proceed as directed in the *Assay*.

**Standard preparation**—Transfer 2.0 mL of the *Stock impurity solution*, prepared as directed in the *Assay*, to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix. Each mL of this *Standard preparation* contains 0.002 mg of 4-chlorobenzoic acid and 0.002 mg of 5-methoxy-2-methyl-3-indoleacetic acid.

**Test preparation**—Use the stock solution used to prepare the *Assay preparation* as directed in the *Assay*.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation*, record the chromatograms for 25 minutes, and measure the response for the peaks having retention times corresponding to the major peaks obtained in the chromatogram of the *Standard preparation*. The relative standard deviation for replicate injections of the *Standard preparation* is not more than

5.0%. Calculate the percentages of 4-chlorobenzoic acid and of 5-methoxy-2-methyl-3-indoleacetic acid in the portion of Indomethacin Sodium taken by the formula:

$$20(r_U / r_S) / [W_U(1.00 - 0.01L)]$$

in which *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the corresponding analytes obtained from the *Test preparation* and the *Standard preparation*, respectively; *W<sub>U</sub>* is the quantity, in mg, of Indomethacin Sodium taken to prepare the *Assay preparation*, as described in the *Assay*; and *L* is the percentage of weight loss obtained in the test for *Loss on drying*: the sum of the percentages of 4-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid does not exceed 0.2%. Calculate the percentage of each peak other than the solvent peak, the main indomethacin peak, the 4-chlorobenzoic acid peak, and the 5-methoxy-2-methyl-3-indoleacetic acid peak in the chromatogram of the *Test preparation* taken by the formula:

$$100r_i / r_t$$

in which *r<sub>i</sub>* is the response of each other peak; and *r<sub>t</sub>* is the sum of the responses of all the peaks, excluding that of the solvent peak: not more than 0.5% of any individual peak is found, and the sum of these individual peaks is not more than 1.0%.

**Other requirements**—Where the label states that Indomethacin Sodium is sterile, it meets the requirements for *Sterility Tests* (71) and for *Pyrogen* under *Indomethacin for Injection*. Where the label states that Indomethacin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Pyrogen* under *Indomethacin for Injection*.

**Assay—**

**Mobile phase**—Prepare a suitable filtered and degassed mixture of methanol, water, acetonitrile, and phosphoric acid (550: 300:150:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a sufficient quantity of a mixture of acetonitrile and water (3:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Indomethacin RS quantitatively in *Diluent* to obtain a stock solution containing 0.80 mg per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Diluent* to obtain a solution containing 0.16 mg per mL (*Standard preparation*).

**Assay preparation**—Transfer about 100 mg of Indomethacin Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this stock solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Stock impurity solution**—Dissolve accurately weighed quantities of 4-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid quantitatively in *Diluent* to obtain a solution containing 0.20 mg of each per mL.

**Resolution solution**—Prepare a mixture of the stock solution used to prepare the *Diluent*, the *Standard preparation*, and the *Stock impurity solution* (7:2:1).

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1, and is maintained at a temperature of 35 ± 1°. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the capacity factor, *K'*, for the indomethacin peak is not less than 2.5, the column efficiency determined from the indomethacin peak is not less than 3500 theoretical plates, the tailing factor for the indomethacin peak is not more than 1.3, and the resolution, *R*, between the 4-chlorobenzoic acid peak and the 5-methoxy-2-methyl-3-indoleacetic acid peak is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak re-

sponses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of indomethacin sodium ( $C_{19}H_{15}ClNNaO_4$ ) in the portion of Indomethacin Sodium taken by the formula:

$$(379.78 / 357.79)(500C)(r_U / r_S)$$

in which 379.78 and 357.79 are the molecular weights of anhydrous indomethacin sodium and indomethacin, respectively;  $C$  is the concentration, in mg per mL, of USP Indomethacin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Indomethacin for Injection

» Indomethacin for Injection contains an amount of Indomethacin Sodium equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of indomethacin ( $C_{19}H_{16}ClNO_4$ ).

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections* (1).

**USP Reference standards** (11)—

USP Endotoxin RS

USP Indomethacin RS

**Constituted solution**—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution*—Dissolve Indomethacin for Injection in methanol to obtain a solution having a concentration of about 5 mg of indomethacin per mL.

*Standard solution:* 5 mg per mL in methanol.

*Developing solvent system:* a mixture of chloroform and glacial acetic acid (19:1).

*Procedure*—Proceed as directed in the chapter, except to dry the spots with the aid of a current of air. The intensity and  $R_f$  value of the principal spot obtained from the *Test solution* correspond to those obtained from the *Standard solution*.

**Bacterial endotoxins**—Using a test solution, prepared by dissolving Indomethacin for Injection in LAL Reagent Water to obtain a concentration of 1.0 mg of indomethacin per mL, proceed as directed under *Bacterial Endotoxins Test* (85). It contains not more than 20.0 USP Endotoxin Units per mg of indomethacin.

**pH** (791): between 5.0 and 7.0, in a solution in water (1 in 2000) containing 0.3 mL of saturated potassium chloride solution per 100 mL.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Limit of 4-chlorobenzoic acid**—

*Mobile phase and Solvent mixture*—Prepare as directed in the *Assay*.

*Standard preparation*—Dissolve a suitable quantity of 4-chlorobenzoic acid, accurately weighed, in acetonitrile to

obtain a solution having a known concentration of about 0.22 mg per mL. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 150 mL of acetonitrile, dilute with water to volume, and mix. This solution contains about 0.44  $\mu$ g of 4-chlorobenzoic acid per mL.

*Test preparation*—Use the *Assay preparation*.

*Chromatographic system*—Prepare as directed in the *Assay*. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , for the 4-chlorobenzoic acid peak is not less than 1.0.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the 4-chlorobenzoic acid peak areas. Calculate the percentage of 4-chlorobenzoic acid in the portion of Indomethacin for Injection taken by the formula:

$$10(C_4 / NC_A)(r_U / r_S)$$

in which  $C_4$  is the concentration, in  $\mu$ g per mL, of 4-chlorobenzoic acid in the *Standard preparation*;  $N$  is the number of containers of Indomethacin for Injection taken;  $C_A$  is the quantity, in mg, of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in each container of Indomethacin for Injection taken, determined as directed herein; and  $r_U$  and  $r_S$  are the 4-chlorobenzoic acid peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 2.2%, equivalent to not more than 5.0%, calculated as indomethacin, is found.

**Other requirements**—It meets the requirements under *Sterility Tests* (71), *Uniformity of Dosage Units* (905), and for *Labeling* under *Injections* (1).

**Assay**—

*Mobile phase*—Prepare a suitable mixture of methanol, water, and phosphoric acid (600:400:1), and pass through a suitable filter having a 0.5- $\mu$ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Solvent mixture*—Prepare a mixture of water, acetonitrile, and phosphoric acid (700:300:1).

*Standard preparation*—Transfer about 20 mg of USP Indomethacin RS, accurately weighed, to a 200-mL volumetric flask, and dissolve in 60 mL of acetonitrile. Dilute with water to volume, and mix.

*Assay preparation*—Select an accurately counted number of containers of Indomethacin for Injection, equivalent to a total of about 10 mg of indomethacin, and constitute each with a volume of *Solvent mixture* sufficient to obtain solutions containing the equivalent of about 0.5 mg of indomethacin per mL. Wash the contents of these containers with the aid of the *Solvent mixture* into a 100-mL volumetric flask. Dilute with *Solvent mixture* to volume, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Use the filtrate as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the indomethacin peak is not less than 1500 theoretical plates; the capacity factor,  $k'$ , for the indomethacin peak is not less than 3.5; the tailing factor for the indomethacin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity,  $C_A$ ,

in mg, of indomethacin ( $C_{19}H_{16}ClNO_4$ ) in each container of Indomethacin for Injection taken by the formula:

$$100(C/N)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Indomethacin RS in the *Standard preparation*;  $N$  is the number of containers of Indomethacin for Injection taken; and  $r_U$  and  $r_S$  are the indomethacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Insulin



$C_{256}H_{381}N_{65}O_{76}S_6$  5777.54  
Insulin (pig) [12584-58-6].



$C_{254}H_{377}N_{65}O_{75}S_6$  5733.49  
Insulin (ox) [11070-73-8].

» Insulin is a protein that affects the metabolism of glucose. It is obtained from the pancreas of healthy bovine or porcine animals, or both, used for food by humans. Its potency, calculated on the dried basis, is not less than 26.5 USP Insulin Units in each mg; Insulin labeled as purified contains not less than 27.0 USP Insulin Units in each mg, calculated on the dried basis. The proinsulin content, determined by a validated method, is not more than 10 ppm.

NOTE—One USP Insulin Unit is equivalent to 0.0342 mg of pure Insulin derived from beef or 0.0345 mg of pure Insulin derived from pork.

**Packaging and storage**—Preserve in tight containers. Store, protected from light, in a freezer.

**Labeling**—Label it to indicate the one or more animal species to which it is related, as pork, as beef, or as a mixture of pork and beef. If the Insulin is purified, label it as such.

### USP Reference standards (11)—

USP Endotoxin RS  
USP Insulin RS  
USP Insulin (Beef) RS  
USP Insulin (Pork) RS

### Identification—

**A:** The retention time of the insulin peak in the chromatogram of the *Assay preparation* corresponds to the retention time of the appropriate species in the chromatogram of the *Identification preparation*, as obtained in the *Assay*. [NOTE—It may be necessary to inject a mixture of *Assay preparation* and *Identification preparation*.]

**B:** Proceed as directed for *Identification test B* under *Insulin Human*, except to use 1 mg of USP Insulin Reference Standard of the appropriate species to prepare the *Standard digest solution*, to use 1 mg of Insulin to prepare the *Test digest solution*, and to obtain a resolution,  $R$ , between digest fragments II and III of not less than 1.9: meets the requirements.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—The total bacterial count does

not exceed 300 per g, the test being made on a portion of about 0.2 g, accurately weighed.

**Bioidentity**—It meets the requirements of the *Bioidentity test* under *Insulin Assays* (121).

**Bacterial endotoxins (85)**—It contains not more than 10 USP Endotoxin Units in each mg.

**Loss on drying (731)**—Dry about 200 mg, accurately weighed, at 105° for 16 hours: it loses not more than 10.0% of its weight.

### Related compounds—

**Solvent**—Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, adjust, if necessary, with ethanolamine to a pH of 2.3, and mix.

**Solution A**—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (82:18).

**Solution B**—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (50:50).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Proceed as directed in the *Assay*.

**Standard solution A**—Dissolve an accurately weighed quantity of USP Insulin RS of the appropriate species in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 3.75 mg per mL.

**Standard solution B**—Pipet 1 mL of *Standard solution A* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Standard solution C**—Pipet 1 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. [NOTE—These three *Standard solutions* may be stored at room temperature for up to 12 hours and in a refrigerator for up to 48 hours.]

**Test solution**—Transfer about 7.5 mg of Insulin to a suitable capped vial, and add 2.0 mL of 0.01 N hydrochloric acid. Cap the vial, and shake gently to dissolve. Store this solution for not more than 2 hours at room temperature or for not more than 12 hours in a refrigerator.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	81	19	equilibration
0–60	81	19	isocratic
60–85	81→36	19→64	linear gradient
85–91	36	64	isocratic
91–92	36→81	64→19	linear gradient

Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 31 minutes for insulin, with the A-21 desamido insulin eluting just prior to the start of the gradient elution phase. Chromatograph *Standard solutions A, B, and C*, record the chromatograms, and measure the peak responses as directed for *Procedure*: calculate the factor  $X_1$  by the formula:

$$10(r_B / r_A)$$

in which  $r_B$  and  $r_A$  are the areas of the peak responses obtained for *Standard solution B* and *Standard solution A*, re-

spectively. The value of  $X_1$  is between 0.91 and 1.09. Calculate the factor  $X_2$  by the formula:

$$100(r_C / r_A)$$

in which  $r_C$  and  $r_A$  are the areas of the peak responses obtained for *Standard solution C* and *Standard solution A*, respectively. The value of  $X_2$  is between 0.7 and 1.3. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between insulin and A-21 desamido insulin is not less than 2.0; and the tailing factor for the insulin peak is not more than 1.8.

**Procedure**—Inject a volume (about 20  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas of the responses for the main insulin peak, the A-21 desamido insulin peak, and the peaks from any other impurities. Calculate the percentage of insulin, %, in the portion of Insulin taken by the formula:

$$100(r_I / r_S)$$

in which  $r_I$  is the peak response for insulin, and  $r_S$  is the sum of the responses for all of the peaks. Calculate the percentage of A-21 desamido insulin, %D, in the portion of Insulin taken by the formula:

$$100(r_D / r_S)$$

in which  $r_D$  is the peak response for A-21 desamido insulin, and  $r_S$  is the sum of the responses for all of the peaks. Calculate the percentage of other insulin related compounds in the portion of Insulin taken by the formula:

$$100 - (\%I + \%D).$$

Not more than 10.0% of A-21 desamido insulin is found, and not more than 5.0% of other insulin related compounds is found. For Insulin derived from a single species, measure the responses of any peaks corresponding to beef or pork insulin, and calculate their concentration as a percentage of  $r_S$ : the amount of cross-contamination is not more than 1.0%.

#### Limit of high molecular weight proteins—

**Arginine solution**—Prepare a solution of L-arginine in water containing 1 mg per mL.

**Mobile phase**—Prepare a filtered and degassed mixture of *Arginine solution*, acetonitrile, and glacial acetic acid (65:20:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Dissolve 4 mg of Insulin containing more than 0.4% high molecular weight proteins in 1 mL of 0.01 N hydrochloric acid. Store this solution in a refrigerator, and use within 7 days. [NOTE—Insulin containing the indicated percentage of high molecular weight proteins may be prepared by allowing Insulin to stand at room temperature for about 5 days.]

**Test solution**—Transfer about 4 mg of Insulin to a small vial, add 1 mL of 0.01 N hydrochloric acid, and mix to dissolve. Store in a refrigerator, and use within 7 days.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 7.8-mm  $\times$  30-cm column that contains packing L20. The flow rate is about 0.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the retention times are between 13 and 17 minutes for the polymeric insulin complexes, about 17.5 minutes for the covalent insulin dimer, and between 18 and 22 minutes for the insulin monomer, with salts eluting after the insulin monomer; and the ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak is not less than 2.0.

**Procedure**—Inject a volume (about 100  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas of the peak responses, disregarding any peaks having retention times greater than that of the insulin monomer. Calculate the percentage of high molecular weight proteins in the portion of Insulin taken by the formula:

$$100\Sigma r_H / (\Sigma r_H + r_M)$$

in which  $\Sigma r_H$  is the sum of the responses for all peaks having retention times less than that of the insulin monomer, and  $r_M$  is the peak response of the insulin monomer: not more than 1.0% is found.

**Zinc content** (591)—Determine the zinc content of about 10 mg of it, accurately weighed: not more than 1.0% is found, calculated on the dried basis.

#### Assay—

**Mobile phase**—Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3 if necessary. Prepare a filtered and degassed mixture of this solution and acetonitrile (74:26). The acetonitrile is warmed to a temperature equal to or higher than 20° to avoid precipitation. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve about 1.5 mg of Insulin in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for not less than 3 days to obtain a solution containing not less than 5% of A-21 desamido insulin.

NOTE—The following *Identification preparation*, *Standard preparation*, and *Assay preparation* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours.

**Identification preparation**—Prepare a solution of USP Insulin (Pork) RS and USP Insulin (Beef) RS in 0.01 N hydrochloric acid containing about 0.6 mg of each per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Insulin RS of the appropriate species in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 1.5 mg per mL.

**Assay preparation**—Transfer about 15 mg of Insulin, accurately weighed, to a 10-mL volumetric flask, and dissolve in and dilute with 0.01 N hydrochloric acid to obtain a solution having a concentration of about 1.5 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.6%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between insulin and A-21 desamido insulin is not less than 2.0; and the tailing factor for the insulin peak is not more than 1.8.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Assay preparation*, the *Identification preparation*, and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the *Identification preparation* to identify the insulin peaks. For Insulin derived from a single species, calculate the potency on the undried basis, in USP Insulin Units per mg, of the Insulin in the *Assay preparation* by the formula:

$$(C_S / C_U)(\Sigma r_U / \Sigma r_S)$$

in which  $C_S$  is the concentration, in USP Insulin Units per mL, of USP Insulin RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Insulin in the *Assay preparation*; and  $\Sigma r_U$  and  $\Sigma r_S$  are the sums of the areas of the insulin



and A-21 desamido insulin peaks obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively. From the value obtained in the test for *Loss on drying*, calculate the potency on the dried basis. For Insulin derived from a mixture of beef and pork, calculate the total potency as the sum of the potencies of the beef- and pork-derived insulins, determined separately.

## Insulin Injection

» Insulin Injection is an isotonic, sterile solution of Insulin. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repack. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate the one or more animal species to which it is related, as pork, as beef, or as a mixture of pork and beef. If the Insulin Injection is made from Insulin that is purified, label it as such. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units per mL.

### USP Reference standards (11)—

USP Endotoxin RS  
USP Insulin RS  
USP Insulin (Beef) RS  
USP Insulin (Pork) RS

**Identification**—The retention time of the insulin peak in the chromatogram of the *Assay preparation* corresponds to the retention time of the appropriate species in the chromatogram of the *Identification preparation*, as obtained in the *Assay*. [NOTE—It may be necessary to inject a mixture of *Assay preparation* and *Identification preparation*.]

**Bacterial endotoxins** (85)—It contains not more than 80 USP Endotoxin Units for each 100 USP Insulin Units.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.0 and 7.8, determined potentiometrically.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Zinc content** (591): between 10 and 40 µg for each 100 USP Insulin Units of appropriate species.

### Limit of high molecular weight proteins—

*Arginine solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin*.

*Test solution*—Quantitatively add 4 µL of 6 N hydrochloric acid per mL of an accurately measured volume of Injection, and mix.

*Procedure*—Proceed as directed for *Procedure* in the test for *Limit of high molecular weight proteins* under *Insulin*. Not more than 2.0% is found.

**Other requirements**—It meets the requirements under *Injections* (1).

### Assay—

*Mobile phase, Identification preparation, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Insulin*.

NOTE—The *Identification preparation*, *Standard preparation*, and *Assay preparation* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours.

*Assay preparation 1* (for Injection labeled as containing 40 USP Insulin Units per mL)—Add 2.5 µL of 9.6 N hydrochloric acid per mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix.

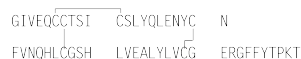
*Assay preparation 2* (for Injection labeled as containing 100 USP Insulin Units per mL)—Add 2.5 µL of 9.6 N hydrochloric acid per mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the test specimen.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

*Procedure*—Separately inject equal volumes (about 20 µL) of the appropriate *Assay preparation*, the *Identification preparation*, and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the *Identification preparation* to identify the insulin peaks. For Insulin Injection prepared from a single species, calculate the potency, in USP Insulin Units per mL, of the Injection taken by the formula:

$$(CD)(\Sigma r_U / \Sigma r_S)$$

in which C is the concentration, in USP Insulin Units per mL, of USP Insulin RS in the *Standard preparation*; D is the dilution factor; and  $\Sigma r_U$  and  $\Sigma r_S$  are the sums of the areas of the insulin and A-21 desamido insulin peaks obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively. For Injection prepared from a mixture of beef and pork insulins, calculate the total potency as the sum of the potencies of both beef and pork insulins, determined as directed above.

## Insulin Human



C<sub>257</sub>H<sub>383</sub>N<sub>65</sub>O<sub>77</sub>S<sub>6</sub> 5807.57  
Insulin (human) [11061-68-0].

» Insulin Human is a protein corresponding to the active principle elaborated in the human pancreas that affects the metabolism of carbohydrate (particularly glucose), fat, and protein. It is derived by enzymatic modification of insulin from pork pancreas in order to change its amino acid sequence appropriately, or produced by microbial synthesis via a recombinant DNA process. Its potency, calculated on the dried basis, is not less than 27.5 USP Insulin Human Units in each mg. The proinsulin content of Insulin Human derived from pork, determined by a validated method, is not more than 10 ppm. The host cell derived proteins content of Insulin Human derived from a recombinant DNA process, determined by an appropriate and validated method, is not more than 10 ppm. The host cell or vector derived DNA content and limit of Insulin Human derived from a recombinant DNA process that utilizes eukaryotic host cells are determined by a validated method.

NOTE—One USP Insulin Human Unit is equivalent to 0.0347 mg of pure Insulin Human.

**Packaging and storage**—Preserve in tight containers. Store in a freezer, and protect from light.

**Labeling**—Label it to indicate that it has been prepared by microbial synthesis or that it is derived by enzymatic modification of insulin from pork pancreas.

**USP Reference standards** (11)—  
USP Endotoxin RS  
USP Insulin Human RS

**Identification—**

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Determine the peptide fragments, using the following peptide mapping procedure.

*Sulfate buffer*—Mix equal volumes of 2.0 M ammonium sulfate and 0.5 M sulfuric acid, and filter.

*Enzyme solution*—Prepare a solution of *Staphylococcus aureus* V-8 protease in water having an activity of 500 units per mL.

*HEPES buffer*—Dissolve 2.38 g of HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) in about 90 mL of water in a 100-mL volumetric flask. Adjust with 5 M sodium hydroxide to a pH of 7.5, dilute with water to volume, and mix.

*Solution A*—Prepare a filtered and degassed mixture of 100 mL of acetonitrile, 700 mL of water, and 200 mL of *Sulfate buffer*.

*Solution B*—Prepare a filtered and degassed mixture of 400 mL of acetonitrile, 400 mL of water, and 200 mL of *Sulfate buffer*.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard digest solution*—Dissolve about 6 mg of USP Insulin Human RS in 3 mL of 0.01 N hydrochloric acid, and transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 hours. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

*Test digest solution*—To 1 mg of Insulin Human add 500 µL of 0.01 N hydrochloric acid, and mix to dissolve. Proceed as directed for *Standard digest solution*, beginning with "Add 2.0 mL of *HEPES buffer*".

*Chromatographic system* (see *Chromatography* (621))—A liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration
0–60	90→30	10→70	linear gradient
60–65	30→0	70→100	linear gradient
65–70	0	100	isocratic
70–71	0→90	100→10	linear gradient
71–86	90	10	re-equilibration

Chromatograph the *Standard digest solution*, and record the peak responses as directed for *Procedure*: the chromatogram of the *Standard digest solution* corresponds to that of the standard chromatogram provided with USP Insulin Human RS. For the chromatogram of the *Standard digest* the tailing

factor is not greater than 1.5; and the resolution, *R*, is not less than 3.4 for digest fragments II and III.

[NOTE\*—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in all insulins; and Fragment III elutes at the same time in insulin derived from beef and pork.]

*Procedure*—Using the gradient program, run a blank. Inject equal volumes of the *Standard digest solution* and the *Test digest solution* into the chromatograph, and record the chromatograms. The chromatographic profile of the *Test digest solution* corresponds to that of the *Standard digest solution*.

**Bioidentity**—It meets the requirements of the *Bioidentity test* under *Insulin*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 300 cfu per g, the test being performed on a portion of about 0.2 g, accurately weighed.

**Bacterial endotoxins** (85)—It contains not more than 10 USP Endotoxin Units in each mg.

**Loss on drying** (731)—Dry about 200 mg, accurately weighed, at 105° for 16 hours: it loses not more than 10.0% of its weight.

**Related compounds**—Proceed as directed for the *Related compounds test* under *Insulin* except to use the following gradient elution program. The program initially calls for isocratic elution for about 36 minutes with a *Mobile phase* consisting of a mixture of 78% *Solution A* and 22% *Solution B*. Following the gradient elution phase, the system is returned to the initial conditions of 78% *Solution A* and 22% *Solution B*. Adjust the composition of the *Mobile phase* so that the retention time of the main insulin human peak is between 15 and 25 minutes. The content of A-21 desamido insulin and of other insulin related compounds is not more than 2.0% each of the total amount of insulin and total related compounds.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin*. Not more than 1.0% is found.

**Other requirements**—It meets the requirements for *Zinc content* under *Insulin*.

**Assay—**

*Mobile phase*, *Standard preparation*, *Assay preparation*, *System suitability solution*, *Chromatographic system*, and *Procedure*—Proceed as directed in the *Assay* under *Insulin* except to use USP Insulin Human RS and otherwise substitute Insulin Human for Insulin throughout.

**Insulin Human Injection**

» Insulin Human Injection is an isotonic sterile solution of Insulin Human in Water for Injection. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

**Packaging and storage**—Preserve in a refrigerator. Protect from sunlight. Avoid freezing. Dispense it in the unopened, multiple-dose container in which it was placed by the manufacturer.

**Labeling**—The labeling states that it has been prepared either with Insulin Human derived by enzyme modification of

\*Fragment I consists of amino acids A5 to A17 and B1 to B13. Fragment II consists of amino acids A18 to A21 and B14 to B21. Fragment III consists of amino acids B22 to B30. Fragment IV consists of amino acids A1 to A4. A refers to the A-chain of Insulin Human, and B refers to the B-chain of Insulin Human.

pork pancreas Insulin or with Insulin Human obtained from microbial synthesis, whichever is applicable. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units per mL.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin Human RS

USP Insulin (Pork) RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 80 USP Endotoxin Units for each 100 USP Insulin Human Units.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 1.7% is found.

**Other requirements**—It meets the requirements under *Injections* <1> and for *pH* and *Zinc content* under *Insulin Injection*.

**Assay**—

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Insulin*.

*Standard preparation*—Prepare as directed in the *Assay* under *Insulin Human*.

*Assay preparations*—Prepare as directed in the *Assay* under *Insulin Injection*.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the appropriate *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin. Calculate the potency, in USP Insulin Human Units per mL, of the *Injection* taken by the formula:

$$(CD)(\Sigma r_U / \Sigma r_S)$$

in which *C* is the concentration, in USP Insulin Human Units per mL, of USP Insulin Human RS in the *Standard preparation*; *D* is the dilution factor; and  $\Sigma r_U$  and  $\Sigma r_S$  are the sums of the areas of the insulin and A-21 desamido insulin peaks obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively.

## Human Insulin Isophane Suspension and Human Insulin Injection

» Human Insulin Isophane Suspension and Human Insulin Injection is a sterile buffered suspension of Insulin Human, complexed with Protamine Sulfate, in a solution of Insulin Human. Its potency, based on the sum of its insulin and desamido insulin components, as determined in the *Assay*, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

**Packaging and storage**—Preserve in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—The *Injection* container label states that the *Injection* is to be properly resuspended before use. Label it to indicate that it has been prepared with Insulin Human of semisynthetic origin (i.e., derived by enzyme modification of pork pancreas insulin) or with Insulin Human of recombinant DNA origin (i.e., obtained from microbial synthesis), whichever is applicable. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units per mL and the percent ratio of human insulin isophane suspension to soluble human insulin *Injection*.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin Human RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Human Units.

**Sterility** <71>—It meets the requirements of the test for *Sterility* under *Isophane Insulin Suspension*.

**pH** <791>: between 7.0 and 7.8, determined potentiometrically.

**Zinc content** <591>: between 0.02 mg and 0.04 mg for each 100 USP Insulin Human Units.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 3.0% is found.

**Soluble insulin human content**—[NOTE—Use one of the two methods listed below.]

METHOD 1—

*Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Insulin*.

*Soluble insulin test solution*—Maintain the temperature at  $25 \pm 1^\circ$  throughout the procedure. Transfer 5.0 mL of the *Injection* to a centrifuge tube. Add 20  $\mu$ L of 1 N sodium hydroxide, and adjust with 0.05 N hydrochloric acid or 0.05 N sodium hydroxide to a pH of  $8.20 \pm 0.02$  if the total zinc concentration is approximately 20  $\mu$ g per mL or adjust to a pH of  $8.35 \pm 0.02$  if the total zinc concentration is approximately 30  $\mu$ g per mL. Record the volume, in  $\mu$ L, of acid or base needed to adjust the pH. Mix, and allow to stand for 1 hour. Centrifuge, transfer the supernatant to another centrifuge tube, and repeat the centrifugation. Transfer 2 mL of the supernatant to another tube, add 5  $\mu$ L of 9.6 N hydrochloric acid, and mix.

*Total insulin test solution*—Transfer 2 mL of *Injection* to a suitable vessel, add 5  $\mu$ L of 9.6 N hydrochloric acid, and allow the suspension to clarify. Dilute the resulting solution with 0.01 N hydrochloric acid to the same theoretical concentration of insulin as the *Soluble insulin test solution* (e.g., if the *Injection* is labeled to contain 20% soluble insulin, the dilution factor is  $100/20 = 5$ ).

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Soluble insulin test solution* and *Total insulin test solution* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin. Calculate the amount of soluble insulin human as a percent of the total insulin content of the *Injection* by the formula:

$$(100/D)[(5020 + V_A)/5000](r_S / r_T)$$

in which *D* is the dilution factor for the *Total insulin test solution*; *V<sub>A</sub>* is the number of  $\mu$ L added to adjust the pH of the *Soluble insulin test solution*; and *r<sub>S</sub>* and *r<sub>T</sub>* are the responses of the *Soluble insulin test solution* and the *Total insulin test solution*, respectively. The percent of soluble insulin human is in the range  $L \pm 5$ , where *L* is the percent of soluble insulin human stated on the product label.

## METHOD 2—

**Mobile phase**—Proceed as directed in the Assay under Insulin.

**0.1 M Tris buffer solution**—Dissolve  $3.54 \pm 0.01$  g of Tris(hydroxymethyl)aminomethane hydrochloride and  $3.34 \pm 0.01$  g of Tris(hydroxymethyl)aminomethane in 500 mL of water. The pH of the 0.1 M Tris buffer solution must be between 8.15 and 8.35. If the pH is outside of this range, discard the solution and prepare fresh; do not adjust the pH.

**System suitability solution**—Dissolve about 0.14 mg of Insulin in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for not less than 3 days to obtain a solution containing not less than 5% of A-21 desamido insulin.

**Soluble insulin test solution**—Dilute a suitable volume of Injection with 0.1 M Tris buffer solution to obtain a solution containing about 6 USP Insulin Human Units of soluble insulin per mL (e.g., 2 mL of 70/30 Human Insulin Isophane Suspension and Human Insulin Injection containing 100 USP Insulin Human Units per mL would be diluted with 8 mL of 0.1 M Tris buffer solution to obtain a filtrate that contains 6 USP Insulin Human Units of soluble insulin per mL). Immerse the container in a water bath at  $25 \pm 1^\circ$  for  $30 \pm 2$  minutes. Immediately pass this solution through a 0.2- $\mu$ m filter using a disposable syringe. Transfer 2 parts of the filtrate to a suitable vessel, and add 1 part 0.2 N hydrochloric acid (e.g., the dilution factor for the Soluble insulin test solution that contains 30% soluble insulin is  $5 \times 3/2 = 7.5$ ).

**Total insulin test solution**—For each mL of Injection, add 3.0  $\mu$ L of 9.6 N hydrochloric acid, mix, and allow the suspension to clarify. Dilute the resulting solution with 0.01 N hydrochloric acid to 4 USP Insulin Human Units per mL (e.g., if the product is labeled to contain a total of 100 USP Insulin Human Units per mL, the dilution factor is 25).

**Chromatographic system**—Proceed as directed in the Assay under Insulin. Make adjustments as necessary (see Chromatography <621>) to obtain a retention time for human insulin between 10 and 17 minutes. Chromatograph five replicate injections of the System suitability solution, and record the peak responses as directed for Procedure: the resolution,  $R$ , between human insulin and A-21 desamido human insulin is not less than 2.0; the tailing factor for the human insulin peak is between 0.8 and 1.5; and the relative standard deviation for replicate injections is not more than 1.6%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the Soluble insulin test solution and the Total insulin test solution, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin. Calculate the amount of soluble insulin human as a percent of the total human insulin content of the Injection by the formula:

$$(100D_s / D_T)(r_s / r_T)$$

in which  $D_s$  and  $D_T$  are the dilution factors for the Soluble insulin test solution and Total insulin test solution, respectively; and  $r_s$  and  $r_T$  are the human insulin peak responses obtained from the Soluble insulin test solution and the Total insulin test solution, respectively. The percent of soluble insulin human is in the range  $L \pm 5$ , where  $L$  is the percent of soluble insulin human stated on the product label.

**Assay**—Proceed as directed in the Assay under Insulin Human Injection.

for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* Linné (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units in each mL.

## IDENTIFICATION

- A.** The retention time of the insulin peak in the chromatogram of the Sample solution corresponds to the retention time of the appropriate species in the chromatogram of the Identification solution, as obtained in the Assay. [NOTE—It may be necessary to inject a mixture of Sample solution A or Sample solution B and Identification solution.]

## ASSAY

## • PROCEDURE

**Solution A:** Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, and adjust, if necessary, with ethanolic ammonia to a pH of 2.3.

**Mobile phase:** Acetonitrile and Solution A (26:74).

[NOTE—The acetonitrile is warmed to a temperature equal to or higher than  $20^\circ$  to avoid precipitation.]

**System suitability solution:** Dissolve about 1.5 mg of insulin in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

**Identification solution:** Prepare a solution of USP Insulin (Pork) RS and USP Insulin (Beef) RS in 0.01 N hydrochloric acid containing about 0.6 mg/mL of each.

**Standard solution:** 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid. [NOTE—The Identification solution, Standard solution, and Sample solutions may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

**Sample solution A** (for Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5  $\mu$ L of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension to clarify, and mix.

**Sample solution B** (for Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5  $\mu$ L of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain a sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Column temperature:**  $40^\circ$

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 2.0 between the insulin and A-21 desamido insulin peaks, System suitability solution

**Tailing factor:** NMT 1.8 for the insulin peak, System suitability solution

**Relative standard deviation:** NMT 1.6% for replicate injections of the Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution A or Sample solution B

**Isophane Insulin Suspension****DEFINITION**

Isophane Insulin Suspension is a sterile suspension of zinc-insulin crystals and Protamine Sulfate in buffered Water

From the measured chromatographic responses for insulin and A-21 desamido insulin, calculate the potency, in USP Insulin Units/mL, of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times (C \times D)$$

$\Sigma r_U$  = sum of the peak areas of insulin and A-21 desamido insulin from the *Sample solution*

$\Sigma r_S$  = sum of the peak areas of insulin and A-21 desamido insulin from the *Standard solution*

$C$  = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Units/mL)

$D$  = dilution factor

For Suspension prepared from a mixture of beef and pork insulins, calculate the total potency as the sum of the potencies of both beef and pork insulins, determined as directed above.

**Acceptance criteria:** 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units in each mL

## OTHER COMPONENTS

- **ZINC DETERMINATION (591):** 10–40 µg for each 100 USP Insulin Units

## IMPURITIES

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

**Solution A:** 1 mg/mL of L-arginine in water

**Mobile phase:** Acetonitrile, *Solution A*, and glacial acetic acid (20:65:15)

**Resolution solution:** Dissolve 4 mg of insulin containing more than 0.4% high molecular weight proteins in 1 mL of 0.01 N hydrochloric acid. Store this solution in a refrigerator, and use within 7 days. [NOTE—Insulin containing the indicated percentage of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

**Sample solution:** Quantitatively add 4 µL of 6 N hydrochloric acid per mL of a volume of Suspension, and mix.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 7.8-mm × 30-cm; packing L20

**Flow rate:** About 0.5 mL/min

**Injection volume:** 100 µL

### System suitability

**Sample:** *Resolution solution*

[NOTE—The relative retention times for the polymeric insulin complexes, for the covalent insulin dimer, and for the insulin monomer, with salts eluting after the insulin monomer, are 13–17, about 17.5, and 18–22 min, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 for the ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak

### Analysis

**Sample:** *Sample solution*

Inject the *Sample solution*, and measure the peak area responses, disregarding any peaks having retention times greater than the insulin monomer.

Calculate the percentage of high molecular weight proteins in the portion of Suspension taken:

$$\text{Result} = 100 \Sigma r_H / (\Sigma r_H + r_M)$$

$\Sigma r_H$  = sum of the peak responses for all peaks having retention times less than that of the insulin monomer

$r_M$  = peak response of the insulin monomer

**Acceptance criteria:** NMT 3.0% for the insulin monomer

## SPECIFIC TESTS

### INSULIN IN THE SUPERNATANT

**Sample solution:** Centrifuge 10 mL of the Suspension at  $1500 \times g$  for 10 min. Use the supernatant.

**Analysis:** Determine the insulin content of the *Sample solution* by a suitable method.

**Acceptance criteria:** NMT 1.0 USP Insulin Unit/mL

- **PH <791>:** 7.0–7.8, determined potentiometrically

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 80 USP Endotoxin Units/100 USP Insulin Units.

### Change to read:

- **STERILITY TESTS <71>:** It meets the requirements, when tested as directed for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent. • (RB 1-Jul-2012)

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

- **LABELING:** Label it to indicate the one or more animal species to which it is related, as porcine, as bovine, or as a mixture of porcine and bovine. Where it is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. The label states the potency in USP Insulin Units/mL. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided.

- **USP REFERENCE STANDARDS <11>**

USP Endotoxin RS

USP Insulin (Beef) RS

USP Insulin (Pork) RS

## Isophane Insulin Human Suspension

### DEFINITION

Isophane Insulin Human Suspension is a sterile suspension of zinc–insulin human crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin human, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* Linné (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, as in the Assay, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

### IDENTIFICATION

- **A.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, and adjust, if necessary, with ethanolamine to a pH of 2.3.

**Mobile phase:** Acetonitrile and *Solution A* (26:74).

[NOTE—The acetonitrile is warmed to a temperature equal to or higher than 20° to avoid precipitation.]

**System suitability solution:** Dissolve about 1.5 mg of insulin in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

**Standard solution:** 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid

**Sample solution A** (for Suspension labeled as containing 40 USP Insulin Human Units/mL): Add 2.5  $\mu$ L of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension to clarify, and mix.

**Sample solution B** (for Suspension labeled as containing 100 USP Insulin Human Units/mL): Add 2.5  $\mu$ L of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

[NOTE—The *Standard solution* and *Sample solutions* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between the insulin and A-21 desamido insulin peaks, *System suitability solution*

**Tailing factor:** NMT 1.8 for the insulin peak, *System suitability solution*

**Relative standard deviation:** NMT 1.6%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution A* or *Sample solution B*

From the measured chromatographic responses for insulin and A-21 desamido insulin, calculate the potency, in USP Insulin Human Units/mL, of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times (C \times D)$$

$\Sigma r_U$  = sum of the peak areas of insulin and A-21 desamido insulin from the *Sample solution*

$\Sigma r_S$  = sum of the peak areas of insulin and A-21 desamido insulin from the *Standard solution*

$C$  = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)

$D$  = dilution factor

**Acceptance criteria:** 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Human Units in each mL

#### OTHER COMPONENTS

- **ZINC DETERMINATION** <591>: 0.021–0.04 mg for each 100 USP Insulin Human Units

#### IMPURITIES

##### LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS

**Solution A:** 1 mg/mL of L-arginine in water

**Mobile phase:** Acetonitrile, *Solution A*, and glacial acetic acid (20:65:15)

**Resolution solution:** Dissolve 4 mg of insulin containing more than 0.4% high molecular weight proteins in 1 mL of 0.01 N hydrochloric acid. Store this solution in a refrigerator, and use within 7 days. [NOTE—Insulin containing the indicated percentage of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

**Sample solution:** Quantitatively add 4  $\mu$ L of 6 N hydrochloric acid per mL of a volume of Suspension, and mix.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 7.8-mm  $\times$  30-cm; packing L20

**Flow rate:** About 0.5 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Resolution solution*

[NOTE—The relative retention times for the polymeric insulin complexes, for the covalent insulin dimer, and for the insulin monomer, with salts eluting after the insulin monomer, are 13–17, about 17.5, and 18–22 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 for the ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak

#### Analysis

**Sample:** *Sample solution*

Inject the *Sample solution*, and measure the peak area responses, disregarding any peaks having retention times greater than the insulin monomer.

Calculate the percentage of high molecular weight proteins in the portion of Suspension taken:

$$\text{Result} = 100 \Sigma r_H / (\Sigma r_H + r_M)$$

$\Sigma r_H$  = sum of the peak responses for all peaks having retention times less than that of the insulin monomer

$r_M$  = peak response of the insulin monomer

**Acceptance criteria:** NMT 3.0% for insulin monomer

#### SPECIFIC TESTS

##### INSULIN IN THE SUPERNATANT

**Sample solution:** Centrifuge 10 mL of the Suspension at 1500  $\times g$  for 10 min. Use the supernatant.

**Analysis:** Determine the insulin content of the *Sample solution* by a suitable method.

**Acceptance criteria:** NMT 1.0 USP Insulin Human Unit/mL

##### PH <791>: 7.0–7.5, determined potentiometrically

##### BACTERIAL ENDOTOXINS TEST <85>: It contains NMT 80 USP Endotoxin Units/100 USP Insulin Human Units.

#### Change to read:

- **STERILITY TESTS** <71>: It meets the requirements, when tested as directed for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent. (RB 1-Jul-2012)

#### ADDITIONAL REQUIREMENTS

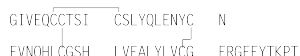
- **PACKAGING AND STORAGE:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

- **LABELING:** The Suspension container label states that the Suspension is to be shaken carefully before use. The labeling states also that it has been prepared with insulin human of semisynthetic origin (i.e., derived by enzyme modification of pork pancreas insulin) or with insulin human of recombinant DNA origin (i.e., obtained from microbial synthesis), whichever is applicable. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS  
USP Insulin (Pork) RS  
USP Insulin Human RS

## Insulin Lispro



C<sub>257</sub>H<sub>383</sub>N<sub>65</sub>O<sub>77</sub>S<sub>6</sub> 5807.57

Insulin (human), 28<sup>B</sup>-L-lysine-29<sup>B</sup>-L-proline-

28<sup>B</sup>-L-Lysine-29<sup>B</sup>-L-prolineinsulin (human) [133107-64-9].

» Insulin Lispro is identical in structure to Insulin Human, except that it has lysine and proline at positions 28 and 29, respectively, of chain B, whereas this sequence is reversed in Insulin Human. Insulin Lispro is produced by microbial synthesis via a recombinant DNA process. Its potency is not less than 27.0 USP Insulin Lispro Units per mg, calculated on the dried basis. The proinsulin content of Insulin Lispro, determined by an appropriate and validated method, is not more than 10 ppm. The host cell-derived protein content, determined by an appropriate and validated method, is not more than 10 ppm.

NOTE—One USP Insulin Lispro Unit is equivalent to 0.0347 mg of pure Insulin Lispro.

**Packaging and storage**—Preserve in tight containers, protected from light, and store in a freezer.

**Labeling**—Label it to indicate that it has been prepared by microbial synthesis.

**USP Reference standards** (11)—

USP Endotoxin RS  
USP Insulin Lispro RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Determine the peptide fragments, using the following peptide mapping procedure.

*Sulfate buffer, HEPES buffer, Mobile phase, Test digest solution, and Procedure*—Proceed as directed for *Identification test B* under *Insulin Human*.

*Standard digest solution*—Proceed as directed for *Identification test B* under *Insulin Human*, except to use USP Insulin Lispro RS instead of USP Insulin Human RS.

*Chromatographic system*—Proceed as directed for *Identification test B* under *Insulin Human*, except to use the following elution program.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–3	95	5	isocratic
3–30	95→41	5→59	linear gradient
30–35	41→20	59→80	linear gradient
35–40	20→95	80→5	return to initial
40–50	95	5	re-equilibration

The flow rate is about 0.8 mL per minute.

**Bioidentity**—Proceed as directed for *Bioidentity Test* under *Insulin Assays* (121), except to obtain the first blood speci-

men at 45 minutes, instead of 1 hour, after the time of injection: meets the requirements.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 100 per g, a portion of about 0.3 g, accurately weighed, being used.

**Bacterial endotoxins** (85): not more than 10 USP Endotoxin Units per mg, the kinetic-chromogenic method under *Photometric Techniques* being used.

**Loss on drying** (731)—Dry about 300 mg, accurately weighed, at 105° for 16 hours: it loses not more than 10.0% of its weight.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin*: not more than 0.25% is found.

**Related compounds**—

*Solvent*—Proceed as directed in the *Assay*.

*Solution A*—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (82:18).

*Solution B*—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (50:50).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve an accurately weighed quantity of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution containing about 3.5 mg per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% A-21 desamido insulin lispro.

*Test solution*—Dissolve about 3.5 mg of Insulin Lispro in 1.0 mL of 0.01 N hydrochloric acid. Store this solution for not more than 56 hours in a refrigerator.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–60	81	19	isocratic
60–83	81→51	19→49	linear gradient
83–84	51→81	49→19	linear gradient
84–94	81	19	re-equilibration

Adjust the *Mobile phase* composition and duration of the isocratic elution to obtain a retention time of about 41 minutes for insulin lispro, with A-21 desamido insulin lispro eluting near the start of the linear gradient phase. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between insulin lispro and A-21 desamido insulin lispro is not less than 2.5; and the tailing factor for the insulin lispro peak is not more than 2.0.

*Procedure*—Proceed as directed for *Procedure* in the test for *Related compounds* under *Insulin*: not more than 1.00% of A-21 desamido insulin lispro is found; not more than 0.50% of any other individual insulin lispro related compound is found; and not more than 2.00% of total impurities, excluding A-21 desamido insulin lispro, is found.

**Zinc content** (591)—Determine the zinc content of about 20 mg of Insulin Lispro, accurately weighed: between 0.30% and 0.60% is found, calculated on the dried basis.

**Assay**—

*Solvent*—Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, mix, and adjust with phosphoric acid to a pH of 2.3.

**Mobile phase**—Mix 745 mL of *Solvent* and 255 mL of acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve an accurately weighed quantity of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 1 mg per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% A-21 desamido insulin lispro.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Insulin Lispro RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 0.7 mg per mL.

**Assay preparation**—Dissolve an accurately weighed portion of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.8 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 0.8 mL per minute. Adjust the *Mobile phase* to provide a retention time of about 24 minutes for the main insulin lispro peak. Chromatograph three replicate injections of the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between insulin lispro and A-21 desamido insulin lispro is not less than 3.0; the tailing factor for the insulin lispro peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.1%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the potency, in USP Insulin Lispro Units per mg, on the as-is basis by the formula:

$$(C_S / C_U)(r_U / r_S)$$

in which *C<sub>S</sub>* is the concentration, in USP Insulin Lispro Units per mL, of USP Insulin Lispro RS in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of Insulin Lispro in the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the insulin lispro peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. From the value obtained in the test for *Loss on drying*, calculate the potency on the dried basis.

## Insulin Lispro Injection

» Insulin Lispro Injection is an isotonic, sterile solution of Insulin Lispro in Water for Injection. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed as USP Insulin Lispro Units in each mL.

**Packaging and storage**—Preserve in tight, multiple-dose containers, and store in a refrigerator. Avoid freezing. Protect from sunlight. Dispense it in the unopened, multiple-dose container provided by the manufacturer.

**Labeling**—The labeling states that it has been prepared with Insulin Lispro obtained from microbial synthesis. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Lispro Units per mL.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin Lispro RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** <85>: not more than 80 USP Endotoxin Units per 100 USP Insulin Lispro Units, the kinetic-chromogenic method under *Photometric Techniques* being used.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** <791>: between 7.0 and 7.8.

**Particulate matter** <788>: meets the requirements for small-volume injections.

**Limit of high molecular weight proteins**—

*Arginine solution, Mobile phase, Resolution solution, Test solution, and Chromatographic system*—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*.

*Procedure*—Proceed as directed for *Procedure* in the test for *Limit of high molecular weight proteins* under *Insulin*: not more than 1.50% is found.

**Related compounds**—

*Test solution*—Acidify each mL of Injection with 3 µL of 9.6 N hydrochloric acid.

*Solvent, System suitability solution, Mobile phase, Chromatographic system, and Procedure*—Proceed as directed in the test for *Related compounds* under *Insulin Lispro*. Not more than 1.50% A-21 desamido insulin lispro is found; and not more than 4.00% of total impurities, excluding A-21 desamido insulin lispro, is found.

**Zinc content** <591>: between 14 and 35 µg for each 100 USP Insulin Lispro Units.

**Other requirements**—It meets the requirements under *Injections* <1>.

**Assay**—

*Solvent, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Insulin Lispro*.

*Assay preparation*—Acidify each mL of Injection with 3 µL of 9.6 N hydrochloric acid. Quantitatively dilute a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 20 USP Insulin Lispro Units per mL.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the potency, in USP Insulin Lispro Units, in each mL of the Injection taken by the formula:

$$CD(r_U / r_S)$$

in which *C* is the concentration, in USP Insulin Lispro Units per mL, of USP Insulin Lispro RS in the *Standard preparation*; *D* is the dilution factor used to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the insulin lispro peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Insulin Zinc Suspension

Insulin zinc.

Insulin zinc [8049-62-5].

» Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a



manner such that the solid phase of the suspension consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous material. Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units per mL.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate the one or more animal species to which it is related, as porcine, as bovine, or as a mixture of porcine and bovine. Where it is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. The label states the potency in USP Insulin Units per mL. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Insulin (Beef) RS

USP Insulin (Pork) RS

**Identification**—It meets the requirements of the *Identification* test under *Insulin Injection*.

**Bacterial endotoxins** (85)—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Units.

**pH** (791): between 7.0 and 7.8, determined potentiometrically.

**Zinc content** (591): between 0.12 mg and 0.25 mg for each 100 USP Insulin Units.

**Zinc in the supernatant**—Centrifuge a portion of Suspension sufficient for the test, and determine the zinc content of the clear supernatant as directed under *Zinc Determination* (591): the zinc concentration, in mg per mL, is between 20% and 65% of the zinc concentration of the Suspension.

**Insulin not extracted by buffered acetone solution**—Centrifuge an amount of Suspension representing 1000 USP Insulin Units, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of buffered acetone TS, shake or stir vigorously, and centrifuge within 3 minutes after the addition of the buffered acetone TS. Discard the supernatant, repeat the treatment with water and buffered acetone TS, centrifuge, and discard the supernatant. Dissolve the crystalline residue in 5 mL of dilute hydrochloric acid (1 in 100), transfer to a 25-mL flask, and dilute with water to volume. The insulin concentration, determined by an appropriate method, is between 63% and 77% of the insulin content of an equal amount of the Suspension.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 1.5% is found.

**Other requirements**—It meets the requirements in the tests for *Sterility* and *Insulin in the supernatant* under *Iso-phane Insulin Suspension*.

**Assay**—Proceed as directed in the *Assay* under *Insulin Injection*.

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## Extended Insulin Zinc Suspension

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» Extended Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is predominantly crystalline. Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units per mL.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate the one or more animal species to which it is related, as porcine, as bovine, or as a mixture of porcine and bovine. Where it is purified, label it as such. Its container label states that the Suspension is to be shaken carefully before use. The label states the potency in USP Insulin Units per mL. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Insulin (Beef) RS

USP Insulin (Pork) RS

**Identification**—It meets the requirements of the *Identification* test under *Insulin Injection*.

**Bacterial endotoxins** (85)—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Units.

**pH** (791): between 7.0 and 7.8, determined potentiometrically.

**Insulin not extracted by buffered acetone solution**—Proceed as directed under *Insulin Zinc Suspension*: the insulin concentration, determined by an appropriate method, is not less than 90% of the insulin content of an equal amount of the Suspension.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 1.5% is found.

**Other requirements**—It meets the requirements in the tests for *Sterility* and *Insulin in the supernatant* under *Iso-phane Insulin Suspension* and for *Zinc content* and *Zinc in the supernatant* under *Insulin Zinc Suspension*.

**Assay**—Proceed as directed in the *Assay* under *Insulin Injection*.

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## Prompt Insulin Zinc Suspension

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» Prompt Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is amorphous. Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units per mL.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not

repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate the one or more animal species to which it is related, as porcine, as bovine, or as a mixture of porcine and bovine. Where it is purified, label it as such. Its container label states that the Suspension is to be shaken carefully before use. The label states the potency in USP Insulin Units per mL. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin (Beef) RS

USP Insulin (Pork) RS

**Identification**—It meets the requirements of the *Identification* test under *Insulin Injection*.

**Bacterial endotoxins** <85>—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Units.

**pH** <791>: between 7.0 and 7.8, determined potentiometrically.

**Insulin not extracted by buffered acetone solution**—

Centrifuge 15 mL (40-Unit), 8 mL (80-Unit), or 6 mL (100-Unit) of Suspension, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of buffered acetone TS, shake or stir vigorously, and centrifuge within 3 minutes after the addition of the buffered acetone TS. Discard the supernatant, repeat the treatment with water and buffered acetone TS, centrifuge, and discard the supernatant: no crystalline residue remains.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 1.5% is found.

**Other requirements**—It meets the requirements in the tests for *Sterility* and *Insulin in the supernatant* under *Iso-phane Insulin Suspension* and for *Zinc content* and *Zinc in the supernatant* under *Insulin Zinc Suspension*.

**Assay**—Proceed as directed in the *Assay* under *Insulin Injection*.

## Insulin Human Zinc Suspension

» Insulin Human Zinc Suspension is a sterile suspension of Insulin Human in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the Suspension consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous material. Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate that it has been prepared with Insulin Human of semisynthetic origin (i.e., derived by enzyme modification of pork pancreas insulin) or with Insulin Human of recombinant DNA origin (i.e., obtained from microbial synthesis), whichever is applicable. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in

a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units per mL.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin Human RS

USP Insulin (Pork) RS

**Bacterial endotoxins** <85>—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Human Units.

**pH** <791>: between 7.0 and 7.8, determined potentiometrically.

**Limit of high molecular weight proteins**—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection*: not more than 1.5% is found.

**Other requirements**—It meets the requirements in the tests for *Identification*, *Sterility*, and *Insulin in the supernatant* under *Iso-phane Insulin Human Suspension* and for *Zinc content*, *Zinc in the supernatant*, and *Insulin not extracted by buffered acetone solution* under *Insulin Zinc Suspension*.

**Assay**—Proceed as directed in the *Assay* under *Insulin Human Injection*.

## Extended Insulin Human Zinc Suspension

» Extended Insulin Human Zinc Suspension is a sterile suspension of Insulin Human in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the Suspension is predominantly crystalline. Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Human Units per mL.

**Packaging and storage**—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

**Labeling**—Label it to indicate that it has been prepared with Insulin Human of semisynthetic origin (i.e., derived by enzyme modification of pork pancreas insulin) or with Insulin Human of recombinant DNA origin (i.e., obtained from microbial synthesis), whichever is applicable. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units per mL.

**USP Reference standards** <11>—

USP Endotoxin RS

USP Insulin Human RS

USP Insulin (Pork) RS

**Bacterial endotoxins** <85>—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Human Units.

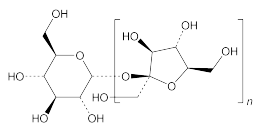
**pH** <791>: between 7.0 and 7.8, determined potentiometrically.

**Limit of high molecular weight proteins**—Proceed as directed in the *Limit of high molecular weight proteins* test under *Insulin Injection*: not more than 1.5% is found.

**Other requirements**—It meets the requirements in the tests for *Identification*, *Sterility*, and *Insulin in the supernatant* under *Iso-phane Insulin Human Suspension*, for *Zinc content* and *Zinc in the supernatant* under *Insulin Zinc Suspension*, and for *Insulin not extracted by buffered acetone solution* under *Extended Insulin Zinc Suspension*.

**Assay**—Proceed as directed in the Assay under *Insulin Human Injection*.

## Inulin



$C_6H_{11}O_5(C_6H_{10}O_5)_nOH$   
Inulin.  
Inulin [9005-80-5].

» Inulin is a polysaccharide which, on hydrolysis, yields mainly fructose. It contains not less than 94.0 percent and not more than 102.0 percent of  $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Dextrose RS  
USP Fructose RS

**Completeness of solution**—Dissolve 10 g in 20 mL of boiling water in a 200-mL volumetric flask, add 150 mL of water, allow to cool, dilute with water to volume, and mix; the solution is clear.

**Specific rotation** (781S): between −32.0° and −40.0°.

*Test solution*: 100 mg per mL, in 0.012 N ammonium hydroxide.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli* and for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*; the total aerobic microbial count is less than 1000 per g.

**Loss on drying** (731): not more than 10.0% after it has been dried at 105° for 2 hours, 2 g of the finely ground powder being used for the test.

**Residue on ignition** (281)—Multiply the percentage of Calcium found by 3.4. The residue on ignition does not exceed this percentage by more than 0.05%.

**pH, Chloride, Iron, and Reducing sugars**—Dissolve 10.0 g in 20 mL of boiling water in a 100-mL volumetric flask, allow to cool, dilute with water to volume, and mix. Use the solution for the following tests.

**pH** (791)—The pH of the solution is between 4.5 and 7.0.

**Chloride** (221)—A 10-mL portion of the solution shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.014%).

**Iron**—To 10 mL of the solution add 0.5 mL of hydrochloric acid and 3 drops of potassium ferrocyanide TS: the solution does not become blue within 1 minute.

**Reducing sugars**—To 2 mL of the solution add 5 mL of alkaline cupric tartrate TS: no reduction occurs at room temperature, and only slight reduction occurs after 1 minute of boiling.

**Calcium**—Heat 10.0 g in 100 mL of water to dissolve. Cool to room temperature, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Not more than 5.0 mL is required: not more than 0.10% calcium is found.

**Sulfate** (221)—A 1.0 g portion shows no more sulfate than corresponds to 0.5 mL of 0.020 N sulfuric acid (0.05%). [NOTE—Inulin should be dissolved in 30 to 40 mL of water with gentle warming, prior to dilution to final volume.]

**Heavy metals** (231)—Dissolve 4.0 g in 20 mL of boiling water, allow to cool, and dilute with water to 25 mL: the limit is 5 ppm.

### Free fructose—

**Blue tetrazolium solution**—Dissolve 50 mg of blue tetrazolium in 10 mL of alcohol, and mix.

**Tetramethylammonium hydroxide solution**—Prepare a mixture of 1 volume of tetramethylammonium hydroxide TS and 9 volumes of alcohol.

**Standard stock solution**—Prepare an aqueous solution having a known concentration of about 250 µg of USP Fructose RS per mL. Store at about 4°.

**Standard preparation**—On the day of use, quantitatively dilute a portion of the *Standard stock solution* with alcohol to obtain a solution having a known concentration of about 2.5 µg per mL. Store at about 4°.

**Test preparation**—Transfer about 2.5 g of Inulin, accurately weighed, to a 100-mL volumetric flask, add about 75 mL of water, heat on a steam bath until solution is complete, cool to room temperature, dilute with water to volume, and mix. Pipet 1 mL into a 100-mL volumetric flask, dilute with alcohol to volume, and mix. If the solution is turbid, pass through fine-porosity filter paper.

**Procedure**—Pipet 10 mL of the *Test preparation* and 10 mL of the *Standard preparation* into separate glass-stoppered centrifuge tubes. Into each of the tubes, and into a similar tube containing 10.0 mL of alcohol to provide the blank, pipet 1 mL of *Blue tetrazolium solution*, and mix. Then into each tube pipet 1 mL of *Tetramethylammonium hydroxide solution*, mix, and allow to stand in the dark for 60 minutes. Without delay, concomitantly determine the absorbances of the solutions from the *Test preparation* and the *Standard preparation* at 530 nm, with a suitable spectrophotometer, against the blank. Calculate the percentage, *F*, of free fructose in the Inulin taken by the formula:

$$F = (C/W)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Fructose RS in the *Standard preparation*; *W* is the quantity, in g, of Inulin taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively. The limit is 2.0%, calculated on the dried basis.

### Content of combined glucose—

**Standard stock solution**—Transfer about 50 mg of USP Dextrose RS, accurately weighed, to a 100-mL volumetric flask, dissolve in a solution of benzoic acid (1.7 in 1000), dilute with the same solution to volume, and mix. Allow to stand at room temperature for not less than 3 hours before using. This solution is stable for 1 month at about 4°.

**Standard preparation**—Pipet 7 mL of *Standard stock solution* into a 100-mL volumetric flask, dilute with water to volume, mix, and use at once.

**Assay preparation**—Transfer about 0.5 g of Inulin, accurately weighed, to a 100-mL volumetric flask, add 5.0 mL of water, dissolve by heating on a steam bath, cool to room temperature, add 0.5 mL of 8 N hydrochloric acid, and mix. Place the flask in a boiling water bath for 5 minutes, cool, dilute with water to volume, and mix. Pipet 2 mL of this solution into a 10-mL volumetric flask, dilute with water to volume, and mix. [NOTE—This solution is used also for preparing the *Assay preparation* in the *Assay for inulin*.]

**Procedure**—Pipet 3-mL portions of glucose oxidase-chromogen TS into 3 separate test tubes, and bring to a temperature of 37 ± 0.5° in a water bath. Pipet 2 mL of *Standard preparation* into one of the tubes, pipet 2 mL of *Assay preparation* into another, and pipet 2 mL of water into

the third tube to provide the blank. Maintain at  $37 \pm 0.5^\circ$  for an additional 10 minutes, then remove the tubes, and allow them to cool. Determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at about 505 nm, with a suitable spectrophotometer, using the reagent blank as a reference. Calculate the percentage of combined glucose,  $G$ , in the Inulin taken by the formula:

$$G = 50(C/W)(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dextrose RS in the *Standard preparation*;  $W$  is the amount, in g, of Inulin taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. Not less than 2.0% and not more than 5.0%, calculated on the dried basis, is found.

#### Assay for inulin—

*Thiobarbituric acid solution*—Dissolve 250 mg of thiobarbituric acid in 100 mL of 8 N hydrochloric acid, and mix. This solution is stable for 2 weeks at a temperature of about  $4^\circ$ .

*Standard stock solution*—Quantitatively dissolve an accurately weighed quantity of USP Fructose RS in an aqueous solution of benzoic acid (1.7 in 1000) to obtain a solution having a known concentration of about 1 mg of USP Fructose RS per mL. [NOTE—This solution is stable for 1 month at about  $4^\circ$ .]

*Standard preparation*—Quantitatively dilute the *Standard stock solution* with water to one-fiftieth of its concentration. Use immediately.

*Assay preparation*—Pipet 4 mL of the *Assay preparation* from the *Content of combined glucose* into a 200-mL volumetric flask, add water to volume, and mix.

*Procedure*—Pipet 1-mL portions of the *Standard preparation* and the *Assay preparation* into separate glass-stoppered tubes. Pipet 1 mL of water into a third tube to provide the blank. Pipet 5-mL portions of *Thiobarbituric acid solution* into each tube, and mix. Place all of the tubes simultaneously in a water bath maintained at a temperature of about  $83^\circ$ , and allow them to stay immersed for 5 minutes, accurately timed. Remove the tubes simultaneously, and allow them to cool in a dark place for 30 minutes. Determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at about 435 nm, with a suitable spectrophotometer, using the reagent blank as a reference. Calculate the percentage of  $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$  in the Inulin taken by the formula:

$$0.900[2.5(C/W)(A_U / A_S) - F] + G$$

in which 0.900 is the ratio of the formula weight of an anhydrofructose unit of inulin to that of fructose;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Fructose RS in the *Standard preparation*;  $W$  is the quantity, in g, of Inulin weighed for the *Content of combined glucose*;  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively;  $F$  is the percentage of free fructose; and  $G$  is the percentage of combined glucose.

### Inulin in Sodium Chloride Injection

» Inulin in Sodium Chloride Injection is a sterile solution, which may be supersaturated, of Inulin and Sodium Chloride in Water for Injection. It may require heating before use if crystallization has occurred. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $(C_6H_{12}O_6)_n$  and not less than

95.0 percent and not more than 105.0 percent of the labeled amount of NaCl. It contains no anti-microbial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type II glass.

#### USP Reference standards (11)—

USP Dextrose RS

USP Endotoxin RS

USP Fructose RS

**Clarity**—If particulate matter is present, heat at  $100^\circ$  for 30 minutes: the resulting solution is free from turbidity and particulate matter.

NOTE—Before applying the following tests, dissolve any solid matter by heating, and cool to room temperature.

**Bacterial endotoxins** (85)—It contains not more than 0.1 USP Endotoxin Unit per mg of inulin.

**pH** (791): between 4.0 and 7.0.

#### Free fructose—

*Blue tetrazolium solution*, *Tetramethylammonium hydroxide solution*, *Standard stock solution*, and *Standard preparation*—Proceed as directed in the test for *Free fructose* under *Inulin*.

*Test preparation*—Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of inulin, to a 100-mL volumetric flask, add water to volume, and mix. Just prior to use, pipet 1 mL of this solution into a 100-mL volumetric flask, dilute with alcohol to volume, mix, and, if the solution is turbid, pass through fine-porosity filter paper.

*Procedure*—Proceed as directed for *Procedure* in the test for *Free fructose* under *Inulin*. Calculate the quantity,  $F$ , in mg, of free fructose in each mL of the Injection taken by the formula:

$$10(C / V)(A_U / A_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Fructose RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively. The limit is 2.2 mg per mL.

**Other requirements**—It meets the other requirements under *Injections* (1).

#### Assay for inulin—

*Content of combined glucose*—Proceed as directed in the *Content of combined glucose* under *Inulin*, but in preparing the *Assay preparation* use, instead of 0.5 g of inulin, an accurately measured volume of the Injection equivalent to about 0.5 g of inulin. Calculate the quantity, in mg, of combined glucose,  $G$ , in each mL of the Injection taken by the formula:

$$500(C / V)(A_U / A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dextrose RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

*Procedure*—Proceed as directed in the *Assay for inulin* under *Inulin*. Calculate the quantity, in mg, of  $(C_6H_{12}O_6)_n$  in each mL of the Injection taken by the formula:

$$0.900[25(C / V)(A_U / A_S) - F] + G$$

in which 0.900 is the ratio of the formula weight of an anhydrofructose unit of inulin to that of fructose;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Fructose RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken;  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively; and  $F$  is the quantity, in mg, of free fructose in each mL of Injection, and the other terms are as defined therein.

**Assay for sodium chloride**—Pipet a volume of Injection, equivalent to about 90 mg of sodium chloride, into a porcelain casserole, and add 140 mL of water and 1 mL of *dichlorofluorescein* TS. Mix, and titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

## Iodine

I<sub>2</sub> 253.81  
Iodine [7553-56-2].

### DEFINITION

Iodine contains NLT 99.8% and NMT 100.5% of I.

### IDENTIFICATION

- **A.** Solutions (1 in 1000) in chloroform and in carbon disulfide have a violet color.
- **B.**  
**Analysis:** To a saturated solution add starch–potassium iodide TS.  
**Acceptance criteria:** A blue color is produced. When the mixture is boiled, the color vanishes but reappears as the mixture cools, unless it has been subjected to prolonged boiling.

### ASSAY

#### PROCEDURE

**Sample:** 500 mg of powdered Iodine

**Analysis:** Place the *Sample* in a tared, glass-stoppered flask, insert the stopper, and add 1 g of potassium iodide dissolved in 5 mL of water. Dilute with water to 50 mL, add 1 mL of 3 N hydrochloric acid, and titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of Iodine (I).

**Acceptance criteria:** 99.8%–100.5%

### IMPURITIES

#### LIMIT OF CHLORIDE OR BROMIDE

**Sample solution:** Triturate 250 mg of finely powdered Iodine with 10 mL of water, and filter the solution.

**Analysis:** To the *Sample solution* add, dropwise, sulfurous acid (free from chloride), previously diluted with several volumes of water, until the iodine color just disappears. Add 5 mL of 6 N ammonium hydroxide, followed by 5 mL of silver nitrate TS in small portions. Filter, and acidify the filtrate with nitric acid.

**Acceptance criteria:** The resulting liquid is not more turbid than a control made with the same quantities of the same reagents to which 0.10 mL of 0.020 N hydrochloric acid has been added, the sulfurous acid being omitted (0.028% as chloride).

#### LIMIT OF NONVOLATILE RESIDUE

**Analysis:** Place 5.0 g in a tared porcelain dish, heat on a steam bath until the iodine has been driven off, and dry at 105° for 1 h.

**Acceptance criteria:** NMT 0.05% of the residue remains.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Iodine Topical Solution

» Iodine Topical Solution contains, in each 100 mL, not less than 1.8 g and not more than 2.2 g of iodine (I), and not less than 2.1 g and not more than 2.6 g of sodium iodide (NaI).

Iodine . . . . .	20 g
Sodium Iodide . . . . .	24 g
Purified Water, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the Iodine and the Sodium Iodide in 50 mL of Purified Water, then add Purified Water to make 1000 mL.

**Packaging and storage**—Preserve in tight, light-resistant containers, at a temperature not exceeding 35°.

### Identification—

**A:** Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water: a deep blue color is produced.

**B:** Evaporate a few mL on a steam bath to dryness: the residue responds to the flame test for *Sodium* (191) and to the tests for *Iodide* (191).

**Assay for iodine**—Transfer 10.0 mL of Topical Solution into a glass-stoppered, 500-mL flask, and dilute with 10 mL of water. Titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of I.

**Assay for sodium iodide**—Transfer 10.0 mL of Topical Solution to a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with 0.05 M potassium iodate VS until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference between the number of mL of 0.05 M potassium iodate used and half the number of mL of 0.1 N sodium thiosulfate used in the *Assay for iodine*, multiplied by 14.99, represents the number of mg of NaI in the volume of Topical Solution taken.

## Strong Iodine Solution

» Strong Iodine Solution contains, in each 100 mL, not less than 4.5 g and not more than 5.5 g of iodine (I), and not less than 9.5 g and not more than 10.5 g of potassium iodide (KI).

Strong Iodine Solution may be prepared by dissolving 50 g of Iodine and 100 g of Potassium Iodide in 100 mL of Purified Water, then adding Purified Water to make the product measure 1000 mL.

**Packaging and storage**—Preserve in tight containers, preferably at a temperature not exceeding 35°.

### Identification—

**A:** Dilute 1 drop with 10 mL of water, and add 1 mL of starch TS: a deep blue color is produced.

**B:** Evaporate a few mL on a steam bath to dryness, and ignite gently to volatilize any free iodine: the residue responds to the tests for *Potassium* (191) and for *Iodide* (191).

**Assay for iodine**—Transfer 10.0 mL of Solution into a glass-stoppered, 500-mL flask, add 10 mL of water, and titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of I.

**Assay for potassium iodide**—Transfer 10.0 mL of Solution to a glass-stoppered, 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with 0.05 M potassium iodate VS until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference between the number of mL of 0.05 M potassium iodate used and half the number of mL of 0.1 N sodium thiosulfate used in the *Assay for iodine*, multiplied by 16.60, represents the number of mg of KI in the volume of the Solution taken.

## Iodine Tincture

» Iodine Tincture contains, in each 100 mL, not less than 1.8 g and not more than 2.2 g of iodine (I), and not less than 2.1 g and not more than 2.6 g of sodium iodide (NaI).

Iodine Tincture may be prepared by dissolving 20 g of Iodine and 24 g of Sodium Iodide in 500 mL of Alcohol and then adding Purified Water to make the product measure 1000 mL.

**Packaging and storage**—Preserve in tight containers.

### Identification—

**A:** Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water: a deep blue color is produced.

**B:** Evaporate a few mL on a steam bath to dryness: the residue responds to the flame test for *Sodium* <191>, and to the tests for *Iodide* <191>.

**Alcohol content** <611>: between 44.0% and 50.0% of C<sub>2</sub>H<sub>5</sub>OH.

**Assay for iodine**—Proceed as directed in the *Assay for iodine* under *Iodine Topical Solution*, using 10 mL of Iodine Tincture.

**Assay for sodium iodide**—Proceed as directed in the *Assay for sodium iodide* under *Iodine Topical Solution*, using 10 mL of Iodine Tincture. The difference between the number of mL of 0.05 M potassium iodate used and half the number of mL of 0.1 N sodium thiosulfate used in the *Assay for iodine*, multiplied by 14.99, represents the number of mg of NaI in the volume of Iodine Tincture taken.

## Strong Iodine Tincture

» Strong Iodine Tincture contains, in each 100 mL, not less than 6.8 g and not more than 7.5 g of iodine (I), and not less than 4.7 g and not more than 5.5 g of potassium iodide (KI).

Strong Iodine Tincture may be prepared by dissolving 50 g of Potassium Iodide in 50 mL of Purified Water, adding 70 g of Iodine, and agitating until solution is effected, and then adding Alcohol to make the product measure 1000 mL.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### Identification—

**A:** Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water: a deep blue color is produced.

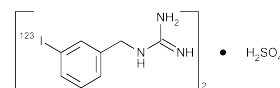
**B:** Evaporate a few mL on a steam bath to dryness: the residue responds to the flame test for *Potassium* <191> and to the tests for *Iodide* <191>.

**Alcohol content** <611>: between 82.5% and 88.5% of C<sub>2</sub>H<sub>5</sub>OH.

**Assay for iodine**—Proceed as directed in the *Assay for iodine* under *Strong Iodine Solution*, using 10 mL of Strong Iodine Tincture.

**Assay for potassium iodide**—Proceed as directed in the *Assay for potassium iodide* under *Strong Iodine Solution*, using 10 mL of Strong Iodine Tincture. The difference between the number of mL of 0.05 M potassium iodate used and half the number of mL of 0.1 N sodium thiosulfate used in the *Assay for iodine*, multiplied by 16.60, represents the number of mg of KI in the volume of Strong Iodine Tincture taken.

## Iobenguane I 123 Injection



(C<sub>8</sub>H<sub>10</sub><sup>123</sup>I<sub>2</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>

[[3-(Iodo-<sup>123</sup>I)-phenyl]methyl]guanidine sulfate (2:1).

(*m*-Iodo-<sup>123</sup>I)-benzyl]guanidine sulfate (2:1) [139755-80-9].

» Iobenguane I 123 Injection is a sterile solution containing iobenguane sulfate in which a portion of the molecules contain radioactive iodine (<sup>123</sup>I) in the molecular structure. Iobenguane I 123 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of I 123 as iobenguane expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of iobenguane. It may contain preservatives or stabilizers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded. Store in a freezer.

**Labeling**—Label it to include the following: the time and date of calibration; the amount of <sup>123</sup>I as iobenguane expressed as total megabecquerels (or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; the expiration time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>123</sup>I is 13.2 hours.

**USP Reference standards** <11>—

USP Endotoxin RS

**Radionuclidic identification** (see *Radioactivity* <821>)—Its gamma-ray spectrum is identical to that of a specimen of <sup>123</sup>I of known purity that exhibits a major photopeak having an energy of 159 KeV.

**Bacterial endotoxins** <85>—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration time.

**pH** (791): between 6.0 and 7.5.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radionuclidic purity of the Injection: not less than 97% of the total radioactivity is present as  $^{131}\text{I}$ .

**Radiochemical purity**—

**METHOD 1** (High-pressure liquid chromatographic method)—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay for iobenguane sulfate*, except that the liquid chromatograph is also equipped with a collimated radiation detector (see *Radioactivity* (821)).

*Test preparation*—Use *Iobenguane I 123 Injection*.

*Procedure*—Inject a volume (about 20  $\mu\text{L}$ ) of Injection, equivalent to 1.6 to 2.7 MBq (44 to 64  $\mu\text{Ci}$ ) of the Injection into the chromatograph, record the chromatogram, and measure the areas for the major peaks. The radioactivity of the iobenguane  $^{123}\text{I}$  peak is not less than 90% of the total radioactivity measured, and its retention time is within 10% of that in the chromatogram of the *Standard preparation* obtained in the *Assay for iobenguane sulfate*.

**METHOD 2** (Thin-layer chromatographic method)—Apply 0.2 to 0.4  $\mu\text{L}$  of the Injection 10 mm from one end of a 20- $\times$  200-mm silica gel glass plate (see *Chromatography* (621)), and allow to dry. Develop the chromatogram by ascending chromatography until the solvent front has moved 100 mm from the origin (about 20 minutes), using a solvent system consisting of a mixture of alcohol, ethyl acetate, and ammonium hydroxide (20:20:1). Air-dry the chromatogram, and determine the radioactivity distribution by scanning the chromatogram with a collimated radiation detector: not less than 90% of the total radioactivity is found as  $^{123}\text{I}$  at the origin.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility* (71), the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for iobenguane sulfate**—

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (900:100). Add 3.04 g of triethylamine per liter, and adjust with phosphoric acid to a pH of 4. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Using an accurately weighed quantity of iobenguane sulfate, prepare a solution in water having a known concentration of about 1 mg per mL.

*Assay preparation*—Use the Injection, which has not yet been brought to full volume with bacteriostatic saline.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm  $\times$  25-cm column that contains 10- $\mu\text{m}$  packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 1.2, and the relative standard deviation for replicate injections is not more than 1.5%.

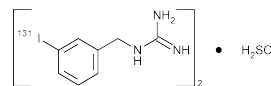
*Procedure*—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of iobenguane sulfate in each mL of the Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of iobenguane sulfate in the *Standard preparation*; and  $r_U$  and  $r_S$  are the iobenguane peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for radioactivity**—Using a counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ), per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Iobenguane I 131 Injection



$(\text{C}_8\text{H}_{10}^{131}\text{IN}_3)_2 \cdot \text{H}_2\text{SO}_4$   
[[3-(Iodo- $^{131}\text{I}$ )-phenyl]methyl]guanidine sulfate (2:1).  
[(*m*-Iodo- $^{131}\text{I}$ )-benzyl]guanidine sulfate (2:1).

» Iobenguane I 131 Injection is a sterile solution containing iobenguane sulfate in which a portion of the molecules contain radioactive iodine ( $^{131}\text{I}$ ) in the molecular structure. Iobenguane I 131 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{131}\text{I}$  as iobenguane sulfate expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It may contain preservatives or stabilizers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded. Store in a freezer.

**Labeling**—Label it to include the following: the date of calibration; the amount of  $^{131}\text{I}$  as iobenguane sulfate expressed as total megabecquerels (or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; the expiration date; and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.04 days.

**USP Reference standards** (11)—

USP Endotoxin RS

**Radionuclidic identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{131}\text{I}$  of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 4.5 and 7.5.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radionuclidic purity of the Injection: not less than 99% of the total radioactivity is present as  $^{131}\text{I}$ .

**Radiochemical purity**—

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (900:100). Add 3.04 g of triethylamine per liter, and adjust with phosphoric acid to a pH of 4.0  $\pm$  0.4. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of iobenguane sulfate in water to obtain a solution having a known concentration of about 1 mg per mL.

*Test preparation*—Use Iobenguane I 131 Injection.

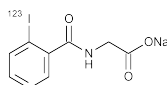
*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 229-nm detector, a collimated radiation detector (see *Radioactivity* <821>), and a 3.9-mm × 30-cm column that contains 10-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 1.2, and the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Inject a volume (about 20 μL) of Injection, equivalent to between 1.6 and 2.7 MBq (or between 44 and 64 μCi) into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The radioactivity of the iobenguane <sup>131</sup>I peak is not less than 90% of the total radioactivity measured, and its retention time is within 10% of that of the *Standard preparation*, similarly chromatographed. The retention time of the iobenguane <sup>131</sup>I peak obtained from the Injection is also within 10% of that obtained from the *Standard preparation*.

**Other requirements**—It meets the requirements under *Injections* <1>, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility* <71>, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation for *Container Content*.

**Assay for radioactivity**—Using a counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radioactivity, in MBq (or μCi), per mL, of Injection by use of a calibrated system as directed under *Radioactivity* <821>.

## Iodohippurate Sodium I 123 Injection



C<sub>9</sub>H<sub>7</sub><sup>123</sup>INNaO<sub>3</sub>

Glycine, N-[2-(iodo-<sup>123</sup>I)benzoyl]-, monosodium salt.  
Sodium o-iodo-<sup>123</sup>I-hippurate [56254-07-0].

» Iodohippurate Sodium I 123 Injection is a sterile, aqueous solution containing o-iodohippurate sodium in which a portion of the molecules contain radioactive iodine (<sup>123</sup>I) in the molecular structure. It may contain a preservative or stabilizer.

Iodohippurate Sodium I 123 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of I 123 as iodohippurate sodium expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of o-iodohippuric acid. Other chemical forms of radioactivity do not exceed 3.0 percent of total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* <1>: the time and date of calibration; the amount of I 123 as iodohippurate sodium expressed as total megabecquerels

(microcuries or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; the expiration time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of I 123 is 13.2 hours.

### USP Reference standards <11>—

USP Endotoxin RS

USP o-Iodohippuric Acid RS

C<sub>9</sub>H<sub>8</sub>INO<sub>3</sub> 305.07

**Radionuclidic identification** (see *Radioactivity* <821>)—Its gamma-ray spectrum is identical to that of a specimen of I 123 of known purity that exhibits a major photopeak having an energy of 159 keV.

**Bacterial endotoxins** <85>—The limit of endotoxin content is not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration time.

**pH** <791>: between 7.0 and 8.5.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radionuclidic purity of the Injection: not less than 85% of the total radioactivity is present as I 123.

### Radiochemical purity—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay for o-iodohippuric acid*, except to provide that the liquid chromatograph is also equipped with a radioactivity detector (see *Radioactivity* <821>).

*Procedure*—Inject about 50 μL equivalent to 1.8 to 3.7 MBq (50 to 100 μCi) of Injection into the chromatograph, record the chromatogram, and measure the area of all radioactivity peaks. The radioactivity under the o-iodohippuric acid I 123 peak is not less than 97.0% of the total area of all peaks observed, and its retention time is within ±10% of the value obtained for USP o-Iodohippuric Acid RS.

**Biological distribution**—Inject intravenously between 0.75 and 22 MBq (20 and 600 μCi) of the Injection, in a volume of 0.10 mL to 0.15 mL, into the caudal vein of each of three 125-g to 225-g anesthetized rats. Clamp the opening of the urethra with a hemostat. Sacrifice the animals 1 hour after administration, and carefully remove the intact bladder and contents and thyroid by dissection. Place each organ and remaining carcass (excluding the tail) in separate, suitable counting containers, and determine the radioactivity, in counts per minute, in each container with an appropriate detector using the same counting geometry. Determine the percentage of radioactivity in each organ: not less than 75% of the administered dose is found in the bladder, and not more than 3% of the administered dose is found in the thyroid in two of the rats.

**Other requirements**—It meets the requirements under *Injections* <1>, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

### Assay for o-iodohippuric acid—

*Mobile phase*—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (75:25:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Using an accurately weighed quantity of USP o-Iodohippuric Acid RS, prepare a solution in water having a known concentration of about 2 mg per mL.

*Assay preparation*—Use Iodohippurate Sodium I 123 Injection.



**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 265-nm detector and an 8-mm × 10-cm column that contains packing L11. The flow rate is about 5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 500 theoretical plates, the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the quantity, in mg, of o-iodohippuric acid in each mL of Injection taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP o-iodohippuric Acid RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the o-iodohippuric acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radioactivity in MBq (µCi or mCi) per mL of Injection by use of a calibrated system as directed under *Radioactivity* <821>.

## Sodium Iodide I 123 Capsules

Sodium iodide (Na<sup>123</sup>I).

Sodium iodide (Na<sup>123</sup>I) [41927-88-2].

» Sodium Iodide I 123 Capsules contain radioactive iodine (<sup>123</sup>I) processed in the form of Sodium Iodide obtained from the bombardment of enriched tellurium 124 with protons or of enriched tellurium 122 with deuterons, or by proton irradiation of enriched xenon 124, or by the decay of xenon 123 in such manner that it is carrier-free. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>123</sup>I as iodide expressed in megabecquerels (microcuries or millicuries) at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Capsules may contain a stabilizer.

**Packaging and storage**—Preserve in well-closed containers that are adequately shielded.

**Labeling**—Label the Capsules to include the following: the name of the Capsules; the name, address, and batch or lot number of the manufacturer; the time and date of calibration; the amount of <sup>123</sup>I as iodide expressed in megabecquerels (microcuries or millicuries) per Capsule at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement indicating that the Capsules are for oral use only; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>123</sup>I is 13.2 hours.

**Radionuclide identification**—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclide identification* under *Sodium Iodide I 123 Solution*.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—Determine the instrument response of each of 20 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the mean radioactivity value per Capsule: the requirements of the test are met if not less than 19 of the Capsules are within the limits of 96.5% and 103.5% of the mean radioactivity value.

**Radionuclidic purity**—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclidic purity* under *Sodium Iodide I 123 Solution*.

**Radiochemical purity**—Homogenize 1 Capsule in 3 mL of water, add 3 mL of methanol, and centrifuge: the supernatant so obtained meets the requirements of the test for *Radiochemical purity* under *Sodium Iodide I 123 Solution*.

**Other requirements**—A solution or suspension prepared by homogenizing 1 or more Capsules in water to yield a concentration of not less than 1 MBq (25 µCi) per mL meets the requirements of the *Assay for radioactivity* under *Sodium Iodide I 123 Solution*.

## Sodium Iodide I 123 Solution

Sodium iodide (Na<sup>123</sup>I).

Sodium iodide (Na<sup>123</sup>I) [41927-88-2].

» Sodium Iodide I 123 Solution is a solution, suitable for oral or for intravenous administration, containing radioactive iodine (<sup>123</sup>I) processed in the form of Sodium Iodide, obtained from the bombardment of enriched tellurium 124 with protons or of enriched tellurium 122 with deuterons, or by proton irradiation of enriched xenon 124, or by the decay of xenon 123 in such manner that it is carrier-free.

Sodium Iodide I 123 Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>123</sup>I as iodide expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Solution may contain a preservative or stabilizer.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption, if necessary.

**Labeling**—Label it to include the following: the time and date of calibration; the amount of <sup>123</sup>I as iodide expressed as total megabecquerels (microcuries or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement to indicate whether the contents are intended for oral or for intravenous use; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>123</sup>I is 13.2 hours.

**USP Reference standards** <11>—

USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* <821>)—Its gamma-ray spectrum is identical to that of a specimen of <sup>123</sup>I of known purity that exhibits a major photoelectric peak having an energy of 0.159 MeV.

**Radionuclidic purity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radionuclidic purity of the Solution: not less than 90% of the total radioactivity is present as I 123.

**Bacterial endotoxins** <85>—Solution intended for intravenous use meets the requirements of the *Bacterial Endotoxins Test* <85>; and the limit of endotoxin content is not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** <791>: between 7.5 and 9.0 for solutions intended for intravenous administration; between 7.5 and 10.0 for solutions intended for oral administration.

**Radiochemical purity**—Place a measured volume of a solution, containing 100 mg of potassium iodide, 200 mg of potassium iodate, and 1 g of sodium bicarbonate in each 100 mL, 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* <621>), and allow to dry. To the same area add a similar volume of appropriately diluted Solution such that it provides a count rate of about 20,000 counts per minute, and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7 in 10). Dry the chromatogram in air, and determine the radioactivity distribution by scanning with a suitable collimated radiation detector: the radioactivity of the iodide <sup>123</sup>I band is not less than 95.0% of the total radioactivity, and its *R<sub>f</sub>* value falls within ±5.0% of the value found for sodium iodide when determined under similar conditions. Confirmation of the identity of the iodide band is made by the addition to the suspected iodide band of 6 drops of acidified hydrogen peroxide solution (prepared by adding 6 drops of 1 N hydrochloric acid to 10 mL of hydrogen peroxide solution) followed by the dropwise addition of starch TS: the development of a blue color indicates the presence of iodide.

**Other requirements**—Solution intended for intravenous use meets the requirements under *Injections* <1>, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radioactivity, in MBq (μCi) per mL, of Solution by use of a calibrated system as directed under *Radioactivity* <821>.

## Iodinated I 125 Albumin Injection

Albumin labeled with iodine-125.

» Iodinated I 125 Albumin Injection is a sterile, buffered, isotonic solution containing normal human albumin adjusted to provide not more than 37 megabecquerels (1 millicurie) of radioactivity per mL. It is derived by mild iodination of normal human albumin with the use of radioactive iodine (<sup>125</sup>I) to introduce not more than one gram-atom of iodine for each gram-molecule (60,000 g) of albumin.

Iodinated I 125 Albumin Injection contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of <sup>125</sup>I as iodinated albumin, expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. Other forms of radioac-

tivity do not exceed 3 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* <1041> and *Radioactivity* <821>).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, at a temperature between 2° and 8°.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* <1>: the date of calibration; the amount of <sup>125</sup>I as iodinated albumin, expressed as total megabecquerels (microcuries or millicuries), and concentration as megabecquerels (microcuries or millicuries) per mL on the date of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>125</sup>I is 60 days.

**USP Reference standards** <11>—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* <821>)—Its gamma-ray spectrum is identical to that of a specimen of <sup>125</sup>I of known purity that exhibits a major photopeak having an energy of 0.0355 MeV.

**Bacterial endotoxins** <85>—The limit of endotoxin content is not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** <791>: between 7.0 and 8.5.

**Radiochemical purity**—Place a measured volume, diluted with a suitable diluent so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* <621>), and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7 in 10), and air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector: not less than 97.0% of the total activity is found as albumin (at the point of application).

**Other requirements**—It meets the requirements under *Biologics* <1041> and under *Injections* <1>, except that it is not subject to the recommendation on *Container Content*. It meets all other applicable requirements of the FDA.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* <821>), determine the radioactivity, in MBq (μCi) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* <821>.

## Iothalamate Sodium I 125 Injection

» Iothalamate Sodium I 125 Injection is a sterile solution of Iothalamic Acid in Water for Injection prepared with the aid of Sodium Bicarbonate. A portion of the molecules contain radioactive iodine (<sup>125</sup>I) in the molecular structure. It may contain small amounts of suitable buffers or a stabilizer.

Iothalamate Sodium I 125 Injection contains not less than 90.0 percent and not more than 110.0 percent of the concentration of Iothalamate Sodium and of the labeled amount of <sup>125</sup>I as Iothalamate Sodium expressed in kilobecquerels (or in microcuries) per mL at the time indi-

cated in the labeling. Other chemical forms of radioactivity do not exceed 2.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers that are adequately shielded.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{125}\text{I}$  as iohalamate sodium expressed as total megabecquerels (microcuries or millicuries equivalent) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{125}\text{I}$  is 60 days.

**Bacterial endotoxins** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.0 and 8.5.

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{125}\text{I}$  of known purity that exhibits a major photoelectric peak having an energy of 0.035 MeV.

**Radiochemical purity**—Place a measured volume of Injection, diluted with a suitable diluent so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* (621)), and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using methanol and ammonium hydroxide (100:1.5) adjusted with 2 N sulfuric acid to a pH of 3 to 6, and air-dry. Determine the radioactivity distribution with a suitable collimated radiation detector. The radioactivity under the free radioiodide peak is not more than 2% of the total area of all peaks: not less than 98% of the total activity is found at the point of application (as iohalamate sodium).

**Other requirements**—It meets the requirements under *Injections* (1), except that it is not subject to the recommendation in *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in kilobecquerels (or  $\mu\text{Ci}$ ) per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* (821).

### Iodinated I 131 Albumin Injection

Albumin labeled with iodine-131.

» Iodinated I 131 Albumin Injection is a sterile, buffered, isotonic solution containing normal human albumin adjusted to provide not more than 37 MBq (1 mCi) of radioactivity per mL. It is derived by mild iodination of normal human albumin with the use of radioactive iodine ( $^{131}\text{I}$ ) to introduce not more than one gram-atom of iodine for each gram-molecule (60,000 g) of albumin.

Iodinated I 131 Albumin Injection contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $^{131}\text{I}$  as iodinated albumin, expressed in megabecquerels

(microcuries or in millicuries) per mL at the time indicated in the labeling. Other forms of radioactivity do not exceed 3 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* (1041) and *Radioactivity* (821)).

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the date of calibration; the amount of  $^{131}\text{I}$  as iodinated albumin, expressed as total megabecquerels (millicuries or microcuries), and concentration as megabecquerels (microcuries or millicuries) per mL on the date of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{131}\text{I}$  of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**Other requirements**—It meets the requirements for *Packaging and storage*, *Bacterial endotoxins*, *pH*, *Radiochemical purity*, and *Assay for radioactivity* under *Iodinated I 125 Albumin Injection*. It meets also the requirements under *Biologics* (1041), and the requirements under *Injections* (1), except that it is not subject to the recommendation on *Container Content*. It meets all other applicable requirements of the FDA.

### Iodinated I 131 Albumin Aggregated Injection

Albumin labeled with iodine-131.

» Iodinated I 131 Albumin Aggregated Injection is a sterile aqueous suspension of Albumin Human that has been iodinated with  $^{131}\text{I}$  and denatured to produce aggregates of controlled particle size. Each mL of the suspension contains not less than 300  $\mu\text{g}$  and not more than 3.0 mg of aggregated albumin with a specific activity of not less than 7.4 megabecquerels (200 microcuries) per mg and not more than 44.4 megabecquerels (1.2 millicuries) per mg of aggregated albumin. Iodinated I 131 Albumin Aggregated Injection contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $^{131}\text{I}$ , as aggregated albumin, expressed in megabecquerels (microcuries) per mL or megabecquerels (millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 6 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* (1041) and *Radioactivity* (821)).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, at a temperature between 2° and 8°.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{131}\text{I}$  as aggregated albumin expressed as total megabecquerels (microcuries or millicuries) and as aggregated albumin in mg per mL on the date of calibration; the expiration date; and the statement “Caution—Radioactive Material.” The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days; in addition, the labeling states that it is not to be used if clumping of the albumin is observed and directs that the container be agitated before the contents are withdrawn into a syringe.

**USP Reference standards** (11)—  
USP Endotoxin RS

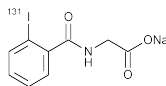
**Radionuclide identification**—Its gamma-ray spectrum is identical to that of a specimen of  $^{131}\text{I}$  of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**pH** (791): between 5.0 and 6.0.

**Other requirements**—It meets the requirements under *Biologics* (1041) and under *Injections* (1), except that it is not subject to the recommendation on *Container Content*. It meets also the requirements for *Particle size*, *Bacterial endotoxins*, and *Radiochemical purity* under *Technetium Tc 99m Albumin Aggregated Injection*, except that in the test for *Radiochemical purity*, not more than 6% of the radioactivity is found in the supernatant following centrifugation.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Iodohippurate Sodium I 131 Injection



$\text{C}_9\text{H}_7^{131}\text{INNaO}_3$

Glycine, *N*-[2-(iodo- $^{131}\text{I}$ -benzoyl)-], monosodium salt.  
Monosodium *o*-iodo- $^{131}\text{I}$ -hippurate [881-17-4].

» Iodohippurate Sodium I 131 Injection is a sterile solution containing *o*-iodohippurate sodium in which a portion of the molecules contain radioactive iodine ( $^{131}\text{I}$ ) in the molecular structure.

Iodohippurate Sodium I 131 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{131}\text{I}$  as iodohippurate sodium expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 3.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{131}\text{I}$  as iodohippurate sodium expressed as total megabecquerels (microcuries or millicuries) and as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the expiration date; and the statement “Caution—Radioactive Material.” The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay,

and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of iodine-131 of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**Bacterial endotoxins** (85)—The limit of endotoxin content is not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

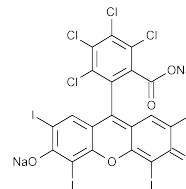
**pH** (791): between 7.0 and 8.5.

**Radiochemical purity**—Place 1 drop of a solution containing 0.2 percent potassium iodide, 1.0 percent sodium bicarbonate, and 1.0 percent sodium thiosulfate, about 45 mm from one end of each of two 25- × 300-mm strips of chromatographic paper (see *Chromatography* (621)), and allow to dry. Superimpose on one of the spots a measured volume of Injection, appropriately diluted, such that it provides a count rate of about 20,000 counts per minute, on this point of application, and allow to air-dry. Superimpose on the second spot 100  $\mu\text{L}$  of a solution prepared by dissolving 50 mg of non-radioactive iodohippurate sodium in 10 mL of alcohol, and allow to air-dry. Develop the chromatogram over a period of about 2½ hours by descending chromatography, using the upper layer obtained by shaking together 2 volumes of benzene, 2 volumes of glacial acetic acid, and 1 volume of water. Use the aqueous layer to equilibrate the apparatus prior to the start of development. Dry the chromatogram in air, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. Locate the position of the non-radioactive spot by viewing the chromatogram under short-wavelength UV light. The radioactivity under the iodohippuric acid band is not less than 97.0% of the total radioactivity, and its  $R_f$  value is within  $\pm 10\%$  of that of the non-radioactive spot.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Rose Bengal Sodium I 131 Injection



$\text{C}_{20}\text{H}_2\text{Cl}_4^{131}\text{I}_4\text{Na}_2\text{O}_5$

Spiro[isobenzofuran-1(3*H*),9'-[9*H*]-xanthene]-3-one, 4,5,6,7-tetrachloro-3',6'-dihydroxy-2',4',5',7'-tetraiodo-, disodium salt, labeled with iodine-131.

4,5,6,7-Tetrachloro-2',4',5',7'-tetraiodofluorescein disodium salt- $^{131}\text{I}$  [24916-55-0; 50291-21-9; 15251-14-6].

» Rose Bengal Sodium I 131 Injection is a sterile solution containing rose bengal sodium in which a portion of the molecules contain radioactive iodine ( $^{131}\text{I}$ ) in the molecular structure. It may contain a suitable buffer.

Rose Bengal Sodium I 131 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{131}\text{I}$  as rose bengal sodium expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. The rose bengal sodium content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of  $^{131}\text{I}$  as rose bengal sodium expressed as total megabecquerels (microcuries or millicuries) and as megabecquerels (microcuries or millicuries) per mL on the date of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{131}\text{I}$  of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**Bacterial endotoxins** (85)—The limit of endotoxin content is not more than  $175/V$  USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which  $V$  is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.0 and 8.5.

**Radiochemical purity**—Place a measured volume of a solution containing 100 mg of potassium iodide, 200 mg of potassium iodate, and 1 g of sodium bicarbonate in each 100 mL about 25 mm from one end of a  $25 \times 300$ -mm strip of chromatographic paper (see *Chromatography* (621)), and allow to dry. To the same area add a similar volume of appropriately diluted Injection such that it provides a count rate of about 20,000 counts per minute, and allow to dry. Develop the chromatogram over a period of about 2 hours by ascending chromatography, using 1 N acetic acid. Dry the chromatogram in air, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity under the rose bengal band is not less than 90.0% of the total radioactivity. The rose bengal band is at the point of application.

**Other requirements**—It meets the requirements under *Injections* (1), except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for rose bengal sodium**—Determine the absorbance of Injection, appropriately diluted, in a 1-cm cell at 550 nm, with a suitable spectrophotometer, using a sodium bicarbonate solution adjusted to a pH of 8.0 as the blank.

Calculate the quantity, in mg, of rose bengal sodium per mL of the Injection taken by the formula:

$$0.004D(A_U / A_S)$$

in which  $D$  is the dilution factor;  $A_U$  is the absorbance of the solution; and  $A_S$  is the absorbance, similarly determined, of a solution of rose bengal sodium adjusted to a pH of 8.0 by the addition of sodium bicarbonate and containing 4  $\mu\text{g}$  of rose bengal sodium per mL.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Sodium Iodide I 131 Capsules

Sodium iodide ( $\text{Na}^{131}\text{I}$ ).

Sodium iodide ( $\text{Na}^{131}\text{I}$ ) [7790-26-3].

» Sodium Iodide I 131 Capsules contain radioactive iodine ( $^{131}\text{I}$ ) processed in the form of Sodium Iodide from products of uranium fission or the neutron bombardment of tellurium in such a manner that it is essentially carrier-free and contains only minute amounts of naturally occurring iodine 127. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{131}\text{I}$  as iodide expressed in megabecquerels (microcuries or millicuries) at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Capsules may contain a stabilizing agent.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label the Capsules to include the following: the date of calibration; the amount of  $^{131}\text{I}$  as iodide expressed in megabecquerels (microcuries or millicuries) per Capsule at the time of calibration; a statement of whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days.

**Radionuclide identification**—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclide identification* under *Sodium Iodide I 131 Solution*.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Determine the instrument response of each of 20 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the mean radioactivity value per Capsule. The requirements are met if not fewer than 19 of the Capsules are within the limits of 96.5% and 103.5% of the mean radioactivity value.

**Radiochemical purity**—Homogenize 1 Capsule in 3 mL of water, add 3 mL of methanol, and centrifuge. The supernatant meets the requirements of the test for *Radiochemical purity* under *Sodium Iodide I 131 Solution*.

**Other requirements**—A solution or suspension prepared by homogenizing 1 or more Capsules in sufficient water to yield a concentration of not less than 1 MBq (25  $\mu\text{Ci}$ ) per

mL meets the requirements of the *Assay for radioactivity* under *Sodium Iodide I 131 Solution*.

## Sodium Iodide I 131 Solution

Sodium iodide ( $\text{Na}^{131}\text{I}$ ).  
Sodium iodide ( $\text{Na}^{131}\text{I}$ ) [7790-26-3].

» Sodium Iodide I 131 Solution is a solution suitable for either oral or intravenous administration, containing radioactive iodine ( $^{131}\text{I}$ ) processed in the form of Sodium Iodide from the products of uranium fission or the neutron bombardment of tellurium in such a manner that it is essentially carrier-free and contains only minute amounts of naturally occurring iodine 127.

Sodium Iodide I 131 Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{131}\text{I}$  as iodide expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Solution may contain a preservative or stabilizer.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption.

**Labeling**—Label it to include the following: the time and date of calibration; the amount of  $^{131}\text{I}$  as iodide expressed as total megabecquerels (microcuries or millicuries) and as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement of the intended use, whether oral or intravenous; a statement of whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{131}\text{I}$  is 8.08 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of iodine 131 of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

**Bacterial endotoxins** (85)—Solution intended for intravenous use meets the requirements of the *Bacterial Endotoxins Test* (85), the limit of endotoxin content being not more than 175/V USP Endotoxin Unit per mL of the Solution, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.5 and 9.0 for solutions intended for intravenous administration; between 7.5 and 10.0 for solutions intended for oral administration.

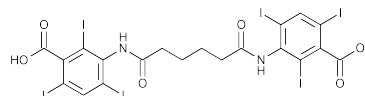
**Radiochemical purity**—Place a measured volume of a solution containing 100 mg of potassium iodide, 200 mg of potassium iodate, and 1 g of sodium bicarbonate in each 100 mL, 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* (621)), and allow to dry. To the same area add a similar volume of appropriately diluted Solution such that it provides a count rate of about 20,000 counts per minute, and allow to dry. Develop the chromatogram over a period of about 4 hours by as-

cending chromatography, using dilute methanol (7.0 in 10). Dry the chromatogram in air, and determine the radioactivity distribution by scanning with a suitable collimated radiation detector: the radioactivity of the iodide  $^{131}\text{I}$  band is not less than 95% of the total radioactivity, and its  $R_f$  value falls within  $\pm 5\%$  of the value found for sodium iodide when determined under parallel conditions. Confirmation of the identity of the iodide band is made by the addition to the suspected iodide band of 6 drops of acidified hydrogen peroxide solution (prepared by adding 6 drops of 1 N hydrochloric acid to 10 mL of hydrogen peroxide solution) followed by the dropwise addition of starch TS; the development of a blue color indicates presence of iodide.

**Other requirements**—Solution intended for intravenous use meets the requirements under *Injections* (1), except that the Solution may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

**Assay for radioactivity**—Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Solution by use of a calibrated system as directed under *Radioactivity* (821).

## Iodipamide



$\text{C}_{20}\text{H}_{14}\text{I}_6\text{N}_2\text{O}_6$  1139.76  
Benzoic acid, 3,3'-[(1,6-dioxo-1,6-hexanediyl)diimino]bis  
[2,4,6-triiodo-  
3,3'-(Adipoyl)diimino]bis[2,4,6-triiodobenzoic acid]  
[606-17-7].

» Iodipamide contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{20}\text{H}_{14}\text{I}_6\text{N}_2\text{O}_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP 3-Amino-2,4,6-triiodobenzoic Acid RS

$\text{C}_7\text{H}_4\text{I}_3\text{NO}_2$  514.83

USP Iodipamide RS

**Identification**—

**A:** It responds to the *Thin-Layer Chromatographic Identification Test* (201), the test solution and the Standard solution being prepared at a concentration of 1 mg per mL in an 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

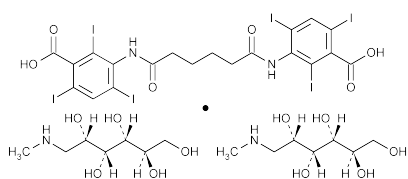
**Free aromatic amine**—Transfer 1.0 g to a 50-mL volumetric flask, and add 12.5 mL of water and 2.5 mL of 1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP 3-Amino-2,4,6-triiodobenzoic Acid RS in 0.1 N sodium hydroxide (use 0.2 mL of the 0.1 N sodium hydroxide for each 5.0 mg of the Reference standard) and

by diluting with water to obtain a solution having a known concentration of 500 µg per mL. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a third 50-mL volumetric flask add 5 mL of water."

**Other requirements**—It meets the requirements of the tests for *Iodine and iodide* and *Heavy metals* under *Diatrizoic Acid*.

**Assay**—Transfer about 300 mg of Iodipamide, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 9.498 mg of  $C_{20}H_{14}I_6N_2O_6$ .

## Iodipamide Meglumine Injection



$C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$  1530.19

Benzoic acid, 3,3'-[(1,6-dioxo-1,6-hexanedyl)diimino]bis [2,4,6-triiodo-, compd. with 1-deoxy-1-(methylamino)-D-glucitol(1:2).

1-Deoxy-1-(methylamino)-D-glucitol 3,3'-(adipoyldiimino)bis [2,4,6-triiodobenzoate] (2:1) (salt) [3521-84-4].

» Iodipamide Meglumine Injection is a sterile solution of Iodipamide in Water for Injection, prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iodipamide meglumine ( $C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iodipamide Meglumine Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I or Type III glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP 3-Amino-2,4,6-triiodobenzoic Acid RS  
 $C_7H_4I_3NO_2$  514.83

USP Endotoxin RS

USP Iodipamide RS

### Identification—

**A:** Dilute a volume of the Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* (201), the Standard solution being prepared at a concentration of 1 mg of USP Iodipamide RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

**B:** Evaporate a volume of Injection, equivalent to about 500 mg of iodipamide, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 3.6 USP Endotoxin Units per mL.

**pH** (791): between 6.5 and 7.7.

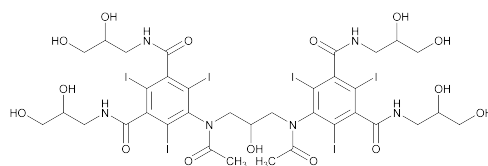
**Free aromatic amine**—Transfer an accurately measured volume of Injection, equivalent to 1 g of iodipamide meglumine, to a 50-mL volumetric flask, dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask add 10 mL of 0.1 N sodium hydroxide, 4 mL of water, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP 3-Amino-2,4,6-triiodobenzoic Acid RS in 0.1 N sodium hydroxide (use 0.2 mL of the 0.1 N sodium hydroxide for each 5.0 mg of the Reference Standard) and diluting with water to obtain a solution having a known concentration of 500 µg per mL. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine*, beginning with "To a third 50-mL volumetric flask add 5 mL of water."

**Meglumine content**—Proceed as directed in the test for *Meglumine content* under *Diatrizoate Meglumine Injection*. The meglumine content is not less than 23.5% and not more than 26.8% of the labeled amount of iodipamide meglumine.

**Other requirements**—It meets the requirements of the tests for *Iodine and iodide* and *Heavy metals* under *Diatrizoate Meglumine Injection*. It meets also the requirements under *Injections* (1).

**Assay**—Pipet a volume of Injection, equivalent to about 5 g of iodipamide meglumine, into a 100-mL volumetric flask, add 1.25 N sodium hydroxide to volume, and mix. Pipet 10 mL of the solution into a glass-stoppered, 250-mL flask, add 20 mL of the sodium hydroxide solution and 500 mg of powdered zinc, and proceed as directed in the Assay under *Diatrizoate Meglumine Injection*, beginning with "connect the flask to a reflux condenser." Each mL of 0.05 N silver nitrate is equivalent to 12.75 mg of  $C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$ .

## Iodixanol



$C_{35}H_{44}I_6N_6O_{15}$  1550.18

1,3-Benzenedicarboxamide, 5,5'-[(2-hydroxy-1,3-propanediyl)bis(acetylimino)]bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-].

5,5'-[(2-Hydroxytrimethylene)bis(acetylimino)]bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide] [92339-11-2].

» Iodixanol contains not less than 98.6 percent and not more than 101.0 percent of  $C_{35}H_{44}I_6N_6O_{15}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Iodixanol RS

USP Iohexol Related Compound B RS

5-Amino-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iodixanol Related Compound C RS

5-[Acetyl[3-[[3,5-bis[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl]amino]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iodixanol Related Compound D RS

5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iodixanol Related Compound E RS

5-[[3-[[3-[(2,3-Dihydroxypropyl)amino]carbonyl]-5-[[amino]carbonyl]-2,4,6-triiodophenyl](acetylmino)]-2-hydroxypropyl]-(acetylmino)]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention times of the two principal peaks in the chromatogram of the *Test solution* correspond to those in the chromatogram of *Standard solution 2*, as obtained in *Related compounds, Test 2*. [NOTE—A third isomer may appear as a minor peak.]

**C:** Heat 0.5 g in a crucible: violet vapors are evolved.

**Specific rotation** (781S): between −0.5° and +0.5°.

*Test solution:* 50 mg per mL.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—Proceed as directed for *Plate Method under Total Aerobic Microbial Count*: not more than 100 cfu per g.

**Water, Method I** (921): not more than 4.0%.

**Heavy metals, Method I** (231): 0.001%.

**Free iodine**—Transfer 2 g to a glass-stoppered tube, add about 20 mL of water, 5 mL of toluene, and 5 mL of 2 N sulfuric acid, shake vigorously, and allow the phases to separate: the toluene layer shows no red or pink color.

**Limit of free iodide**—Transfer 5.0 g to a suitable container, add about 30 mL of water, and titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of iodine. Not more than 0.39 mL of 0.001 N silver nitrate is required: not more than 10 µg of iodide per g is found.

**Limit of free aromatic amine**—

*N*-(1-Naphthyl)ethylenediamine dihydrochloride solution—Prepare a fresh solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride (3 in 1000) in a mixture of propylene glycol and water (70:30).

*Blank solution*—Add 15 mL of water to a 25-mL volumetric flask.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Iohexol Related Compound B RS, and quantitatively dilute with water to obtain a solution having a known concentration of about 10 µg per mL.

*Standard solution*—Transfer 10.0 mL of the *Standard stock solution* and 5 mL of water to a 25-mL volumetric flask.

*Test solution*—Transfer about 200 mg of Iodixanol, accurately weighed, to a 25-mL volumetric flask, add 15 mL of water, and mix.

**Procedure**—Treat the *Standard solution*, the *Test solution*, and the *Blank solution* as follows. Place the flask in an ice bath for 5 minutes. Add 1.5 mL of 6 N hydrochloric acid, mix by swirling, add 1.0 mL of sodium nitrite solution (2 in 100), mix, and allow to stand in the ice bath for 4 minutes. Remove the flask from the ice bath, add 1.0 mL of 4% sulfamic acid solution, and swirl gently until gas evolution ceases. Add 1.0 mL of *N*-(1-Naphthyl)ethylenediamine dihydrochloride solution, mix, dilute with water to volume, mix, and allow to stand for 5 minutes. Transfer the solution obtained from the *Test solution* and the solution obtained from the *Standard solution* to separate color-comparison tubes. The solution obtained from the *Test solution* is lighter than the solution obtained from the *Standard solution*: not more than 0.05% is found. If the solution obtained from the *Test solution* is about the same color or darker than the solution obtained from the *Standard solution*, proceed as follows. Concomitantly determine the absorbances of the solution obtained from the *Test solution*, the solution obtained from the *Standard solution*, and the solution obtained from the *Blank solution* in 5-cm cells, at the wavelength of maximum absorbance at about 495 nm, using the solution obtained from the *Blank solution* to zero the spectrophotometer. Calculate the percentage of free aromatic amine in the portion of Iodixanol taken by the formula:

$$(C/W)[(A_U - A_B)/(A_S - A_B)]$$

in which *C* is the concentration, in µg per mL, of USP Iohexol Related Compound B RS in the *Standard solution*; *W* is the weight, in mg, of Iodixanol taken to prepare the *Test solution*; and *A<sub>U</sub>*, *A<sub>B</sub>*, and *A<sub>S</sub>* are the absorbances of the final solutions obtained from the *Test solution*, the *Blank solution*, and the *Standard solution*, respectively: not more than 0.05% is found.

**Limit of calcium**—

*Internal standard solution*—Accurately weigh about 3.067 g of scandium oxide, and dissolve in 1 L of water (each mL contains 2.0 mg of scandium). Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Blank solution*—Transfer 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with water to volume, and mix.

*Standard solutions*—Prepare a solution having a concentration of 10 µg of calcium per mL. Add 0.5, 2.5, 5.0, and 10.0 mL of this solution to separate 50-mL volumetric flasks. To each flask, add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Test solution*—Transfer about 2 g of Iodixanol, accurately weighed, to a 20-mL volumetric flask, add about 10 mL of water, and mix. Add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of each *Standard solution* and the *Test solution* at 393.366 nm, the calcium emission line, and at 361.38 nm, the scandium emission line, with an atomic absorption spectrophotometer, using the *Blank solution* as the blank. Plot a standard curve of the ratio of the calcium absorption to the scandium absorption versus the respective calcium concentrations. From the graph so obtained, determine the calcium concentration, *C*, in µg per mL, in the *Test solution*. Calculate the content of calcium, in µg per g, in the portion of Iodixanol taken by the formula:

$$20(C/W)$$

in which *C* is as obtained above; and *W* is the weight, in g, of Iodixanol taken to prepare the *Test solution*: not more than 5 µg per g is found.

**Limit of ionic compounds**—[NOTE—Rinse all glassware with water.] Prepare a solution of Iodixanol in water (2 in 100). The specific conductance of this solution is not greater



than that of a sodium chloride solution having a concentration of 4 µg per mL (equivalent to not more than 0.02% of ionic compounds, as NaCl).

**Limit of methanol, isopropyl alcohol, and methoxyethanol—**

*Internal standard stock solution*—Transfer about 500 mg of secondary butyl alcohol to a 500-mL volumetric flask, dilute with water to volume, and mix.

*Internal standard solution*—Transfer 1.0 mL of *Internal standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution*—Transfer about 500 mg of methanol, accurately weighed, and about 1000 mg each of isopropyl alcohol and methoxyethanol, both accurately weighed, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution and 1.0 mL of *Internal standard stock solution* to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL to a headspace vial, and seal the vial with a septum and a crimp cap. This solution contains about 0.005 mg of methanol and about 0.01 mg each of isopropyl alcohol and methoxyethanol per mL.

*Test solution*—Transfer about 250 mg of Iodixanol, accurately weighed, to a headspace vial. Add 1.0 mL of *Internal standard solution*, seal the vial with a septum and a crimp cap, and mix until dissolved.

*Chromatographic system* (see *Chromatography* <621>)—The gas chromatograph is equipped with a headspace injector and a 0.54-mm × 30-m capillary column coated with a 1-µm layer of phase G16. The carrier gas is helium, flowing at a rate of about 11 mL per minute. The column temperature is maintained at 40° for 3.0 minutes, then it is increased linearly at a rate of 8° per minute to 100°, and is maintained at 100° for 1 minute. The temperatures of the headspace injector and the detector are maintained at 150° and 200°, respectively. The *Standard solution* and the *Test solution* are maintained at about 105°, and the needle temperature and transfer temperature are maintained at about 130° and 140°, respectively. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the elution order is methanol, isopropyl alcohol, secondary butyl alcohol, and methoxyethanol; the resolution, *R*<sub>s</sub> between methanol and isopropyl alcohol is not less than 1.0; and the relative standard deviation, determined from peak area ratios, for replicate injections is not more than 5% for methanol and isopropyl alcohol and not more than 10% for methoxyethanol.

*Procedure*—Using a heated, gas-tight syringe, separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of methanol, isopropyl alcohol, and methoxyethanol in the portion of Iodixanol taken by the formula:

$$100(C/W)(R_i / R_s)$$

in which *C* is the concentration, in mg per mL, of the relevant analyte in the *Standard solution*; *W* is the weight, in mg, of Iodixanol taken to prepare the *Test solution*; and *R*<sub>i</sub> and *R*<sub>s</sub> are the peak area ratios of the corresponding analyte to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not more than 50 µg per g each of methanol, isopropyl alcohol, or methoxyethanol is found.

**Related compounds—**

TEST 1—

*Solution A*—Prepare a solution containing 500 mL of acetonitrile and 500 mL of water, and degas.

*Solution B*—Use 1000 mL of degassed water.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Blank solution*—Use water.

*Standard stock solution 1*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol RS in water to obtain a solution having a known concentration of about 12.5 mg of anhydrous iodixanol per mL.

*Standard stock solution 2*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol Related Compound C RS in water to obtain a solution having a known concentration of about 0.25 mg of anhydrous iodixanol related compound C per mL.

*Standard stock solution 3*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol Related Compound D RS in water to obtain a solution having a known concentration of about 0.025 mg of anhydrous iodixanol related compound D per mL.

*Standard solution 1*—Dilute 2.0 mL of *Standard stock solution 1* in water to 10.0 mL, and mix.

*Standard solution 2*—Transfer 5.0 mL of *Standard stock solution 1*, 2.5 mL of *Standard stock solution 2*, and 2.5 mL of *Standard stock solution 3* to a 25-mL volumetric flask, dilute with water to volume, and mix.

*Test solution 1*—Dissolve an amount of Iodixanol, equivalent to about 500 mg of anhydrous iodixanol, in water, dilute with water to 20.0 mL, and mix.

*Test solution 2*—Dilute 5.0 mL of *Test solution 1* with water to 50.0 mL.

*Control solution*—Dilute 5.0 mL of *Test solution 1* and 2.5 mL of *Standard stock solution 2* with water to 50 mL.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	6	94	equilibration (for about 20 minutes)
0–30	6→20	94→80	linear gradient
30–70	20→100	80→0	linear gradient
70–80	100	0	isocratic
80–81	100→6	0→94	linear gradient
81–90	6	94	isocratic

Chromatograph the solutions as directed for *Procedure*, in the following injection sequence: *Blank solution*, *Standard solution 1*, *Control solution*, and at least three replicates of *Standard solution 2*.

The chromatogram obtained from *Standard solution 1* exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are about 60% and 40%. If the chromatogram exhibits three principal peaks, their relative areas are about 60%, 38%, and 2%. The chromatogram obtained from *Standard solution 2* exhibits two resolved peaks due to iodixanol related compound D that elute before the iodixanol peaks and one iodixanol related compound C peak between the two principal iodixanol peaks. The area of the two iodixanol related compound D peaks is between 0.075% and 0.125% of the total area. [NOTE—Disregard any peak due to the solvent front and any peak corresponding to those obtained from the *Blank solution*.] Add the areas of the two isomer peaks for iodixanol related compound D from each of the three injections of *Standard solution 2*, and calculate the relative standard deviation for the three summed areas: the relative standard deviation is not more than 5%. Measure

the height of the iodixanol related compound C peak, and, if necessary, adjust the sensitivity of the amplifier to obtain a peak height between 80% and 100% of the full scale. Measure the height,  $A$ , above the baseline of the iodixanol related compound C peak and the height,  $B$ , above the baseline of the lowest part of the curve separating this peak from the first principal iodixanol peak:  $A$  is not less than 1.3 $B$ . In the chromatogram obtained from the *Control solution*, iodixanol related compound C exhibits a measurable peak.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Blank solution*, *Test solution 1*, and *Test solution 2*.

**HIGH-LOW CHROMATOGRAPHY**—Where it is specified to proceed as directed for *High-low chromatography*, for the chromatogram obtained from *Test solution 1*, calculate the percentage of each specified related compound in the portion of iodixanol taken by the formula:

$$(10X)/(0.1Y + Z)$$

in which  $X$  is the peak area for each of the specified related compounds obtained from *Test solution 1*;  $Y$  is the total area of all the peaks eluted before and after iodixanol obtained from *Test solution 1*, disregarding any peaks due to injection noise or solvent; and  $Z$  is the sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks obtained from *Test solution 2*.

**IOHEXOL**—If iohehexol is present, it exhibits two peaks, with retention times of about 0.37 and 0.39 relative to the main iodixanol peak, in the chromatogram obtained from *Test solution 1*. Draw a baseline at the height of the baseline obtained from the *Blank solution*. Calculate the total area of the two peaks and the percentage of iohehexol in the portion of iodixanol taken as directed for *High-low chromatography*.

**IODIXANOL RELATED COMPOUND B<sup>1</sup>**—If iodixanol related compound B is present, it elutes as a single peak with a retention time of about 0.34 relative to the main iodixanol peak, in the chromatogram obtained from *Test solution 1*. Draw a baseline at the height of the baseline obtained from the *Blank solution*. Calculate the area of the peak and the percentage of iodixanol related compound B in the portion of iodixanol taken as directed for *High-low chromatography*.

**IODIXANOL RELATED COMPOUND C**—If iodixanol related compound C is present, only the first and larger peak, with a retention time of about 1.07 relative to the main iodixanol peak, is seen between the two principal iodixanol peaks in the chromatogram obtained from *Test solution 1*; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to about 80% of the total area of iodixanol related compound C. Draw a vertical line through the minimum before the first and larger peak. Draw a horizontal baseline at the minimum after the first and larger peak. This encompasses the iodixanol related compound C peak area,  $X_2$ . Calculate the percentage of iodixanol related compound C in the portion of iodixanol taken by the formula:

$$12.5X_2/(0.1Y + Z)$$

in which  $Y$  and  $Z$  are as defined for *High-low chromatography*.

**IODIXANOL RELATED COMPOUND F<sup>2</sup>**—If iodixanol related compound F is present, only the first and smaller peak with a retention time of about 0.8 relative to the main iodixanol peak, can be seen in the chromatogram obtained from *Test solution 1*; the second peak co-elutes with iodixanol. The area of the first and smaller peak corresponds to about 25%

of the total area of iodixanol related compound F. Draw the baseline at the height of the baseline obtained from the *Blank solution*. Calculate the percentage of iodixanol related compound F in the portion of iodixanol taken by the formula:

$$40X_1/(0.1Y + Z)$$

in which  $X_1$  is the actual observed area of the peak of iodixanol related compound F obtained from *Test solution 1*; and  $Y$  and  $Z$  are as defined for *High-low chromatography*.

**IODIXANOL RELATED COMPOUND G<sup>3</sup>**—If iodixanol related compound G is present, the second and larger peak, with a retention time of about 1.18 relative to the last iodixanol peak, is seen in the chromatogram obtained from *Test solution 1*; the first peak co-elutes with iodixanol. The area of the second peak corresponds to about 85% of the total area of iodixanol related compound G. Draw the baseline at the height of the baseline obtained from the *Blank solution*. Calculate the percentage of iodixanol related compound G in the portion of iodixanol taken by the formula:

$$10X_3/[0.85(0.1Y + Z)]$$

in which  $X_3$  is the peak area of iodixanol related compound G; and  $Y$  and  $Z$  are as defined for *High-low chromatography*.

**OVERALKYLATED RELATED COMPOUNDS**—These compounds elute after iodixanol related compound G, with a retention time greater than 1.18 relative to the last iodixanol peak. Draw the baseline at the height of the baseline obtained from the *Blank solution*, and determine the peak areas. Calculate the percentage of overalkylated related compounds as directed for *High-low chromatography*.

**UNSPECIFIED RELATED COMPOUNDS**—Examine the chromatograms obtained from *Test solution 1* and the area of each peak eluting before or after iodixanol, other than those of iodixanol, specified related compounds, specified impurities, and overalkylated related compounds. Draw the baseline at the height of the baseline obtained from the *Blank solution*. Calculate the percentage of the largest of these peaks as directed for *High-low chromatography*.

**OTHER UNSPECIFIED RELATED COMPOUNDS**—Determine the area of any unspecified peak eluting between those of iodixanol. Draw the baseline between minima, and calculate the percentage as directed for *High-low chromatography*.

Not more than 0.2% of iodixanol related compound B is found; not more than 0.4% of iodixanol related compound C is found; not more than 0.2% of iodixanol related compound F is found; not more than 0.2% of iodixanol related compound G is found; not more than 0.6% of iohehexol is found; not more than 1.0% of overalkylated related compounds is found; not more than 0.2% of any individual unspecified related compound is found; not more than 0.5% of total unspecified related compounds is found; and not more than 1.5% of total related compounds is found.

**TOTAL RELATED COMPOUNDS**—From each of the chromatograms obtained from *Test solution 1*, calculate the percentage of all related compounds as the sum of the results for the peaks appearing between the two principal iodixanol peaks, and the area percent obtained by the formula:

$$[100(Y - X_1 - X_3 + X_1/0.25 + X_2/0.8 + X_3/0.85)]/10(0.1Y + Z)$$

in which the variables are as defined above.

#### TEST 2—

**Solution A**—Use 1000 mL of filtered and degassed acetonitrile.

**Solution B and Mobile phase**—Proceed as directed for *Test 1*.

<sup>1</sup>5-(acetylamino)- $N,N'$ -bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide

<sup>2</sup>2-[[acetyl[3,5-bis[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]- $N,N'$ -bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4 $H$ -1,4-benzoxazine-6,8-dicarboxamide

<sup>3</sup>4-acetyl-2-[[acetyl[3,5-bis[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]- $N,N'$ -bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4 $H$ -1,4-benzoxazine-6,8-dicarboxamide

*Blank solution*—Use water.

*Standard stock solution 1*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol RS in water to obtain a solution having a known concentration of about 12.5 mg of anhydrous iodixanol per mL.

*Standard stock solution 2*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol Related Compound D RS, equivalent to about 0.125 g of anhydrous iodixanol related compound D, in water, and dilute with water to 100.0 mL. Dilute 2.0 mL of this solution with water to 100.0 mL.

*Standard stock solution 3*—Quantitatively dissolve an accurately weighed quantity of USP Iodixanol Related Compound E RS in water to obtain a solution having a known concentration of about 2.5 mg per mL.

*Standard solution 1*—Dilute 2.0 mL of *Standard stock solution 1* with water to 10.0 mL.

*Standard solution 2*—Transfer 5.0 mL of *Standard stock solution 1* and 2.5 mL of *Standard stock solution 2* to a 25-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution 3*—Transfer 1.0 mL of *Standard solution 1* and 1.0 mL of *Standard stock solution 3* to a 10-mL volumetric flask, dilute with water to volume, and mix.

*Test solution*—Dissolve an accurately weighed quantity of Iodixanol, equivalent to about 125 mg of anhydrous iodixanol, in water, dilute with water to 50.0 mL, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L8. The flow rate is about 2.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	85	15	equilibration
0–25	85→66	15→34	linear gradient

Chromatograph the solutions as directed for *Procedure*. The chromatogram obtained from *Standard solution 1* exhibits three principal unresolved peaks: the relative areas are about 62%, 35%, and 3%; and the retention time of the last iodixanol peak is not more than 14 minutes.

The chromatogram obtained from *Standard solution 2* exhibits two partially unresolved peaks due to iodixanol related compound D, with relative retention times of about 0.33 and 0.39, that elute before the iodixanol peaks: the peak area of iodixanol related compound D is between 0.075% and 0.125% of the total area. Disregard any peak due to the solvent.

Determine the sum of the peak areas of the two isomers of iodixanol related compound D for each of the three chromatograms obtained from *Standard solution 2*: the relative standard deviation for replicate injections is not more than 5%.

The chromatogram obtained from *Standard solution 3* exhibits two unresolved peaks due to iodixanol related compound E, with relative retention times of about 0.67 and 0.72, that elute before the iodixanol peaks. Adjust the sensitivity of the amplifier so that the peak heights are between 90% and 100% of full scale of the highest peak: the resolution, *R*, between the first and largest iodixanol related compound E peak and the first principal iodixanol peak is not less than 5.0.

*Procedure*—Separately inject equal volumes (about 10 μL) of *Standard solution 1*, three times *Standard solution 2*, *Standard solution 3*, and the *Test solution*. For the first chromatogram obtained from *Standard solution 2*, adjust the sensitivity of the amplifier to obtain a peak height of about 15% of the first and larger peak that corresponds to iodixanol related compound D. Use this sensitivity setting for the subsequent injections.

Compare the retention times of the peaks obtained from *Standard solution 3* to those obtained from the *Test solution*. Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with another peak; use only the area of the first and larger peak, which corresponds to about 60% of the total area of iodixanol related compound E. Draw a baseline at the height of the baseline obtained from the *Blank solution*. Calculate the percentage of iodixanol related compound E by dividing the area obtained from the *Test solution* by 0.6 and using area percent.

Iodixanol related compound H<sup>4</sup> appears as a single peak, with a shoulder, on the tail of the iodixanol peak. Calculate the percentage of iodixanol related compound H by using area percent. Not more than 0.3% of iodixanol related compound E is found; and not more than 0.6% of iodixanol related compound H is found.

**Assay**—Transfer about 500 mg of Iodixanol, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 25 mL of a sodium hydroxide solution (5 in 100) and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux for 1 hour. Cool the flask to room temperature, and rinse the condenser with 20 mL of water, adding the rinsing to the refluxed solution. Filter the mixture, rinsing the flask and the filter with several small portions of water, and adding the filtered rinsings to the filtrate. Add 5 mL of glacial acetic acid, titrate with 0.1 N silver nitrate VS, and determine the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of C<sub>35</sub>H<sub>44</sub>I<sub>6</sub>N<sub>6</sub>O<sub>15</sub>.

## Iodixanol Injection

### DEFINITION

Iodixanol Injection is a sterile solution of Iodixanol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iodixanol (C<sub>35</sub>H<sub>44</sub>I<sub>6</sub>N<sub>6</sub>O<sub>15</sub>), as organically bound iodine. It may contain stabilizers and buffers. It contains no antimicrobial agents.

### IDENTIFICATION

- A.** The retention times of the two major peaks of the *Sample solution* correspond to those of *Standard solution B*, as obtained in *Organic Impurities, Procedure 2*.

### ASSAY

#### PROCEDURE

**Sample solution:** Transfer 2 mL of the Injection to a glass-stoppered, 125-mL conical flask, add 50 mL of 5% sodium hydroxide solution and 1.0 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, and rinse the condenser with 20 mL of water, adding the rinsing to the refluxed solution. Filter the mixture, rinsing the flask and the filter with several small portions of water, and adding the filtered rinsings to the filtrate. Add 20 mL of glacial acetic acid, dilute with water to 200.0 mL, and transfer 100.0 mL of this solution to a 250-mL conical flask.

**Analysis:** Titrate with 0.1 N silver nitrate VS using autotitration. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of C<sub>35</sub>H<sub>44</sub>I<sub>6</sub>N<sub>6</sub>O<sub>15</sub>. [NOTE—The result must be corrected for any inorganic halides that may be present due to added stabilizers or buffers.]

<sup>45</sup>-[[[3-[[[3-[[[3-[[[3,5-bis-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide

Acceptance criteria: 95.0%–105.0%

## IMPURITIES

### • ORGANIC IMPURITIES, PROCEDURE 1

**Solution A:** Acetonitrile and water (1:1)

**Solution B:** Water

**Blank solution:** Water

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	6	94
30	20	80
70	100	0
80	100	0
81	6	94
90	6	94

**Standard stock solution A:** 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS in water

**Standard stock solution B:** 0.25 mg/mL of anhydrous iodixanol related compound C from USP Iodixanol Related Compound C RS in water

**Standard stock solution C:** 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS in water

**Standard solution A:** 2.5 mg/mL of *Standard stock solution A* in water

**Standard solution B:** *Standard stock solution A*, *Standard stock solution B*, *Standard stock solution C*, and water (5: 2.5: 2.5: 15)

**Sample solution A:** 25 mg/mL of iodixanol obtained from a volume of Injection in water

**Sample solution B:** 2.5 mg/mL of iodixanol obtained from a volume of Injection in water

**Control solution:** *Sample solution A*, *Standard stock solution B*, and water (5: 2.5: 42.5)

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

### System suitability

**Samples:** *Blank solution*, *Standard solution A*, at least three replicates of *Standard solution B*, and *Control solution*

Chromatograph the solutions as mentioned above. The chromatogram from *Standard solution A* exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are 60% and 40%. If the chromatogram exhibits three principal peaks, their relative areas are 60%, 38%, and 2%. The chromatogram from *Standard solution B* exhibits two resolved peaks due to iodixanol related compound D that elute before the iodixanol peaks and one iodixanol related compound C peak between the two principal iodixanol peaks. The area of the two iodixanol related compound D peaks is between 0.075% and 0.125% of the total area. Add the areas of the two isomer peaks for iodixanol related compound D from each of the three injections of *Standard solution B*, and calculate the relative standard deviation for the three summed areas: the relative standard deviation is NMT 5%. Measure the height of the iodixanol related compound C peak, and, if necessary, adjust the sensitivity of the amplifier to obtain a peak height between 80% and 100% of the full scale. Measure the height, A, above the baseline of the iodixanol related compound C peak and the height, B, above the baseline of the lowest part of the curve sep-

arating this peak from the first principal iodixanol peak: A is NLT 1.3B. In the chromatogram from the *Control solution*, iodixanol related compound C exhibits a measurable peak.

### Analysis

**Samples:** *Blank solution*, *Sample solution A*, and *Sample solution B*

**High-low chromatography:** Where it is specified to proceed as directed for *High-low chromatography*, for the chromatogram from *Sample solution A*, calculate the percentage of each specified related compound in the portion of Injection taken:

$$\text{Result} = (10X)/(0.1Y + Z)$$

X = peak area for each of the specified related compounds from *Sample solution A*

Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

**Iohexol:** If iohexol is present, it exhibits two peaks, with retention times of 0.37 and 0.39 relative to the main iodixanol peak, in the chromatogram from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*.

Calculate the total area of the two peaks and the percentage of iohexol in the portion of Injection taken as directed for *High-low chromatography*.

**Iohexol related compound A:** If iohexol related compound A is present, it elutes as a single peak with a retention time of 0.34 relative to the main iodixanol peak, in the chromatogram from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*.

Calculate the area of the peak and the percentage of iohexol related compound A in the portion of Injection taken as directed for *High-low chromatography*.

**Iodixanol related compound C:** If iodixanol related compound C is present, only the first and larger peak, with a retention time of 1.07 relative to the main iodixanol peak, is seen between the two principal iodixanol peaks in the chromatogram from *Sample solution A*; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to 80% of the total area of iodixanol related compound C. Draw a vertical line through the minimum before the first and larger peak. Draw a horizontal baseline at the minimum after the first and larger peak.

Calculate the percentage of iodixanol related compound C in the portion of Injection taken:

$$\text{Result} = 12.5X_2/(0.1Y + Z)$$

X<sub>2</sub> = iodixanol related compound C peak area

Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent

Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

**Iodixanol related compound F:** If iodixanol related compound F is present, only the first and smaller peak with a retention time of 0.8 relative to the main iox-

<sup>1</sup> 5-(Acetyl(amino))-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

<sup>2</sup> 2-[[Acetyl[3,5-bis[[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-dicarboxamide.

anol peak can be seen in the chromatogram from *Sample solution A*; the second peak co-elutes with iodixanol. The area of the first and smaller peak corresponds to 25% of the total area of iodixanol related compound F. Draw the baseline at the height of the baseline from the *Blank solution*.

Calculate the percentage of iodixanol related compound F in the portion of Injection taken:

$$\text{Result} = 40X_1 / (0.1Y + Z)$$

- $X_1$  = actual observed area of the peak of iodixanol related compound F from *Sample solution A*  
 $Y$  = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent  
 $Z$  = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

**Iodixanol related compound G<sup>3</sup>:** If iodixanol related compound G is present, the second and larger peak, with a retention time of 1.18 relative to the last iodixanol peak, is seen in the chromatogram from *Sample solution A*; the first peak co-elutes with iodixanol. The area of the second peak corresponds to 85% of the total area of iodixanol related compound G. Draw the baseline at the height of the baseline from the *Blank solution*.

Calculate the percentage of iodixanol related compound G in the portion of Injection taken:

$$\text{Result} = 10X_3 / [0.85(0.1Y + Z)]$$

- $X_3$  = peak area of iodixanol related compound G  
 $Y$  = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent  
 $Z$  = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

**Overalkylated related compounds:** These compounds elute after iodixanol related compound G, with a retention time greater than 1.18 relative to the last iodixanol peak. Draw the baseline at the height of the baseline from the *Blank solution*, and determine the peak areas.

Calculate the percentage of overalkylated related compounds as directed for *High-low chromatography*.

**Unspecified related compounds:** Examine the chromatograms from *Sample solution A* and the area of each peak eluting before or after iodixanol, other than those of iodixanol, specified related compounds, specified impurities, and overalkylated related compounds. Draw the baseline at the height of the baseline from the *Blank solution*.

Calculate the percentage of the largest of these peaks as directed for *High-low chromatography*.

**Other unspecified related compounds:** Determine the area of any unspecified peak eluting between those of iodixanol. Draw the baseline between minima, and calculate the percentage as directed for *High-low chromatography*.

<sup>3</sup> 4-Acetyl-2-[[acetyl[[3,5-bis[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-dicarboxamide.

**Individual impurities:** See *Table 2*.

**Table 2**

Name	Acceptance Criteria, NMT (%)
Iohexol related compound A	0.2
Iodixanol related compound C	0.4
Iodixanol related compound F	0.2
Iodixanol related compound G	0.2
Iohexol	0.6
Overalkylated related compounds	1.0
Any individual unspecified related compound	0.2
Total unspecified related compounds	0.5

**Total related compounds:** From each of the chromatograms from *Sample solution A*, calculate the percentage of all related compounds as the sum of the results for the peaks appearing between the two principal iodixanol peaks, and the area percent:

$$\text{Result} = [100(Y - X_1 - X_3 + X_1/0.25 + X_2/0.8 + X_3/0.85)] / 10(0.1Y + Z)$$

- $Y$  = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent  
 $X_1$  = actual observed area of the peak of iodixanol related compound F from *Sample solution A*  
 $X_3$  = peak area of iodixanol related compound G  
 $X_2$  = iodixanol related compound C peak area  
 $Z$  = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

**Total impurities:** NMT 1.5%

• **ORGANIC IMPURITIES, PROCEDURE 2**

**Solution A:** Acetonitrile

**Solution B:** Water

**Blank solution:** Water

**Mobile phase:** See *Table 3*.

**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	85	15
25	66	34

**Standard stock solution A:** 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS

**Standard stock solution B:** 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS

**Standard stock solution C:** 2.5 mg/mL of anhydrous iodixanol related compound E from USP Iodixanol Related Compound E RS

**Standard solution A:** 2.5 mg/mL of anhydrous iodixanol from *Standard stock solution A* diluted with water

**Standard solution B:** 2.5 mg/mL of anhydrous iodixanol and 0.0025 mg/mL of anhydrous iodixanol related compound D from *Standard stock solution A* and *Standard stock solution B*, respectively, diluted with water

**Standard solution C:** 2.5 mg/mL of anhydrous iodixanol and 0.25 mg/mL of anhydrous iodixanol related compound E from *Standard stock solution A* and *Standard stock solution C*, respectively, diluted with water

**Sample solution:** 2.5 mg/mL of iodixanol from a volume of Injection

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L8**Flow rate:** 2.5 mL/min**Injection volume:** 10 μL**System suitability**

**Samples:** *Standard solution A*, three times of *Standard solution B*, *Standard solution C*, and *Sample solution*

The chromatogram from *Standard solution A* exhibits three principal unresolved peaks: the relative areas are 62%, 35%, and 3%; and the retention time of the last iodixanol peak is NMT 14 min. The chromatogram from *Standard solution B* exhibits two partially unresolved peaks due to iodixanol related compound D, with relative retention times of 0.33 and 0.39, that elute before the iodixanol peaks: the peak area of iodixanol related compound D is between 0.075% and 0.125% of the total area. Disregard any peak due to the solvent.

Determine the sum of the peak areas of the two isomers of iodixanol related compound D for each of the three chromatograms from *Standard solution B*.

**Relative standard deviation:** NMT 5%

The chromatogram from *Standard solution C* exhibits two unresolved peaks due to iodixanol related compound E, with relative retention times of 0.67 and 0.72, that elute before the iodixanol peaks. Adjust the sensitivity of the amplifier so that the peak heights are between 90% and 100% of full scale of the highest peak.

**Resolution, R:** NLT 5.0 between the first and largest iodixanol related compound E peak and the first principal iodixanol peak

**Analysis**

**Samples:** *Standard solution A*, three times of *Standard solution B*, *Standard solution C*, and *Sample solution*

For the first chromatogram from *Standard solution B*, adjust the sensitivity of the amplifier to obtain a peak height of 15% of the first and larger peak that corresponds to iodixanol related compound D. Use this sensitivity setting for the subsequent injections.

Compare the retention times of the peaks from *Standard solution C* to those from the *Sample solution*. Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with another peak; use only the area of the first and larger peak, which corresponds to 60% of the total area of iodixanol related compound E. Draw a baseline at the height of the baseline from the *Blank solution*.

Calculate the percentage of iodixanol related compound E by dividing the area from the *Sample solution* by 0.6 and using area percent.

**Iodixanol related compound H<sup>4</sup>:** Appears as a single peak, with a shoulder, on the tail of the iodixanol peak.

Calculate the percentage of iodixanol related compound H by using area percent.

**Acceptance criteria:** NMT 0.3% of iodixanol related compound E is found and NMT 0.6% of iodixanol related compound H is found.

**SPECIFIC TESTS**

- **pH** <791>: 6.8–7.7
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.2 USP Endotoxin Unit/50 mg of iodine
- **OSMOLALITY AND OSMOLARITY**, *Osmolality* <785>: 270–310 mOsmol/kg

<sup>4</sup> 5-[[3-[[[3-[[[3-[[[3-5-Bis-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide.

- **INJECTIONS** <1>: Meets the requirements

**• LIMIT OF FREE IODIDE****Sample:** 5.0 mL of Injection

**Analysis:** Transfer *Sample* to a suitable container, add 2.0 mL of acetic acid solution (1:5 glacial acetic acid in water) and 30 mL of water, and titrate with 0.001 N silver nitrate VS. Each mL of 0.001 N silver nitrate is equivalent to 0.1269 mg of iodine. NMT 15.0 mL of 0.001 N silver nitrate is required.

**Acceptance criteria:** NMT 0.02% based on the content of iodixanol

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multi-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
- **LABELING** Label containers of Injection to direct the user to discard any unused portion. The labeling states also that it is not to be used if it is discolored or contains a precipitate. Label it to state its routes of administration. Label it to indicate "not approved for intrathecal use".
- **USP REFERENCE STANDARDS** <11>
  - USP Iodixanol RS
  - USP Iodixanol Related Compound C RS
 

5-[Acetyl[3-[[[3,5-bis[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl]amino]N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
  - USP Iodixanol Related Compound D RS
 

5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
  - USP Iodixanol Related Compound E RS
 

5-[[3-[[[3-[[[2,3-Dihydroxypropyl]amino]carbonyl]-5-[[[amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl]-(acetylimino)]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

**Iodoform**CHI<sub>3</sub>

Triiodomethane 393.73 [75-47-8].

» Iodoform, previously dried over silica gel for 24 hours, contains not less than 99.0 percent and not more than 100.5 percent of CHI<sub>3</sub>.

**Packaging and storage**—Preserve in tight, light-resistant containers, store at controlled room temperature, and prevent exposure to excessive heat.

**Labeling**—Label it to indicate that it is intended for use in compounding dosage forms for topical, periodontal, nasal, and intracavitary use only.

**Identification**—Heat 0.1 g: a purple gas is evolved.

**Loss on drying** <731>—Dry it over silica gel for 24 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Coloring matter, acids, and alkalis**—Shake about 2 g of it with 5 mL of water for 1 minute, and filter: the filtrate is colorless and is neutral to litmus.

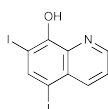
**Chloride** <221>—Shake 3.0 g of powdered Iodoform with 75 mL of water for 1 minute, allow to stand, and filter the supernatant. To 25 mL of the filtrate add 6 mL of 3 N nitric acid, and dilute with water to 40 mL. This solution shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid (0.011%).

**Sulfate** <221>—To 25 mL of the filtrate obtained in the test for *Chloride* add 1 mL of 3 N hydrochloric acid, and dilute with water to 40 mL. This solution shows no more sulfate

than corresponds to 0.17 mL of 0.020 N sulfuric acid (0.017%).

**Assay**—Transfer about 200 mg of Iodoform, previously dried over silica gel for 24 hours and accurately weighed, to a 500-mL glass-stoppered conical flask. Add 20 mL of alcohol, and swirl to dissolve. Add 30.0 mL of 0.1 N silver nitrate VS and 10 mL of nitric acid, insert the stopper, and allow it to stand in a dark place for 16 hours. Add 150 mL of water and 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Perform a blank determination (see *Titrimetry* <541>). Each mL of 0.1 N silver nitrate is equivalent to 13.124 mg of  $\text{CHI}_3$ .

## Iodoquinol



$\text{C}_9\text{H}_5\text{I}_2\text{NO}$  396.95  
8-Quinolinol, 5,7-diiodo-.  
5,7-Diiodo-8-quinolinol [83-73-8].

» Iodoquinol contains not less than 96.0 percent and not more than 100.5 percent of  $\text{C}_9\text{H}_5\text{I}_2\text{NO}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Iodoquinol RS

### Identification—

**A:** Prepare a 1 in 200 solution in carbon disulfide, warming slightly, if necessary, to effect complete solution: the IR absorption spectrum of this solution, in a 3-mm sodium chloride cell, carbon disulfide being used as the blank, in the region from 7  $\mu\text{m}$  to 11  $\mu\text{m}$  exhibits absorption maxima and minima only at the same wavelengths as that of a similar solution of USP Iodoquinol RS, concomitantly measured.

**B:** Warm a small quantity of it with 1 mL of sulfuric acid: violet vapors of iodine are evolved.

**Loss on drying** <731>—Dry it over silica gel for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Free iodine and iodide**—Shake 1.0 g with 20 mL of water for 30 seconds, allow to stand for 5 minutes, and filter. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid, then add 2 mL of chloroform, and shake: no violet color appears in the chloroform (*free iodine*). To the mixture add 5 mL of 2 N sulfuric acid and 1 mL of potassium dichromate TS, and shake for 15 seconds: the color in the chloroform layer is not deeper than that produced in a control test made in the following manner. Dilute 2 mL of potassium iodide solution (1 in 6000) with water to 10 mL, add 6 mL of 2 N sulfuric acid, 1 mL of potassium dichromate TS, and 2 mL of chloroform, and shake for 15 seconds (0.05% of *iodide*).

**Assay**—Using about 14 mg of Iodoquinol, accurately weighed, proceed as directed under *Oxygen Flask Combustion* <471>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of freshly prepared sodium bisulfite solution (1 in 100) as the absorbing liquid. When the combustion is complete, place a few mL of water around the stopper of the flask, loosen the stopper, then rinse the stopper, the specimen holder, and the sides of the flask with about 20 mL of water, added in small portions. Add 1 mL of an oxidizing solution prepared by adding 5 mL

of bromine to 100 mL of a 1 in 10 solution of sodium acetate in glacial acetic acid. Insert the stopper in the flask, and shake vigorously for 1 minute. Add 0.5 mL of formic acid, replace the stopper, and shake vigorously for 1 minute. Remove the stopper, and rinse the stopper, the specimen holder, and the sides of the flask with several small portions of water. Bubble nitrogen through the flask to remove the oxygen and excess bromine, add 500 mg of potassium iodide, swirl to dissolve, add 3 mL of 2 N sulfuric acid, swirl to mix, and allow to stand for 2 minutes. Titrate with 0.02 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.02 N sodium thiosulfate is equivalent to 0.6616 mg of  $\text{C}_9\text{H}_5\text{I}_2\text{NO}$ .

## Iodoquinol Tablets

» Iodoquinol Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $\text{C}_9\text{H}_5\text{I}_2\text{NO}$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** <11>—  
USP Iodoquinol RS

### Identification—

**A:** Shake a portion of finely powdered Tablets, equivalent to about 5 mg of iodoquinol, with 10 mL of carbon disulfide, and filter: the filtrate responds to *Identification test A* under *Iodoquinol*.

**B:** Place a portion of the powdered Tablets prepared for the Assay, equivalent to about 50 mg of iodoquinol, in a dry test tube, add 1 mL of sulfuric acid, and warm gently: violet vapors of iodine are evolved.

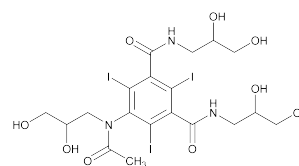
**Disintegration** <701>: 1 hour.

**Uniformity of dosage units** <905>: meet the requirements.

**Soluble iodides**—Digest a quantity of powdered Tablets, equivalent to 100 mg of iodoquinol, with 5 mL of water for 10 minutes, cool, and filter. To the filtrate add 1 mL of 3 N hydrochloric acid, 2 drops of ferric chloride TS, and 2 mL of chloroform, shake gently, and allow to separate: any violet color in the chloroform is not more intense than that in a blank to which 0.2 mg of potassium iodide has been added.

**Assay**—Weigh and finely powder not less than 20 Tablets. Using a portion of the powder, accurately weighed and equivalent to about 14 mg of iodoquinol, proceed as directed in the Assay under *Iodoquinol*. Each mL of 0.02 N sodium thiosulfate is equivalent to 0.6616 mg of  $\text{C}_9\text{H}_5\text{I}_2\text{NO}$ .

## Iohexol



$\text{C}_{19}\text{H}_{26}\text{I}_3\text{N}_3\text{O}_9$  821.14  
1,3-Benzenedicarboxamide, 5-[acetyl(2,3-dihydroxypropyl)amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-N,N'-Bis(2,3-dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodoisophthalamide  
[66108-95-0].

» Iohexol contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{26}I_3N_3O_9$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Iohexol RS

USP Iohexol Related Compound A RS

5-(Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iohexol Related Compound B RS

5-Amino-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iohexol Related Compound C RS

*N,N'*-Bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzenedicarboxamide.

**Color of solution**—Transfer 16.18 g to a 25-mL volumetric flask, dilute with water to volume, and mix. Pass through a filter having a porosity of 0.22  $\mu$ m. The absorbances of this solution, determined in 1-cm cells at 400 nm, 420 nm, and 450 nm, with a suitable spectrophotometer and using water as the blank, are not greater than 0.180, 0.030, and 0.015, respectively.

**Identification**—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Iohexol RS.

**B:** The UV absorption spectrum of a 1 in 100,000 solution in water exhibits a maximum and a minimum at the same wavelengths as that of a similar solution of USP Iohexol RS, concomitantly measured.

**C:** It responds to the *Thin-layer Chromatographic Identification Test* (201), the test solution and the Standard solution of USP Iohexol RS being prepared at a concentration of 10 mg per mL in methanol, the solvent system being a mixture of *n*-butyl alcohol, water, and glacial acetic acid (50:25:11), and short-wavelength UV light being used to locate the spots. The presence of *exo*- and *endo*-isomers in the test solution is shown by the appearance of two spots, each of which corresponds in size and intensity to the corresponding principal spot, at the same  $R_f$  value from the Standard solution. The spot having the  $R_f$  value is the *endo*-isomer.

**D:** Heat about 500 mg in a crucible: violet vapors are evolved.

**Specific rotation** (781S): between  $-0.5^\circ$  and  $+0.5^\circ$ .

*Test solution:* 50 mg per mL, in water.

**Water**, Method I (921): not more than 4.0%.

**Heavy metals**, Method I (231): 0.002%.

**Free aromatic amine**—Transfer 200 mg to a 50-mL volumetric flask, add 15 mL of water, and mix to dissolve. To a second 50-mL volumetric flask transfer 5 mL of water and 10.0 mL of a Standard solution prepared by dissolving an accurately weighed quantity of USP Iohexol Related Compound B RS in water to obtain a solution having a known concentration of 10  $\mu$ g per mL. To a third 50-mL volumetric flask add 15 mL of water to provide a blank. Place the three flasks in an ice bath, and chill for 5 minutes. [NOTE—In conducting the following steps, keep the flasks in the ice bath as much as possible until all of the reagents have been added.] Treat each flask as follows. Add 3.0 mL of 5 N hydrochloric acid, and swirl to mix. Add 2.0 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 4 minutes. Add 2.0 mL of sulfamic acid solution (1 in 25), shake, and allow to stand for 1 minute. [Caution—Considerable pressure is produced.]

Remove the flasks from the ice bath. To each flask add 2.0 mL of a freshly prepared 1 in 1000 solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in dilute pro-

pylene glycol (7 in 10), and mix. Dilute with water to volume, mix, and allow to stand for 5 minutes.

Concomitantly determine the absorbances of the test solution and the Standard solution in 5-cm cells at the wavelength of maximum absorbance at about 495 nm, with a suitable spectrophotometer, against the blank. The absorbance of the solution from the Iohexol is not greater than that of the Standard solution (0.05%).

**Free iodine**—Transfer 2.1 g to a 50-mL centrifuge tube provided with a stopper, add 20 mL of water, and shake vigorously to dissolve. [NOTE—The solution may be heated gently to assist in dissolving the sample. Cool to room temperature before proceeding.] Add 5.0 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge at high speed for 15 minutes: the toluene layer shows no red or pink color.

**Free iodide**—Transfer 5.0 g to a suitable container, add about 20 mL of water to dissolve, and titrate with 0.001 N silver nitrate VS using a silver electrode in combination with an appropriate reference electrode, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9  $\mu$ g of I (0.001%).

**Ionic compounds**—[NOTE—Rinse all glassware five times with distilled water.] Measure the specific resistance, ( $R_{sp}$ ), at 20° of a solution in water (1 in 50), using a suitable water purity meter. Calculate the specific conductance,  $\kappa$ , taken by the formula:

$$(1/R_{sp})10^6.$$

The specific conductance of the solution is not greater than that of a 0.0002% solution of sodium chloride (equivalent to 0.01% ionic compounds).

**Limit of methanol, isopropyl alcohol, and methoxyethanol—**

*Internal standard solution*—Prepare a solution of secondary butyl alcohol in water containing about 0.05 mg per mL.

*Reference solution 1*—Transfer about 0.6 g of methanol, accurately weighed, to a 1000-mL volumetric flask, add about 100 mL of water, and mix. Add about 0.6 g of isopropyl alcohol, accurately weighed, and about 100 mL of water, and mix. Add 0.6 g of methoxyethanol, accurately weighed, and about 100 mL of water, and mix. Dilute with water to volume, and mix.

*Reference solution 2*—Transfer 10.0 mL of *Reference solution 1* to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Reference solution 3*—Transfer 5.0 mL of *Reference solution 1* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Reference solution 4*—Transfer 10.0 mL of *Reference solution 1* to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Reference solution 5*—Transfer 10.0 mL of *Reference solution 4* and 10.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 6.0 mL of the solution so obtained to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95° for 15 minutes.

*Test solution 1*—Transfer about 6.25 g of Iohexol, accurately weighed, to a 25-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Test solution 2*—Transfer 5.0 mL of *Test solution 1* and 1.0 mL of water to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95° for 15 minutes.

*Test solution 3*—Transfer 5.0 mL of *Test solution 1* and 1.0 mL of *Reference solution 2* to a vial fitted with a septum



and crimp cap, and seal. Heat the sealed vial at 95° for 15 minutes.

**Test solution 4**—Transfer 5.0 mL of *Test solution 1* and 1.0 mL of *Reference solution 3* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95° for 15 minutes.

**Test solution 5**—Transfer 5.0 mL of *Test solution 1* and 1.0 mL of *Reference solution 4* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 95° for 15 minutes.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m fused-silica column coated with a 3-μm phase G43. The carrier gas is helium, flowing at a rate of about 14 mL per minute. The chromatograph is programmed as follows. Initially the column temperature is equilibrated at 40° for 5 minutes, then the temperature is increased at a rate of 10° per minute to 100°, and is maintained at that temperature for 1 minute. The injection port and detector temperatures are maintained at 140° and 250°, respectively. Inject the headspace from *Reference solution 5* into the chromatograph, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for methanol, 0.5 for isopropyl alcohol, 1.0 for secondary butyl alcohol, and 1.3 for methoxyethanol; the resolution, *R*, between methanol and isopropyl alcohol is not less than 2.5; and the relative standard deviation determined from individual peak responses from replicate injections is not more than 5%.

**Procedure**—Using a gas-tight syringe, separately inject equal volumes (about 2 mL) of the headspace of *Test solutions 2, 3, 4, 5*, and *Reference solution 5* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the peak area ratio for each analyte to the internal standard. Plot the peak area ratios obtained from the *Test solutions* against the quantity of each individual analyte standard added per g of Iohexol. Extrapolate the line joining the points until it intercepts the concentration axis. The distance between this point and the intersection of the axis is the concentration, in mg per g, of methanol, isopropyl alcohol, or methoxyethanol in the portion of Iohexol taken. Not more than 0.005% each of methanol and isopropyl alcohol is found; and not more than 0.002% of methoxyethanol is found.

#### Limit of 3-chloro-1,2-propanediol—

**Test solution**—Dissolve about 1 g of Iohexol, accurately weighed, in 1.0 mL of water. Extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 2.0 mL, using a warm water bath and a stream of nitrogen. Pass this solution through a membrane filter, and use the clear filtrate.

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of 3-chloro-1,2-propanediol in ethyl acetate to obtain a solution having a known concentration of about 20 μg per mL.

**System suitability solution**—Dissolve 1 g of Iohexol containing less than 5 μg of chloropropanediol in 1 mL of water. Quantitatively dissolve an accurately weighed quantity of 3-chloro-1,2-propanediol in ethyl acetate to obtain a solution having a concentration of about 25 μg per mL. Add 2.0 mL of the ethyl acetate solution to the aqueous solution of Iohexol in a separator, and mix. Transfer the ethyl acetate layer to a separate container, and extract the aqueous layer three additional times with 2 mL of ethyl acetate, combining all four extracts. Dry the combined extracts using anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate and filter as directed under *Test solution*.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m fused-silica capillary column bonded with a 1-μm layer of phase G46. The injection port and detector temperatures are maintained at about 230° and 250°, respectively. The carrier gas is helium under 760 mm Hg pressure. The column temperature is held at 50° for 2 minutes and is programmed to increase at a rate of 10° per minute to 200°. Chromatograph the *Standard solution*, and record the responses as directed for *Procedure*: the relative standard deviation of replicate injections is not more than 10%. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*. Calculate the percentage of 3-chloro-1,2-propanediol in the portion of Iohexol taken by the formula:

$$100(A_{RC} / A_{ST})(C_{ST} / C_{RS})$$

in which  $A_{RC}$  is the response of the analyte peak in the chromatogram obtained from the *System suitability solution*;  $A_{ST}$  is the response of the analyte peak in the chromatogram obtained from the *Standard solution*;  $C_{ST}$  is the concentration of 3-chloro-1,2-propanediol, in μg per mL, in the *Standard solution*; and  $C_{RS}$  is the concentration of 3-chloro-1,2-propanediol, in μg per mL, in the *System suitability solution*: not less than 60% and not more than 90% of 3-chloro-1,2-propanediol is found.

**Procedure**—Inject a volume (about 2 μL) of *Test solution* into the chromatograph, and record the chromatograms. Measure the areas for the major peaks due to the two chloropropanediol isomers, which elute at about 12 and 12.5 minutes. Calculate the quantity, in μg, of 3-chloro-1,2-propanediol in the portion of Iohexol taken by the formula:

$$100(A_{SA} / A_{ST})(2C_{ST} / R)$$

in which  $A_{SA}$  is the total of the peak responses of the two isomers in the chromatograph obtained from the *Test solution*;  $A_{ST}$  is the total of the peak responses of the two isomers in the chromatograph obtained from the *Standard solution*;  $C_{ST}$  is the concentration of 3-chloro-1,2-propanediol, in μg per mL, in the *Standard solution*; and  $R$  is the percentage recovery determined under *Chromatographic system*. Not more than 0.0025% of 3-chloro-1,2-propanediol is found.

#### Related compounds—

**Solution A**—Use acetonitrile.

**Solution B**—Use water.

**Mobile phase**—Use variable mixtures of a degassed mixture of *Solution A* and *Solution B* as directed for *Chromatographic system*.

**System suitability solution**—Dissolve accurately weighed quantities of USP Iohexol RS, USP Iohexol Related Compound A RS, and USP Iohexol Related Compound C RS in water to obtain a solution having known concentrations of about 1.5 mg per mL, 0.0075 mg per mL, and 0.0069 mg per mL, respectively.

**Test solution**—Transfer 75.0 mg of Iohexol, accurately weighed, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm stainless steel column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B*: the percentage of *Solution A* increases from 1% to 13% at a rate of 0.2% per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the retention time for the O-alkylated compounds is between 1.1 and 1.4 relative to 1.0 for the *exo*-isomer of Iohexol; the resolution, *R*, between Iohexol related compound A and Iohexol related compound C is not less than 20.0; and the peak area of

iohexol related compound C is  $0.5\% \pm 0.1\%$  by comparison to the total area of all the peaks in the chromatogram.

**Procedure**—Inject a volume (about 10  $\mu\text{L}$ ) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of O-alkylated compounds and any other individual impurity peak, excluding peaks with a retention time between 0.84 (relative to the *endo*-isomer of iohexol [first main peak]) and the *endo*-isomer of iohexol, in the portion of iohexol taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each impurity; and  $r_s$  is the sum of the responses of all of the peaks: not more than 0.1% of any individual impurity is found; not more than 0.6% of O-alkylated compounds is found; and the sum of all impurities, other than O-alkylated compounds, is not more than 0.3%.

**Assay**—Transfer about 500 mg of iohexol, accurately weighed, to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 27.37 mg of  $\text{C}_{19}\text{H}_{26}\text{I}_3\text{N}_3\text{O}_9$ .

## Iohexol Injection

### DEFINITION

Iohexol Injection is a sterile solution of iohexol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iohexol ( $\text{C}_{19}\text{H}_{26}\text{I}_3\text{N}_3\text{O}_9$ ) as organically bound iodine. It may contain small amounts of suitable buffers and Edetate Calcium Disodium as a stabilizer. Iohexol Injection intended for intravascular or intrathecal use contains no antimicrobial agents.

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

### ASSAY

#### PROCEDURE

**Sample:** A volume of Injection equivalent to 300 mg of iodine

**Analysis:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the solution for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 27.37 mg of  $\text{C}_{19}\text{H}_{26}\text{I}_3\text{N}_3\text{O}_9$ .

**Acceptance criteria:** 95.0%–105.0%

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A:** Acetonitrile

**Solution B:** Water

**Mobile phase:** The percentage of *Solution A* increases from 1% to 13% at a rate of 0.2%/min.

**System suitability solution:** 1.5 mg/mL, 0.0075 mg/mL, and 0.0069 mg/mL each of USP Iohexol RS, USP Iohexol Related Compound A RS, and USP Iohexol Related Compound C RS in water

**Sample solution:** 1.5 mg/mL of Iohexol

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm stainless steel column; packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10  $\mu\text{L}$

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for the *exo*-isomer of iohexol and the O-alkylated compounds are 1.0 and between 1.1 and 1.4, respectively.]

[NOTE—The peak area of iohexol related compound C is  $0.5\% \pm 0.1\%$  compared to the total area of all the peaks in the chromatogram.]

#### Suitability requirements

**Resolution:** NLT 20.0 between iohexol related compound A and iohexol related compound C

#### Analysis

**Sample:** *Sample solution*

Excluding peaks with retention times between 0.84 (relative to the *endo*-isomer of iohexol, which is the first main peak) and 1.0, calculate the percentage of O-alkylated compounds and any other individual impurity peak, in the portion of Iohexol taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak response of each impurity

$r_T$  = sum of all the peak responses

#### Acceptance criteria

**Individual impurity:** NMT 0.6% of O-alkylated compounds; NMT 0.1% of any other individual impurity

**Total impurities:** NMT 0.3%, excluding O-alkylated compounds

### SPECIFIC TESTS

- BACTERIAL ENDOTOXINS TEST (85):** NMT 0.2 USP Endotoxin Unit per 50 mg of iodine
- PH (791):** 6.8–7.7
- PARTICULATE MATTER IN INJECTIONS (788):** The Injection labeled for intrathecal use meets the requirements for small-volume injections.
- FREE IODIDE:** Transfer 5.0 mL of Injection to a suitable container, add 20 mL of water, and titrate with 0.001 N silver nitrate VS using a silver electrode in combination with an appropriate reference electrode. Each mL of 0.001 N silver nitrate is equivalent to 0.1269 mg of iodine.
- Acceptance criteria:** NMT 0.02%, based on the content of iohexol
- INJECTIONS (1):** Meets the requirements

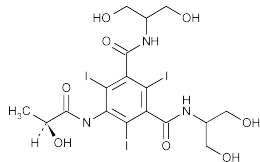
### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve Injection intended for intravascular or intrathecal use in single-dose or multiple-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
- LABELING:** Label containers of Injection to direct the user to discard any unused portion. The labeling states also that it is not to be used if it is discolored or contains a precipitate. Label it also to state its routes of administration. When the specific dose strength is not intended for intrathecal use, label it to indicate "serious injury can occur if given by intrathecal route".

• **USP REFERENCE STANDARDS** (11)

- USP Endotoxin RS
- USP Iohexol RS
- USP Iohexol Related Compound A RS
- 5-(Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
- USP Iohexol Related Compound C RS
- N,N'*-Bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzenedicarboxamide.

## Iopamidol



$C_{17}H_{22}I_3N_3O_8$  777.09

1,3-Benzenedicarboxamide, *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-, (S)-  
(S)-*N,N'*-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodo-5-lactamidoisophthalamide [60166-93-0].

» Iopamidol contains not less than 98.0 percent and not more than 101.0 percent of Iopamidol, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

- USP Iopamidol RS
- USP Iopamidol Related Compound A RS
- N,N'*-Bis-(1,3-dihydroxy-2-propyl)-5-amino-2,4,6-triiodoisophthalamide.
- $C_{14}H_{18}I_3N_3O_6$  705.03
- USP Iopamidol Related Compound C RS
- 4-Chloro-*N'*, *N'*-bis(1,3-dihydroxypropan-2-yl)-5-(S)-lactamido-2,6-diiodoisophthalamide.
- $C_{17}H_{22}ClI_2N_3O_8$  685.63

**Identification**—

**A:** Infrared Absorption (197K).

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**C:** The retention time of the major peak in the chromatogram of the *Identification solution* corresponds to that of the Iopamidol peak observed in the chromatogram of the *System suitability solution*, as obtained in the test for *Related compounds*.

**Specific rotation** (781S): between  $-4.6^\circ$  and  $-5.2^\circ$  ( $t = 20^\circ$ ;  $\lambda = 436$  nm).

**Test solution:** 400 mg per mL, in water, heating on a water bath, if necessary to effect solution, and passing through a membrane filter having a 3- $\mu$ m or finer porosity.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Free aromatic amine**—Transfer 500 mg to a 25-mL volumetric flask, and add 20 mL of water, heating on a water bath, if necessary, to effect solution. To a second 25-mL volumetric flask transfer 18.4 mL of water and 1.6 mL of a Standard solution prepared by dissolving a suitable quantity of USP Iopamidol Related Compound A RS in water and diluting with water to obtain a solution having a concentra-

tion of 62.5  $\mu$ g per mL. To a third 25-mL volumetric flask add 20 mL of water to provide a blank. Treat each flask as follows. Place the flasks in an ice bath, protected from light, for 5 minutes. [NOTE—In conducting the following steps, keep the flasks in the ice bath and protected from light as much as possible until all of the reagents have been added.] Add slowly 1 mL of hydrochloric acid, mix, and allow to stand for 5 minutes. Add 1 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 1 mL of ammonium sulfamate solution (3 in 25), shake, and allow to stand for 5 minutes. [Caution—Considerable pressure is produced.] Add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), and mix. Remove the flasks from the ice bath, and allow to stand in a water bath at about 25° for 10 minutes. Dilute with water to volume, mix, and without delay (about 5 minutes from final dilution), concomitantly determine the absorbances of the solution from the substance under test and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer, against the prepared blank. The absorbance of the solution from the Iopamidol is not greater than that of the Standard solution (0.02%).

**Free iodine**—Transfer 2.0 g to a stoppered, 50-mL centrifuge tube, add sufficient water to dissolve, heating on a water bath, if necessary, to effect solution, and dilute with water to 25 mL. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake well, and centrifuge: the toluene layer shows no red color.

**Limit of free iodide**—Transfer about 6.0 g, accurately weighed, to a suitable container, dissolve in 50 mL of water, and add 2.0 mL of 0.001 M potassium iodide. Titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination, and make any necessary correction. Each mL of 0.001 N silver nitrate is equivalent to 126.9  $\mu$ g of iodide. Not more than 0.001% is found.

**Free acid or alkali**—Dissolve 10.0 g in 100 mL of freshly boiled and cooled water. Using a pH meter and a glass-calomel electrode system, determine the volume of 0.01 N hydrochloric acid VS or 0.01 N sodium hydroxide VS to bring the pH of the test solution to 7.0: not more than 1.37 mL of 0.01 N sodium hydroxide, equivalent to a free acid content of 5 mg of hydrochloric acid per 100 g, or not more than 0.75 mL of 0.01 N hydrochloric acid, equivalent to a free alkali content of 3 mg of sodium hydroxide per 100 g, is required.

**Heavy metals, Method II** (231): not more than 0.001%.

**Related compounds**—

*Solution A*—Use water.

*Solution B*—Prepare a filtered and degassed mixture of water and acetonitrile (1:1).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve accurately weighed quantities of USP Iopamidol RS and USP Iopamidol Related Compound C RS in water, and dilute with water to obtain a solution having concentration of about 20  $\mu$ g per mL of each.

*Standard solution*—Dissolve accurately weighed quantities of USP Iopamidol RS and USP Iopamidol Related Compound C RS in water, and dilute with water to obtain a solution having concentrations of about 20  $\mu$ g per mL and 50  $\mu$ g per mL, respectively.

**Test solution**—Transfer about 0.5 g of Iopamidol, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix.

**Identification solution**—Dilute a suitable volume of the *Test solution* with water to obtain a solution with a concentration of iopamidol of about 20 µg per mL.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 240-nm detector and two 4.6-mm × 25-cm columns that contain packing L11, connected in series. The column temperature is maintained at 60°, and the flow rate is about 2.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–18	100	0	isocratic
18–40	100→62	0→38	linear gradient
40–45	62→50	38→50	linear gradient
45–50	50→100	50→0	linear gradient
50–60	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between iopamidol related compound C and iopamidol is not less than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor for each peak is between 0.7 and 1.5, and the relative standard deviation for replicate injections for either of the two peaks is not more than 2.0%. Chromatograph the *Identification solution*, and record the peak responses as directed for *Procedure* to obtain a chromatogram for *Identification test C*.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Identification solution*, the *System suitability solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses.

Calculate the total percentage of iopamidol related compound C and 2-chloro derivative in the portion of iopamidol taken by the formula:

$$100(C_1V/W)(r_i / r_s)$$

in which *C*<sub>1</sub> is the concentration, in mg per mL, of iopamidol related compound C in the *Standard solution*; *V* is the volume of the *Test solution*; *W* is the weight of iopamidol used to prepare the *Test solution*; *r*<sub>i</sub> is the total peak response for the iopamidol related compound C and 2-chloro derivative obtained from the *Test solution*; and *r*<sub>s</sub> is the peak response for iopamidol related compound C obtained from the *Standard solution*.

Calculate the total percentage of any other impurity in the portion of iopamidol taken by the formula:

$$100(C_2V/W)(r_i / r_s)$$

in which *C*<sub>2</sub> is the concentration of iopamidol, in mg per mL, in the *Standard solution*; *V* and *W* are as previously defined; *r*<sub>i</sub> is the peak response for the individual impurity obtained from the *Test solution*; and *r*<sub>s</sub> is the peak response for iopamidol obtained from the *Standard solution*. In addition to not exceeding the limits for each impurity shown in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 0.20% of total impurities, other than iopamidol related compound C and 2-chloro derivative, is found.

Table 1

Name	Relative Retention Time	Limit (%)
Monocarboxylic acid <sup>1</sup>	0.1	0.1
Iopamidol related compound B <sup>2</sup>	0.6	0.1
Iopamidol related compound C <sup>3</sup> and 2-chloro derivative <sup>4</sup>	0.9	0.5*
Iopamidol	1.0	—
2,3-Dihydroxypropyl isomer <sup>5</sup>	1.1	0.1
Diiodo derivative <sup>6</sup>	1.2	0.1
Acetyl analog <sup>7</sup>	1.3	0.1
Hydroxyethyl derivative <sup>8</sup>	1.5	0.1
O-Acetyl iopamidol <sup>9</sup>	2.2	0.1
<i>N,N</i> -Dimethylamino derivative <sup>10</sup>	2.3	0.1

\*These peaks, appearing at a relative retention time of 0.9, are integrated together to determine conformance.

<sup>1</sup>3-(1,3-Dihydroxypropan-2-ylcarbamoyl)-5-(*S*)-lactamido-2,4,6-triiodobenzoic acid.

<sup>2</sup>5-Glycolamido-*N*<sup>1</sup>,*N*<sup>3</sup>-bis(1,3-dihydroxy-2-propyl)-2,4,6-triiodoisophthalamide.

<sup>3</sup>4-Chloro-*N*<sup>1</sup>,*N*<sup>3</sup>-bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,6-diiodoisophthalamide.

<sup>4</sup>2-Chloro-*N*<sup>1</sup>,*N*<sup>3</sup>-bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-4,6-diiodoisophthalamide.

<sup>5</sup>*N*<sup>1</sup>-(1,3-Dihydroxypropan-2-yl)-*N*<sup>3</sup>-(2,3-dihydroxypropyl)-5-(*S*)-lactamido-2,4,6-triiodoisophthalamide.

<sup>6</sup>*N*<sup>1</sup>,*N*<sup>3</sup>-Bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,4-diiodoisophthalamide.

<sup>7</sup>5-Acetamido-*N*<sup>1</sup>,*N*<sup>3</sup>-bis(1,3-dihydroxy-2-propyl)-2,4,6-triiodoisophthalamide.

<sup>8</sup>*N*<sup>1</sup>-(1,3-Dihydroxypropan-2-yl)-*N*<sup>3</sup>-(2-hydroxyethyl)-5-(*S*)-lactamido-2,4,6-triiodoisophthalamide.

<sup>9</sup>5-(*S*)-(2-Acetoxypentan-2-yl)-*N*<sup>1</sup>,*N*<sup>3</sup>-bis(1,3-dihydroxypropan-2-yl)-carbamoyl)-2,4,6-triiodoisophthalamide.

<sup>10</sup>*N*<sup>1</sup>-(1,3-Dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,4,6-triiodo-*N*<sup>3</sup>,*N*<sup>3</sup>-dimethylisophthalamide.

**Assay**—Transfer about 300 mg of iopamidol, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 40 mL of 1.25 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 25.90 mg of C<sub>17</sub>H<sub>22</sub>I<sub>3</sub>N<sub>3</sub>O<sub>8</sub>.

## Iopamidol Injection

» Iopamidol Injection is a sterile solution of iopamidol in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iopamidol (C<sub>17</sub>H<sub>22</sub>I<sub>3</sub>N<sub>3</sub>O<sub>8</sub>). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. Iopamidol Injection intended for intravascular or intrathecal use contains no antimicrobial agents.

**Packaging and storage**—Preserve Injection intended for intravascular or intrathecal use in single-dose containers, preferably of Type I glass, and protected from light.

**Labeling**—Label containers of Injection to direct the user to discard any unused portion remaining in the container and to check for the presence of particulate matter before using. Label it also to state its routes of administration.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Iopamidol RS

USP Iopamidol Related Compound A RS

*N,N'*-Bis-(1,3-dihydroxy-2-propyl)-5-amino-2,4,6-triiodoisophthalamide.

$C_{14}H_{18}I_3N_3O_6$  705.03

USP Iopamidol Related Compound B RS

5-Glycolamido-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodoisophthalamide.

$C_{16}H_{20}I_3N_3O_7$  747.07

**Identification**—

**A:** Evaporate a volume of Injection, equivalent to about 500 mg of iopamidol, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

**B:** It responds to the *Thin-Layer Chromatographic Identification Test* (201), the test solution and the Standard solution being prepared at a concentration of 0.5 mg per mL in a mixture of methanol and water (9:1), the solvent mixture being chloroform, methanol, ammonium hydroxide, and water (60:30:9:1), and short-wavelength UV light being used to locate the spots.

**Bacterial endotoxins** (85)—It contains not more than 0.6 USP Endotoxin Unit per mg of iodine.

**pH** (791): between 6.5 and 7.5.

**Particulate matter** (788)—The Injection labeled for intrathecal use meets the requirements for small-volume injections.

**Free aromatic amine**—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of iopamidol, to a 25-mL volumetric flask, dilute with water to 20 mL, and mix. To a second 25-mL volumetric flask transfer 16 mL of water and 4.0 mL of Standard solution prepared by dissolving a suitable quantity of USP Iopamidol Related Compound A RS in water and diluting with water to obtain a solution having a concentration of 62.5 µg per mL. Proceed as directed in the test for *Free aromatic amine* under *Iopamidol*, beginning with “to the third 25-mL volumetric flask add 20 mL of water.” The absorbance of the solution from the iopamidol is not greater than that of the Standard solution (0.05%).

**Free iodine**—Transfer a volume of Injection, equivalent to 2.0 g of iopamidol, to a glass-stoppered test tube. Add 2 mL of 2 N sulfuric acid and 1.0 mL of toluene, shake, and allow the layers to separate: the toluene layer shows no red color.

**Limit of free iodide**—Transfer 10.0 mL of Injection to a beaker, add 40 mL of water, and mix. Proceed as directed in the *Limit of free iodide* test under *Iopamidol* beginning with “add 2.0 mL of 0.001 M potassium iodide.” Not more than 3.1 mL of 0.001 N silver nitrate is required (0.04 mg of iodide per mL).

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Solution A*—Use water.

*Solution B*—Prepare a filtered and degassed mixture of water and methanol (3:1).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Resolution solution*—Transfer 10.0 mg of USP Iopamidol Related Compound B RS and 10.0 mg of USP Iopamidol RS to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

*Standard preparation*—Dissolve about 20 mg of USP Iopamidol RS, accurately weighed, in about 10 mL of water,

and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 80 µg of USP Iopamidol RS per mL.

*Assay preparation*—Dilute an accurately measured volume of Injection, equivalent to about 1000 mg of iopamidol, quantitatively and stepwise with water to obtain a solution having a concentration of about 80 µg of iopamidol per mL.

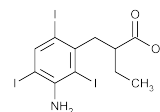
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm stainless steel column that contains 5-µm packing L1. The column temperature is maintained at 35°, and the flow rate is about 1.5 mL per minute. The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B*, the percentage of *Solution B* being 8.0% at the time of injection, and is held at that percentage for 6 minutes, then increased linearly to 35.0% at 18 minutes, after which it is changed to increase linearly to 92.0% at 30 minutes, maintained at that percentage for 4 minutes, and decreased linearly to 8.0% at 36 minutes, where it is held to the end of the run at 40 minutes. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between iopamidol related compound B and iopamidol is not less than 7.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of iopamidol ( $C_{17}H_{22}I_3N_3O_8$ ) in the portion of Injection taken by the formula:

$$12.5C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Iopamidol RS in the *Standard preparation*, and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Iopanoic Acid



$C_{11}H_{12}I_3NO_2$  570.93

Benzenepropanoic acid, 3-amino- $\alpha$ -ethyl-2,4,6-triiodo-, ( $\pm$ )-. ( $\pm$ )-3-Amino- $\alpha$ -ethyl-2,4,6-triiodohydrocinnamic acid [96-83-3].

» Iopanoic Acid contains an amount of iodine equivalent to not less than 97.0 percent and not more than 101.0 percent of  $C_{11}H_{12}I_3NO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—Mix about 100 mg with 500 mg of sodium carbonate in a crucible, and heat until thoroughly charred. Cool, add 5 mL of hot water, heat on a steam bath for 5 minutes, and filter: the solution responds to the tests for *Iodide* (191).

**Melting range** (741): between 152° and 158°, with decomposition.

**Loss on drying** (731)—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Free iodine**—Shake about 200 mg with 2 mL of water and 2 mL of chloroform for 1 minute: the chloroform layer shows no violet color.

**Halide ions**—Place about 500 mg in a glass-stoppered, 50-mL cylinder, add 10 mL of 2 N nitric acid and 15 mL of water, shake for 5 minutes, and filter through paper: 10 mL of the filtrate shows no greater turbidity than corresponds to 0.05 mL of 0.020 N hydrochloric acid (see *Chloride and Sulfate* (221)).

**Heavy metals, Method II** (231): 0.002%.

**Assay**—Transfer about 250 mg of Iopanoic Acid, accurately weighed, to a glass-stoppered, 250-mL conical flask. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, and reflux the mixture for 30 minutes. Cool to room temperature, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the color of the yellow precipitate just changes to green. Each mL of 0.05 N silver nitrate is equivalent to 9.516 mg of  $C_{11}H_{12}I_3NO_2$ .

## Iopanoic Acid Tablets

» Iopanoic Acid Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{11}H_{12}I_3NO_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 1 g of iopanoic acid, with two 10-mL portions of solvent hexane, and decant and discard the liquid. Allow the residue to dry spontaneously, triturate with 15 mL of acetone, and filter. Repeat the trituration with another 15-mL portion of acetone, evaporate the combined filtrates on a steam bath to a volume of not more than 1 mL, add, with constant stirring, 20 mL of water, filter, wash the precipitate with two 5-mL portions of water, and dry at 105° for 2 hours: the iopanoic acid so obtained melts between 150° and 158°, with decomposition, and responds to the *Identification* test under *Iopanoic Acid*.

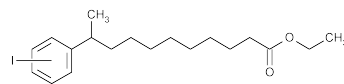
**Disintegration** (701): 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Halide ions**—A portion of the powdered Tablets prepared for the *Assay*, equivalent to about 500 mg of iopanoic acid, meets the requirements of the test for *Halide ions* under *Iopanoic Acid*.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of iopanoic acid, and triturate with 10 mL of solvent hexane. Allow the mixture to settle, decant the hexane through a small filter, repeat the trituration with 10 mL of solvent hexane, filter through the same filter, and discard the filtrates. Warm the residue with 10 mL of neutralized alcohol at 70°, filter through the same filter, and wash the undissolved residue with four 10-mL portions of neutralized alcohol at 70°, passing the washings through the same filter. Cool the combined filtrate and washings to room temperature, add 3 to 5 drops of thymol blue TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 57.09 mg of  $C_{11}H_{12}I_3NO_2$ .

## Iophendylate



$C_{19}H_{29}IO_2$  416.34

Benzenedecanoic acid, iodo-1-methyl-, ethyl ester.  
Ethyl 10-(iodophenyl)undecanoate [1320-11-2].

» Iophendylate is a mixture of isomers of ethyl iodophenylundecanoate, consisting chiefly of ethyl 10-(iodophenyl)undecanoate. It contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{29}IO_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Identification**—Place about 1 mL of Iophendylate, 15 mL of water, and 7 g of potassium dichromate in a round-bottom, 50-mL flask. Carefully add 10 mL of sulfuric acid, moderating the ensuing vigorous reaction by cooling the flask with tap water. When the reaction has subsided, reflux the mixture for 2 hours. Pour the cooled contents of the flask into 25 mL of water, filter the mixture with suction, and wash the precipitate with a small quantity of cold water. Crystallize the precipitate from 10 mL of diluted alcohol, and sublime the solid so obtained: the sublimate of *p*-iodobenzoic acid melts between 268° and 272°.

**Specific gravity** (841): between 1.248 and 1.257.

**Refractive index** (831): between 1.524 and 1.526.

**Residue on ignition** (281): not more than 0.1%.

**Free acids**—Transfer about 4 g, accurately weighed, to a small flask, and add 20 mL of alcohol. Swirl to dissolve the test specimen, add 5 drops of phenolphthalein TS, and titrate with 0.050 N alcoholic potassium hydroxide to a pink color that persists for 30 seconds: not more than 0.60 mL of 0.050 N alcoholic potassium hydroxide is required for neutralization, correction being made for the amount of 0.050 N alcoholic potassium hydroxide consumed by a blank (0.3% as iodophenylundecanoic acid).

**Free iodine**—Determine its absorbance in a 4-cm cell, at 485 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not greater than 0.16 (7.5 ppm).

**Saponification value**—Transfer about 1 g, accurately weighed, to a 250-mL flask, add 25.0 mL of 0.5 N alcoholic potassium hydroxide VS, and reflux the mixture on a steam bath for 1 hour. Cool, add 25 mL of water and 0.7 mL of phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. The saponification value (see *Fats and Fixed Oils* (401)) is between 132 and 142.

**Assay**—Dissolve about 50 mg of Iophendylate, accurately weighed, in 5 mL of toluene contained in a 125-mL separator fitted with a suitable, inert plastic stopcock. Add 15 mL of sodium biphenyl, and shake vigorously for 2 minutes. Extract gently with three 10-mL portions of 5 M phosphoric acid, combining the lower phases in a 125-mL iodine flask. Add 1 N sodium hypochlorite dropwise to the combined extracts until the solution turns brown, and then add an additional 0.5 mL. Shake intermittently for 3 minutes, add 5 mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 minute, accurately timed. Add 1 g of potassium iodide, shake for 30 seconds, and titrate rapidly with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 6.939 mg of  $C_{19}H_{29}IO_2$ .

## Iophendylate Injection

» Iophendylate Injection is sterile Iophendylate.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

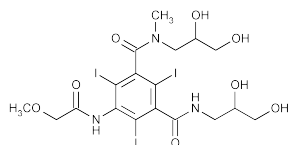
**USP Reference standards** (11)—

USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 0.9 USP Endotoxin Unit per mg of Iophendylate.

**Other requirements**—It conforms to the Definition, responds to the *Identification* test, and meets the requirements for *Specific gravity*, *Refractive index*, *Residue on ignition*, *Free acids*, *Free iodine*, *Saponification value*, and *Assay* under *Iophendylate*. It meets also the requirements under *Injections* (1).

## Iopromide



$C_{18}H_{24}I_3N_3O_8$  791.11

1,3-Benzenedicarboxamide, *N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(methoxyacetyl)amino]-*N*-methyl-*N,N'*-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-*N*-methylisophthalamide [73334-07-3].

» Iopromide contains not less than 97.0 percent and not more than 102.5 percent of  $C_{18}H_{24}I_3N_3O_8$ , calculated on the anhydrous and solvent-free basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Iopromide RS

USP Iopromide Related Compound A RS

USP Iopromide Related Compound B RS

5-(Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-*N*-methyl-1,3-benzenedicarboxamide.

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The  $R_f$  value of the principal spot in the chromatogram, developed with the *Basic eluant*, obtained from the *Test solution* corresponds to that obtained from the *Standard solution* in the *Ordinary impurities* test.

**Water**, *Method I* (921): not more than 1.5%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method I* (231): not more than 0.002%.

**Test solution**—Transfer an accurately weighed quantity, about 1.00 g of Iopromide, to a 20-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Pipet 12.0 mL of this solution into a test tube, add 2.0 mL of pH 3.5 Acetate Buffer, and mix.

**Standard solution**—Pipet 1.0 mL of *Standard Lead Solution* (10 µg of lead), into a test tube, add 9.0 mL of water, 2.0 mL of the *Test solution*, and 2.0 mL of pH 3.5 Acetate Buffer, and mix.

**Thioacetamide-glycerin base solution color-comparison tubes**—Mix 15 mL of 1 N sodium hydroxide and 5 mL of

water, and add 20 mL of glycerin. Pipet 1.0 mL of this solution and 0.20 mL of thioacetamide TS into each of two color-comparison test tubes, and heat in a boiling water bath for 20 seconds. Use these tubes immediately.

**Procedure**—Immediately add the *Test solution* to one of the *Thioacetamide-glycerin base solution color-comparison tubes* and the *Standard solution* to the other. Mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution*, treated in the same manner.

**Free iodine**—Transfer 2.0 g of Iopromide to a glass-stoppered test tube, and dissolve in 20 mL of water. Add 2 mL of toluene and 2 mL of diluted sulfuric acid, and shake vigorously: the toluene layer shows no red color.

**Limit of free iodide**—Transfer 10.0 g of Iopromide to a 150-mL conical flask, and dissolve in 70 mL of water. Adjust with 0.1 N sulfuric acid to a pH of  $3.5 \pm 0.5$ . Titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically, using a silver or platinum electrode in combination with an appropriate reference electrode (see *Titrimetry* (541)). Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of I: the limit is 0.002%.

**Limit of free aromatic amine**—

**Test solution**—Transfer 500 mg of Iopromide to a 25-mL volumetric flask, add 20 mL of water, and mix.

**Standard solution**—Dissolve a suitable quantity of USP Iopromide Related Compound A RS in water, and dilute with water to obtain a stock solution having a known concentration of 0.25 mg per mL. Transfer 2.0 mL of this stock solution to a 25-mL volumetric flask, add 18.0 mL of water, and mix.

**Blank solution**—Transfer 20 mL of water to a 25-mL volumetric flask.

**Procedure**—Treat each flask as follows. Place the flasks in an ice bath, and protect from light. Add slowly 1.0 mL of 8 N hydrochloric acid, mix, and allow to stand for 5 minutes. Add 1.0 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 0.50 mL of freshly prepared sulfamic acid solution, (8 in 100). Shake each flask vigorously several times within the next 5 minutes, venting off the gas that evolves. [Caution—Considerable pressure is produced.] Add 1.0 mL of freshly prepared *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution, (1 in 1000) in a mixture of propylene glycol and water (70:30), and shake. Remove the flasks from the ice bath, and allow to stand in a water bath at about 25° for 10 minutes. Dilute with water to volume, mix, and degas with the aid of sonication for 1 minute. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 495 nm, with a suitable spectrophotometer, using the *Blank solution*, treated in the same manner. The absorbance of the *Standard solution* is not less than 0.40. Calculate the percentage of free aromatic amine in the portion of Iopromide taken by the formula:

$$10(W_s / W_u)(A_u / A_s)$$

in which  $W_s$  is the quantity, in mg, of USP Iopromide Related Compound A RS taken to prepare the *Standard solution*;  $W_u$  is the quantity, in mg, of the Iopromide taken to prepare the *Test solution*; and  $A_u$  and  $A_s$  are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

**Limit of alcohol**—

**Standard solution**—Prepare a solution of alcohol in dimethylformamide to obtain a solution having a known concentration of about 0.050 mg of alcohol ( $C_2H_5OH$ ) per mL.

**Test solution**—Dissolve an accurately weighed portion of Iopromide in dimethylformamide to obtain a concentration of about 50 mg per mL.

**Blank solution**—Use dimethylformamide.

**Chromatographic system** (see *Chromatography* <621>)—The gas chromatograph is equipped with a headspace injector, a flame-ionization detector, and a 0.25-mm × 30-m capillary column, the internal wall of which is coated with a 1.4-μm film of liquid phase G43. The column temperature is programmed according to the following steps: it is held at 40° for 10 minutes, then increased at a rate of 5° per minute to 70°; it is then increased at a rate of 30° per minute to 220°. The injector port is maintained at 160°; the headspace sampler is maintained at 80°; and the detector is maintained at 250°. Helium is used as the carrier gas at a flow rate of about 27 cm per second. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the retention time for alcohol is about 3 minutes; and the relative standard deviation for three injections of the *Standard solution* is not more than 4.0%. Chromatograph the *Blank solution*, and record the peak responses as directed for *Procedure*: the chromatogram shows no peak at the retention time for alcohol.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Transfer 2.0 mL each of the *Test solution*, the *Standard solution*, and the *Blank solution* to separate headspace vials, add 10 μL of 1 N hydrochloric acid to each vial, then seal the vials using a flanged cap so that the cap can no longer be turned. Record the chromatograms, and measure the responses for the alcohol peak. Calculate the concentration of alcohol in the portion of Iopromide taken by the formula:

$$(C/I)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the *Standard solution*; *I* is the quantity, in mg per mL, of Iopromide in the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the alcohol peak responses in the chromatograms obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.4% of alcohol (C<sub>2</sub>H<sub>5</sub>OH) is found. Use the percentage obtained to calculate the Assay result on the solvent-free basis.

**Limit of N-acetyl compound (iopromide related compound B)**—Using the chromatograms obtained in the Assay, calculate the percentage of N-acetyl compound in the Iopromide taken by the formula:

$$20(W_B/W)[(A_{Y1} + A_{Y2}) / (R_{Y1} + R_{Y2})]$$

in which *W<sub>B</sub>* is the quantity, in mg, of USP Iopromide Related Compound B RS taken to prepare the *Related compound B standard solution*; *W<sub>i</sub>* is the quantity, in mg, of Iopromide taken to prepare the *Assay preparation*; *A<sub>Y1</sub>* and *A<sub>Y2</sub>* are the peak responses for the Iopromide related compound B Y1- and Y2-isomers, respectively, in the chromatogram obtained from the *Assay preparation*; and *R<sub>Y1</sub>* and *R<sub>Y2</sub>* are the peak responses for the Iopromide related compound B Y1- and Y2-isomers, respectively, in the chromatogram obtained from the *Related compound B standard solution*: not more than 1.5% of N-acetyl compound is found.

**Ordinary impurities** <466>—

**Test solution**: a mixture of methanol and water (1:1).

**Standard solutions**: a mixture of methanol and water (1:1).

**Visualization solution**—

**SOLUTION A**—Dissolve 2.7 g of ferric chloride in 100 mL of 2.4 N hydrochloric acid. Store this solution in a refrigerator.

**SOLUTION B**—Dissolve 3.5 g of potassium ferricyanide in 100 mL of water. Store this solution in a refrigerator.

**SOLUTION C**—Dissolve 5.0 g of sodium arsenite in 30 mL of 1 N sodium hydroxide solution that has been cooled to 0°. While stirring, mix with 65 mL of 2.4 N hydrochloric acid, and store at room temperature. Use the clear supernatant.

**Procedure**—Mix 10 mL of *Solution A*, 10 mL of *Solution B*, and 2.0 mL of *Solution C*. Use within 30 minutes.

**Basic eluant**: a mixture of dioxane, water, and ammonium hydroxide (85:15:4).

**Acidic eluant**: a mixture of chloroform, methanol, water, and 96 percent formic acid (62:32:6:2).

**Procedure**—Apply 1 μL and 2 μL of the *Test solution* and 1 μL of each of the *Standard solutions* to two separate thin-layer chromatographic plates. Place one plate in a development chamber containing the *Acidic eluant*, and the second plate in a development chamber containing the *Basic eluant*. After the chromatograms have developed, remove the plates from the chambers, and allow to dry at room temperature.

**Visualization**—

**DETECTION 1**—Observe both plates under 254-nm UV light.

**DETECTION 2**—The plate developed with the *Acidic eluant* is exposed to ammonia vapors for 10 to 30 minutes and is air dried. Both plates are exposed to unfiltered 254-nm UV light for several minutes until the principal spots appear yellow. Overspray with *Visualization solution*, and examine the plates under ambient light. Determine the percentage of all secondary spots, except those due to free aromatic amine and to the N-acetyl compound.

**Limit**—The sum of all secondary spots observed in the chromatograms of the *Test solution*, except those due to the free aromatic amine and to the N-acetyl compound in addition to the percentage of N-acetyl compound obtained in the test for *Limit of N-Acetyl compound*, corresponds to not more than 3.0%.

**Isomer distribution**—Using the chromatogram of the Assay preparation obtained in the Assay, calculate the percentage of Iopromide E1- and Z1-isomers in the Iopromide taken by the formula:

$$100(r_{E1} + r_{Z1}) / (r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$

in which *r<sub>E1</sub>*, *r<sub>E2</sub>*, *r<sub>Z1</sub>*, and *r<sub>Z2</sub>* are the peak responses for Iopromide E1-, E2-, Z1-, and Z2-isomers, respectively, in the chromatogram obtained from the Assay preparation: between 40.0% and 51.0% of E1- and Z1-isomers is found. Calculate the percentages of Iopromide E2- and Z2-isomers in the Iopromide taken by the formula:

$$100(r_{E2} + r_{Z2}) / (r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$

between 49.0% and 60.0% E2- and Z2-isomers is found.

**Assay**—

**Diluent**—Prepare a mixture of methanol and water (1:1).

**Mobile phase**—[NOTE—Use chloroform, methanol, and water that have been filtered and degassed.] Mix 6 g of chloroform with 59 g of methanol, then add 900 g of water. Store in a sealed container, and do not stir or sparge the *Mobile phase* during use.

**Standard preparation**—Transfer an accurately weighed quantity of about 38 mg of USP Iopromide RS<sub>1</sub> to a 20-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Related compound B standard solution**—Transfer about 1.9 mg of USP Iopromide Related Compound B RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 38 mg of Iopromide, accurately weighed, to a 20-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.2 mL per minute. The temperature is maintained at a constant temperature of about 20°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative reten-



tion times for iopromide *E1*-isomer, iopromide *E2*-isomer, iopromide *Z1*-isomer, and iopromide *Z2*-isomer are about 0.70, 0.75, 0.85, and 1.0, respectively; the resolution,  $R$ , between iopromide isomers *Z1* and *Z2* is not less than 2.0; and the relative standard deviation for replicate injections for total iopromide area is not more than 2.0%. Chromatograph the *Related compound B standard solution*, and measure the area of the peak responses: the relative retention times for the iopromide related compound B *Y1*- and *Y2*-isomers are about 0.28 and 0.31, respectively; and the signal-to-noise ratio for the iopromide related compound B *Y2*-isomer is not less than 20.

Determine which peaks in the chromatograms correspond to the *E*-isomers as follows. Transfer a portion of the *Standard preparation* to a vial, seal with a crimp-top, and heat to 121° for 15 minutes. Inject the cooled solution. Compare the chromatogram obtained with that of the unheated *Standard preparation*, and note the retention times of the two *E*-isomer peaks, which increase in size after heating.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation*, the *Related compound B standard solution*, and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Allow the *Mobile phase* to flow for not less than 60 minutes between each injection to prevent interference from late-eluting amine peaks. Calculate the quantity of  $C_{18}H_{24}I_3N_3O_8$  in the portion of iopromide taken by the formula:

$$C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Iopromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the sums of the peak responses for iopromide *E1*-isomer, iopromide *E2*-isomer, iopromide *Z1*-isomer, and iopromide *Z2*-isomer in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Iopromide Injection

» Iopromide Injection is a sterile solution of iopromide in Water for Injection. It contains not less than 94.0 percent and not more than 105.0 percent of the labeled amount of iopromide ( $C_{18}H_{24}I_3N_3O_8$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. It contains no anti-microbial agents.

**Packaging and storage**—Preserve in single-dose glass Containers for Injections as described under *Injections* (1), and protect from light. Store at controlled room temperature.

**Labeling**—Label Injection to state that it is not to be used if it contains particulate matter and that after use any unused portion remaining in the container is to be discarded. It is labeled also to state that it is not for intrathecal use.

### USP Reference standards (11)—

USP Endotoxin RS

USP Iopromide RS

USP Iopromide Related Compound A RS

USP Iopromide Related Compound B RS

5-(Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-*N*-methyl-1,3-benzenedicarboxamide.

### Identification—

**A:** Evaporate 3 mL of Injection to dryness, and heat the residue so obtained in a crucible in a hood: violet vapors are evolved.

**B:** The  $R_f$  value of the principal spot in the chromatogram obtained from the *Test solution*, developed with the *Basic eluant*, in the test for *Ordinary impurities* corresponds to that obtained from the *Standard solution* similarly tested.

**Bacterial endotoxins** (85)—It contains not more than 1.25 USP Endotoxin Units per mL of Injection.

**pH** (791): between 6.5 and 8.0.

**Free iodine**—Transfer a volume of Injection, equivalent to 2 g of iopromide, to a 50-mL centrifuge tube. Dilute with water to 24 mL. Add 2 mL of toluene and 2 mL of diluted sulfuric acid solution, and shake: the toluene layer shows no red color.

**Limit of free iodide**—Transfer 10.0 mL of Injection and 50 mL of water to a 150-mL titration vessel, and titrate with 0.001 N silver nitrate VS using a silver or platinum electrode in combination with a reference electrode, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9  $\mu$ g of I. The limit is 80  $\mu$ g of iodide per g of iopromide, based on the labeled content of iopromide.

**Limit of free aromatic amine**—Proceed as directed in the test for *Limit of free aromatic amine* under *Iopromide*, except to prepare the *Test solution* as follows. Transfer an accurately measured volume of Injection, equivalent to about 500 mg of iopromide, to a 25-mL volumetric flask, dilute with water to 20 mL, and mix. Calculate the percentage of free aromatic amine based on the labeled amount of iopromide in the Injection taken by the formula:

$$10(W_S / CV)(A_U / A_S)$$

in which  $W_S$  is the quantity, in mg, of USP Iopromide Related Compound A RS taken to prepare the *Standard solution*;  $C$  is the labeled concentration, in mg per mL, of iopromide in the Injection used to prepare the *Test solution*;  $V$  is the volume, in mL, of Injection to prepare the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 0.2% is found.

**Limit of *N*-acetyl compound** (iopromide related compound B)—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of *N*-acetyl compound in the iopromide in the Injection taken by the formula:

$$(W_B / C)[(A_{Y1} + A_{Y2}) / (R_{Y1} + R_{Y2})]$$

in which  $W_B$  is the quantity, in mg, of USP Iopromide Related Compound B RS taken to prepare the *Related compound B standard solution*;  $C$  is the concentration, in mg of iopromide per mL, in the *Assay preparation* based on the labeled amount and the extent of dilution;  $A_{Y1}$  and  $A_{Y2}$  are the peak responses for iopromide related compound B *Y1*- and *Y2*-isomers, respectively, from the *Assay preparation*; and  $R_{Y1}$  and  $R_{Y2}$  are the peak responses for iopromide related compound B *Y1*- and *Y2*-isomers, respectively, from the *Related compound B standard solution*: not more than 1.5% is found.

**Isomer distribution**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of iopromide isomers in the iopromide in the Injection taken by the formula:

$$100(r_i) / (r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$

in which  $r_i$  is the peak response of each individual iopromide isomer; and  $r_{E1}$ ,  $r_{E2}$ ,  $r_{Z1}$ , and  $r_{Z2}$  are the peak responses for the iopromide *E1*-, *E2*-, *Z1*-, and *Z2*-isomers, respectively, from the *Assay preparation*: between 8.0% and 12.0% of the *E1*-isomer, between 9.0% and 14.0% of the *E2*-isomer, between 32.0% and 40.0% of the *Z1*-isomer, and between 38.0% and 46.0% of the *Z2*-isomer are found.

**Other requirements**—It meets the requirements under *Injections* (1), and meets the requirements for *Ordinary impurities* and *Heavy metals* under *Iopromide*.

**Assay**—

*Diluent, Mobile phase, Standard preparation, Related compound B standard solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Iopromide*.

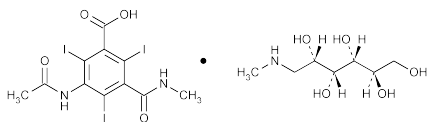
*Assay preparation*—Dilute an accurately measured volume of Injection, quantitatively and stepwise, with *Diluent* to obtain a solution having a final nominal concentration of 1.9 mg of iopromide per mL.

*Procedure*—Proceed as directed in the *Assay* under *Iopromide*. Calculate the quantity, in mg, of iopromide ( $C_{18}H_{24}I_3N_3O_8$ ) in each mL of the Injection taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Iopromide RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of iopromide in each mL of Injection; *D* is the concentration, in mg per mL, of iopromide in the *Assay preparation*, based on the volume of Injection taken and the extent of dilution; and the other factors are as defined therein.

## Iothalamate Meglumine Injection



$C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$  809.13

Benzoic acid, 3-(acetamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-, compd. with 1-deoxy-1-(methylamino)-D-glucitol (1:1).

1-Deoxy-1-(methylamino)-D-glucitol 5-acetamido-2,4,6-triiodo-*N*-methylisophthalamate (salt) [13087-53-1].

» Iothalamate Meglumine Injection is a sterile solution of Iothalamic Acid in Water for Injection, prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iothalamate meglumine ( $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Meglumine Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP 5-Amino-2,4,6-triiodo-*N*-methylisophthalamic Acid RS  
 $C_9H_7I_3N_2O_3$  571.88

USP Endotoxin RS

USP Iothalamic Acid RS

**Identification**—Dilute 3 mL of Injection with water to 100 mL, add an excess of 3 N hydrochloric acid, and filter. Wash the precipitated iothalamic acid on the filter with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamic acid responds to the following tests.

**A:** The IR absorption spectrum of a 0.5% potassium bromide dispersion of the dried acid exhibits maxima only at the same wavelengths as that of a similar preparation of USP Iothalamic Acid RS.

**B:** Heat about 500 mg of the dried acid in a suitable crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 0.9 USP Endotoxin Unit per mL.

**pH** (791): between 6.5 and 7.7.

**Free aromatic amine**—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of iothalamate meglumine per mL. Proceed as directed in the test for *Free aromatic amine* under *Iothalamic Acid*, beginning with "Pipet 5 mL of this solution into a 50-mL volumetric flask."

**Iodine and iodide**—Dilute a volume of Injection, equivalent to 2 g of iothalamate meglumine, with 20 mL of water in a 50-mL beaker, add 5 mL of 2 N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. Proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Iothalamic Acid*, beginning with "To the filtrate add 5 mL of toluene."

**Heavy metals** (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of iothalamate meglumine, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Meglumine content**—Proceed as directed in the test for *Meglumine content* under *Diatrizoate Meglumine Injection*. The meglumine content is not less than 22.9% and not more than 25.3% of the labeled amount of iothalamate meglumine.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Pipet a volume of Injection, equivalent to about 4 g of iothalamate meglumine, into a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ .

## Iothalamate Meglumine and Iothalamate Sodium Injection

» Iothalamate Meglumine and Iothalamate Sodium Injection is a sterile solution of Iothalamic Acid in Water for Injection, prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of iothalamate meglumine ( $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ ) and iothalamate sodium ( $C_{11}H_8I_3N_2NaO_4$ ). It may con-

tain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Meglumine and Iothalamate Sodium Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP 5-Amino-2,4,6-triiodo-*N*-methylisophthamic Acid RS  
 $C_9H_7I_3N_2O_3$  571.88

USP Endotoxin RS

USP Iothalamic Acid RS

**Identification**—Dilute 3 mL of Injection with water to 100 mL, add an excess of 3 N hydrochloric acid, mix, and filter. Wash the precipitate of iothalamic acid so obtained with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamic acid so obtained responds to the following tests.

**A:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Iothalamic Acid RS.

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 3.35 USP Endotoxin Units per mL.

**pH** (791): between 6.5 and 7.7.

**Free aromatic amine**—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of the total of iothalamate meglumine and iothalamate sodium per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask, and add 10 mL of water. Proceed as directed in the test for *Free aromatic amine* under *Iothalamic Acid*, beginning with "In another flask place 15 mL of water. The absorbance of the solution from the Injection is not greater than that of the Standard solution (0.05%)."

**Iodine and iodide**—Dilute a volume of Injection, equivalent to 2 g of the total of iothalamate meglumine and iothalamate sodium, with 20 mL of water in a 50-mL beaker, and proceed as directed for *Procedure* in the test for *Iodine* under *Iothalamic Acid*, beginning with "add 5 mL of 2 N sulfuric acid." The limit of *Iodine and Iodide* is 0.02% of iodide.

**Heavy metals** (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of the total of iothalamate meglumine and iothalamate sodium, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for iothalamate meglumine**—Pipet 5 mL of Injection into a 10-mL volumetric flask, add water to volume, and mix. Determine the angular rotation (see *Optical Rotation* (781)) of the diluted Injection, using a 10-cm cell and a suitable polarimeter. Calculate the quantity, in mg per mL,

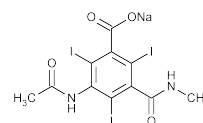
of iothalamate meglumine in the Injection taken by the formula:

$$2000\alpha / 6.01$$

in which  $\alpha$  is the observed angular rotation, in degrees, corrected for the blank; and the factor 6.01 is the specific rotation, in degrees, of iothalamate meglumine.

**Assay for iothalamate sodium**—Transfer an accurately measured volume of Injection, equivalent to about 4 g of iothalamate meglumine and iothalamate sodium, to a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the end-point potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Calculate the volume, in mL, consumed by the iothalamate meglumine in the portion of solution taken, using the value found in the *Assay for iothalamate meglumine*. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of  $C_{11}H_9I_3N_2O_4 \cdot C_7H_7NO_5$ . Subtract this volume from the total volume of 0.05 N silver nitrate consumed. Use the resulting volume to calculate the amount, in mg per mL, of iothalamate sodium in the Injection. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of  $C_{11}H_8I_3N_2NaO_4$ .

## Iothalamate Sodium Injection



$C_{11}H_8I_3N_2NaO_4$  635.90

Benzoic acid, 3-(acetamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-, monosodium salt.

Monosodium 5-acetamido-2,4,6-triiodo-*N*-methylisophthalamate [1225-20-3].

» Iothalamate Sodium Injection is a sterile solution of Iothalamic Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iothalamate sodium ( $C_{11}H_8I_3N_2NaO_4$ ). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Sodium Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label containers of the Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of the Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP 5-Amino-2,4,6-triiodo-*N*-methylisophthamic Acid RS  
 $C_9H_7I_3N_2O_3$  571.88

USP Endotoxin RS

USP Iothalamic Acid RS

**Identification—**

**A:** Dilute 3 mL of Injection with water to 100 mL, add an excess of 3 N hydrochloric acid, and filter. Wash the precipitated iothalamate acid with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamate acid responds to *Identification* tests *A* and *B* under *Iothalamate Meglumine Injection*.

**B:** It responds to the flame test for *Sodium* (191).

**Bacterial endotoxins** (85)—It contains not more than 3.35 USP Endotoxin Units per mL.

**pH** (791): between 6.5 and 7.7.

**Free aromatic amine**—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of iothalamate sodium per mL. Proceed as directed in the test for *Free aromatic amine* under *Iothalamic Acid*, beginning with "Pipet 5 mL of this solution into a 50-mL volumetric flask."

**Iodine and iodide**—Dilute a volume of Injection, equivalent to about 2 g of iothalamate sodium, with 20 mL of water in a 50-mL beaker, add 5 mL of 2 N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. Proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Iothalamic Acid*, beginning with "To the filtrate add 5 mL of toluene."

**Heavy metals** (231)—In a 50-mL color-comparison tube, mix a volume of Injection, equivalent to 1.0 g of iothalamate sodium, with 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

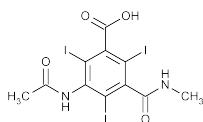
**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—Proceed with Injection as directed in the *Assay* under *Iothalamate Meglumine Injection*. Each mL of 30.05 N silver nitrate is equivalent to 10.60 mg of  $C_{11}H_9I_3N_2NaO_4$ .

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**Iothalamic Acid**


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$C_{11}H_9I_3N_2O_4$  613.91

Benzoic acid, 3-(acetylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-.

5-Acetamido-2,4,6-triiodo-*N*-methylisophthamic acid  
 [2276-90-6].

» Iothalamic Acid contains not less than 98.0 percent and not more than 102.0 percent of  $C_{11}H_9I_3N_2O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP 5-Amino-2,4,6-triiodo-*N*-methylisophthamic Acid RS  
 $C_9H_7I_3N_2O_3$  571.88

USP Iothalamic Acid RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** Heat about 500 mg in a suitable crucible: violet vapors are evolved.

**Water, Method I** (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

**Free aromatic amine**—Dissolve 10.0 g of Iothalamic Acid in a minimal amount of 1 N sodium hydroxide in a 150-mL beaker, add 75 mL of water, and adjust with 1 N sulfuric acid to a pH of  $7 \pm 0.1$ . Transfer the solution to a 100-mL cylinder, dilute with water to 100 mL, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask, and add 10 mL of water. In another flask place 15 mL of water to provide a blank, and to a third flask add 12.5 mL of water and 2.5 mL of a Standard solution prepared as follows. Dissolve 25.0 mg of USP 5-Amino-2,4,6-triiodo-*N*-methylisophthamic Acid RS, accurately weighed, in a mixture of 0.5 mL of 1 N sodium hydroxide and 2.5 mL of water in a 250-mL beaker, swirling to effect solution, then add 225 mL of water, mix, adjust with 1 N sulfuric acid to a pH of  $7 \pm 0.1$ , transfer to a 250-mL volumetric flask, add water to volume, and mix. Place the three flasks containing the solutions from the substance under test, the Standard solution, and the blank, respectively, in an ice bath. [NOTE—In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible, until all of the reagents have been added. Chill all reagents and the diluting water to about 5° prior to addition.] Treat each flask as follows. Add 5 mL of freshly prepared sodium nitrite solution (1 in 200), immediately add 10 mL of 1 N hydrochloric acid, and swirl gently to mix. [NOTE—Disregard any precipitate that may be formed at this point.] Allow to stand for 2 minutes, accurately timed. Add 10 mL of ammonium sulfamate solution (1 in 50), and shake frequently during 5 minutes. Five minutes after the addition of the ammonium sulfamate solution, add 3 drops of a 1 in 10 solution of 1-naphthol in alcohol. Mix, and allow to stand for 1 minute. Add 3.5 mL of a pH 10 buffer (made by dissolving 67.5 g of ammonium chloride in 300 mL of water, adding 570 mL of ammonium hydroxide, and diluting with water to 1 L). Mix, remove from the ice bath, and immediately dilute, with water that has been chilled to 5°, to volume. Within 20 minutes of diluting the contents of all three flasks to 50 mL, concomitantly determine the absorbances of the test solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 485 nm, with a suitable spectrophotometer, versus the prepared blank. The absorbance of the solution from the Iothalamic Acid is not greater than that of the Standard solution (0.05%).

**Iodine and iodide—**

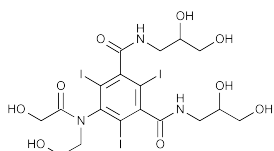
**Test solution**—To 10.0 g in a 50-mL beaker add 16 mL of 1 N sodium hydroxide, and stir until solution is complete. Dilute with water to about 35 mL, and adjust the solution to a pH of between 7.0 and 7.5 with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid. Dilute with water to 50 mL.

**Procedure**—Dilute 10 mL of *Test solution* with 20 mL of water in a 50-mL beaker, add 5 mL of 2 N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. To the filtrate add 5 mL of toluene, and shake: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), and shake: any red color in the toluene layer is not darker than that obtained when a mixture of 2 mL of potassium iodide solution (1 in 4000) and 22 mL of water is substituted for the solution under test (0.02% of iodide).

**Heavy metals** (231)—To a 50-mL color-comparison tube transfer 5.0 mL of solution prepared as directed for *Test solution* in the test for *Iodine and iodide*, add 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation*, proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine*: the limit is 0.002%.

**Assay**—Transfer about 400 mg of Iothalamic Acid, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 10.23 mg of  $C_{11}H_9I_3N_2O_4$ .

## Ioversol



$C_{18}H_{24}I_3N_3O_9$  807.11

1,3-Benzenedicarboxamide, *N,N'*-bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)(2-hydroxyethyl)amino]-2,4,6-triiodo-*N,N'*-Bis(2,3-dihydroxypropyl)-5-*N*-(2-hydroxyethyl)glycol-amido]-2,4,6-triiodoisophthalamide. [87771-40-2].

» Ioversol contains not less than 97.0 percent and not more than 101.0 percent of  $C_{18}H_{24}I_3N_3O_9$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Ioversol RS

USP Ioversol Related Compound A RS

USP Ioversol Related Compound B RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Heat about 500 mg in a crucible: violet vapors are evolved.

**Water**, Method I (921): not more than 5%.

**Residue on ignition** (281): not more than 0.1%.

### Related compounds—

**Mobile phase**—Prepare a degassed mixture of water and acetonitrile (99.5:0.5).

**Standard solution**—Dissolve accurately weighed quantities of USP Ioversol Related Compound A RS and USP Ioversol Related Compound B RS in water to obtain a solution having known concentrations of about 1.0 µg per mL of USP Ioversol Related Compound A RS and 5.0 µg per mL of USP Ioversol Related Compound B RS.

**Test solution**—Transfer about 100 mg of Ioversol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm stainless steel column containing packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 35 ± 0.5°. The system is capable of operating at a column pressure of up to 3000 psi. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between Ioversol related compound A and Ioversol re-

lated compound B is not less than 2.0; and the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each Ioversol related compound in the portion of Ioversol taken by the formula:

$$100(C/W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ioversol Related Compound A RS or USP Ioversol Related Compound B RS in the *Standard solution*; *W* is the concentration, in mg per mL, of Ioversol in the *Test solution*; *r<sub>U</sub>* is the peak response obtained from the *Test solution*; and *r<sub>S</sub>* is the average peak response from the *Standard solution*. Not more than 0.10% of Ioversol related compound A and not more than 0.50% of Ioversol related compound B are found.

**Iodine and iodide**—Dissolve 2.0 g of Ioversol in water in a 50-mL glass-stoppered cylinder, and dilute with water to 15 mL. To this solution add 5 mL each of diluted sulfuric acid and toluene. Shake vigorously, and allow the layers to separate: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), and shake: any red color in the toluene layer is not darker than that obtained from a mixture of 2 mL of potassium iodide solution (1 in 4000) and 13 mL of water, similarly treated (0.02% of iodide).

**Heavy metals**, Method I (231): 0.002%.

**Standard solution**—Into a 50-mL color-comparison tube, pipet 2 mL of *Standard Lead Solution* (20 µg of lead), and dilute with water to 10 mL.

**Test solution**—Dissolve 1 g in 10 mL of water in a 50-mL color-comparison tube.

**Procedure**—For each of the tubes containing the *Standard solution* and the *Test solution*, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH of 3.0 to 4.0 using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix. Add 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, allow to stand for 5 minutes, and view downward over a white surface: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution*, treated in the same manner. The limit is 20 µg per g.

**Assay**—Transfer about 500 mg of Ioversol, accurately weighed, to a glass-stoppered 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride double junction reference electrode and a silver billet electrode. Each mL of 0.05 N silver nitrate is equivalent to 13.45 mg of  $C_{18}H_{24}I_3N_3O_9$ .

## Ioversol Injection

» Ioversol Injection is a sterile solution of Ioversol in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of Ioversol ( $C_{18}H_{24}I_3N_3O_9$ ) and iodine (I). It may contain small amounts of

suitable buffers and Edetate Calcium Disodium as a stabilizer. Ioversol Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Ioversol RS

USP Ioversol Related Compound A RS

USP Ioversol Related Compound B RS

**Identification**—

**A:** The IR absorption spectrum of the test specimen, a zinc sulfide cell having a thickness of 0.01 to 0.02 mm being used, exhibits maxima only at the same wavelength as that of a similar preparation of USP Ioversol RS.

**B:** Heat about 1 mL in a crucible: violet vapors are evolved.

**Bacterial endotoxins** (85)—It contains not more than 1.4 USP Endotoxin Units per mL of Injection.

**pH** (791): between 6.0 and 7.4.

**Heavy metals, Method I** (231)—

**Standard solution**—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 5 mL.

**Test solution**—Into a 50-mL color-comparison tube, pipet a volume of Injection, equivalent to 1 g of Ioversol, and dilute with water to 5 mL.

**Procedure**—For each of the tubes containing the *Standard solution* and the *Test solution*, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Add 5.0 mL of ferrous sulfate solution (1 in 1000), dilute with water to 40 mL, and mix. Add 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, allow to stand for 5 minutes, and view downward over a white surface: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution*, treated in the same manner. The limit is 20 µg per g.

**Other requirements**—It meets the requirements under *Injections* (1).

**Related compounds**—

**Mobile phase and Chromatographic system**—Proceed as directed in the test for *Related compounds* under *Ioversol*.

**Standard solution**—Dissolve accurately weighed quantities of USP Ioversol Related Compound A RS and USP Ioversol Related Compound B RS in water to obtain a solution having known concentrations of 1.5 and 15 µg per mL, respectively.

**Test solution**—Dilute an accurately measured volume of Injection with water to obtain a solution containing 1 mg of Ioversol per mL.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each Ioversol related compound in the volume of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Ioversol Related Compound A RS or USP Ioversol Related Compound B RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of Ioversol in the *Assay preparation*;  $r_U$

is the peak response obtained from the *Test solution*; and  $r_S$  is the average peak response obtained from the *Standard solution*. Not more than 0.15% of Ioversol related compound A and not more than 1.5% of Ioversol related compound B are found.

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 0.5 g of Ioversol, to a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride double junction reference electrode and a silver billet electrode. Each mL of 0.05 N silver nitrate is equivalent to 13.45 mg of  $C_{18}H_{24}I_3N_3O_9$ .

## Ioxaglate Meglumine and Ioxaglate Sodium Injection

» Ioxaglate Meglumine and Ioxaglate Sodium Injection is a sterile solution of Ioxaglic Acid in Water for Injection, prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of Ioxaglate meglumine ( $C_{24}H_{21}I_6N_5O_8 \cdot C_7H_{17}NO_5$ ) and iodine (I). It may contain small amounts of Edetate Calcium Disodium as a stabilizer. Ioxaglate Meglumine and Ioxaglate Sodium Injection intended for intravascular use contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to indicate that the contents are not intended for intravascular injection.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Ioxaglic Acid RS

**Identification**—

**A:** It responds to *Identification test A* under *Ioxaglic Acid*, a solution of 1.7 mL of Injection in 100 mL of water being used as the test solution.

**B:** Evaporate a volume of Injection, equivalent to about 500 mg of Ioxaglate meglumine and Ioxaglate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.

**pH** (791): between 6.0 and 7.6.

**Heavy metals**—

**Test solution**—Transfer a volume of Injection, equivalent to a total of 1.0 g of Ioxaglate meglumine and Ioxaglate sodium, to a 50-mL color-comparison tube, and dilute with water to 5 mL.

**Standard solution**—Transfer 2.0 mL of *Standard Lead Solution* (20 µg of Pb) (see *Heavy Metals* (231)) to a 50-mL color-comparison tube, and dilute with water to 5 mL.

**Procedure**—Add 1.0 mL of ferrous sulfate solution (1 in 1000) to the *Test solution* and the *Standard solution*, adjust the solutions with 1 N acetic acid to a pH between 3 and 4, add 10 mL of hydrogen sulfide TS, mix, allow to stand for 5 minutes, and view downward over a white surface: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution* (0.002%).

#### Free iodine and iodide—

**Test solution**—Transfer a volume of Injection, equivalent to 2 g of the total of ioxaglate meglumine and ioxaglate sodium, to a 50-mL centrifuge tube, add 25 mL of water and 15 mL of 2 N sulfuric acid, and mix thoroughly. Centrifuge for 15 minutes, and decant the supernatant layer into a glass-stoppered 50-mL graduated cylinder. Repeat the sulfuric acid washing and centrifugation once more, adding the supernatant layer into the 50-mL graduated cylinder.

**Procedure**—Proceed as directed for *Procedure* in the test for *Free iodine and iodide* under *Ioxaglic Acid* (0.02% of iodide).

**Other requirements**—It meets the requirements under *Injections* (1).

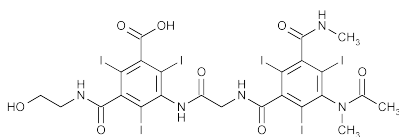
**Assay for ioxaglate meglumine**—Determine the angular rotation (see *Optical Rotation* (781)) of the Injection, using a 10-cm cell and a suitable polarimeter. Calculate the percentage of ioxaglate meglumine in the Injection taken by the formula:

$$100a / 3.32$$

in which *a* is the observed angular rotation, in degrees, corrected for a water blank; and 3.32 is the specific rotation, in degrees, of ioxaglate meglumine.

**Assay for iodine**—Transfer an accurately measured volume of Injection, equivalent to about 5 g (total) of ioxaglate meglumine and ioxaglate sodium, to a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Proceed as directed in the *Assay for iodine* under *Ioxaglic Acid*. Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine (I).

## Ioxaglic Acid



$C_{24}H_{21}I_6N_5O_8$  1268.88

Benzoic acid, 3-[[[3-(acetylmethylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]benzoyl]amino]acetyl]amino]-5-[[[(2-hydroxyethyl)amino]carbonyl]-2,4,6-triiodo-

*N*-(2-Hydroxyethyl)-2,4,6-triiodo-5[[2-[2,4,6-triiodo-3-(*N*-methylacetamido)-5-(methylcarbamoyl)benz-amido]acetamido]isophtalamic acid [59017-64-0].

» Ioxaglic Acid contains not less than 98.5 percent and not more than 101.5 percent of  $C_{24}H_{21}I_6N_5O_8$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

#### USP Reference standards (11)—

USP Ioxaglic Acid RS

#### Identification—

**A: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution**—Dissolve 50 mg in 5 mL of methanol.

**Application volume:** 5  $\mu$ L.

**Developing solvent system:** butyl alcohol, water, and acetic acid (50:25:11).

**Procedure**—Proceed as directed in the chapter. The *R<sub>F</sub>* values of the two spots obtained from the *Test solution* correspond to those obtained from the *Standard solution*.

**B:** Heat about 500 mg in a crucible: violet vapors are evolved.

**Water, Method I** (921): not more than 5%.

**Residue on ignition** (281): not more than 0.1%, the residue being moistened with 2 mL of 35% sulfuric acid and ignited at 600°.

#### Heavy metals—

**Test solution**—Dissolve 2 g of Ioxaglic Acid in 16 mL of 0.1 N sodium hydroxide in a glass-stoppered 25-mL graduated cylinder, dilute with water to 20 mL, and mix. Transfer 15 mL of this solution to a 50-mL color-comparison tube.

**Standard solution**—Transfer 2.0 mL of *Standard Lead Solution* (20  $\mu$ g of Pb) (see *Heavy Metals* (231)) to a 50-mL color-comparison tube, add 5 mL of the *Test solution* and 15 mL of water, and mix.

**Procedure**—Adjust the *Test solution* and the *Standard solution* with 1 N acetic acid to a pH of between 3 and 4. Add 1 mL of a solution of sodium sulfide containing 1.16 mg per mL, mix, allow to stand for 5 minutes, and view downward over a white surface: the color of the solution from the *Test solution* is not darker than that of the solution from the *Standard solution* (0.002%).

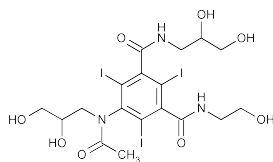
#### Free iodine and iodide—

**Test solution**—Dissolve 2 g of Ioxaglic Acid in 20 mL of 0.1 N sodium hydroxide in a 50-mL centrifuge tube. Add 15 mL of 2 N sulfuric acid, mix on a vortex mixer, centrifuge for 15 minutes, and decant the supernatant layer into a glass-stoppered 100-mL graduated cylinder. Repeat the sulfuric acid washing and centrifugation twice more, decanting each supernatant layer into the 100-mL graduated cylinder.

**Procedure**—Add 5 mL of toluene, shake vigorously, and allow the layers to separate: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and allow the layers to separate: any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (1 in 4000), 25 mL of water, and 15 mL of 2 N sulfuric acid is substituted for the *Test solution* (0.02% of iodide).

**Assay**—Transfer about 500 mg of Ioxaglic Acid, accurately weighed, to a glass-stoppered 125-mL conical flask, and add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc. Connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsing to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the end-point potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 10.57 mg of  $C_{24}H_{21}I_6N_5O_8$ .

## Ioxilan



» Ioxilan contains not less than 98.0 percent and not more than 102.0 percent of  $C_{18}H_{24}I_3N_3O_8$ , calculated on the anhydrous and methanol-free basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Endotoxin RS

USP Ioxilan RS

USP Ioxilan Related Compound A RS

5-Amino-2,4,6-triiodo-3 N-(2-hydroxyethyl)carbamoyl benzoic acid.

$C_{10}H_9I_3N_2O_4$  601.90

### Identification—

**A:** Infrared Absorption (197K).

**B:** UV Absorption (197U)—

Solution: 10 µg per mL.

Medium: water.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per 50 mg of iodine.

**Heavy metals**, Method I (231): 0.002%.

**pH** (791): between 5.0 and 7.5, in a solution (1 in 10).

[NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.]

**Water**, Method I (921): not more than 4.0%.

**Free iodine**—Transfer 2 g to a stoppered flask, add 12 mL of water, and swirl to dissolve. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene, stopper the flask, and shake for 1 minute. Allow the layers to separate: the toluene layer shows no red color. Reserve the contents of the flask for use as the test solution in the test for *Free iodide*.

**Free iodide**—Prepare a solution of potassium iodide in water containing 1000 µg of iodide per mL. Quantitatively dilute portions of this solution with water to obtain standard solutions having concentrations of 100, 50, 25, and 10 µg of iodide per mL. Transfer 2 mL of each standard solution to separate stoppered flasks, and add 12 mL of water to each flask. Add 14 mL of water to an additional flask to serve as the blank. Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene to the flasks containing the standard solutions and the blank, stopper the flasks, and shake for 1 minute. To the test solution obtained in the test for *Free iodine*, each of the standard solutions, and the blank, add 0.5 mL of sodium nitrite solution (2 in 100), stopper the flasks, and shake vigorously for 1 minute. Allow the layers to separate, transfer the toluene layers to separate centrifuge tubes, and centrifuge for 1 minute. Concomitantly determine the absorbances of the test solution and the standard solutions against the blank. From a linear regression equation calculated from the concentrations and absorbances of the iodide standard solutions, determine the content of iodide in the Ioxilan: the limit is 30 µg of iodide per g of Ioxilan.

**Free aromatic amine**—[NOTE—Protect the Standard solution, the test solution, and the blank from light throughout the test.]

**pH 10 Buffer**—To 33.7 g of ammonium chloride add 285 mL of ammonium hydroxide, dilute with water to 500 mL, and mix.

**Procedure**—Transfer 20 mg of USP Ioxilan Related Compound A RS, accurately weighed, to a 200-mL volumetric flask, dissolve in a small volume of hot water, cool, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, add 12 mL of water, and mix. Transfer 0.5 g of Ioxilan to a second 50-mL volumetric flask, add 12 mL of water, and shake to dissolve. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] To a third 50-mL volumetric flask, add 12 mL of water to serve as the blank, and place the flasks containing the Standard solution, the test solution, and the blank in an ice bath. [NOTE—In conducting the following steps, keep the flasks in the ice bath as much as possible until all of the reagents have been added.] Treat each of the flasks as follows. Add 5 mL of sodium nitrite solution (0.5 in 100), and shake. [Caution—Considerable pressure is produced with the addition of each reagent.]

Add 10 mL of 1 N hydrochloric acid, swirl, and allow to stand for 2 minutes. Add 3 drops of a 1 in 10 solution of  $\alpha$ -naphthol in alcohol, swirl, and allow to stand for 2 minutes. Add 3.5 mL of pH 10 Buffer, swirl, and allow to stand out of the ice bath for 2 minutes. Degass all solutions in a water bath at 25° for 10 minutes, dilute with water to 50 mL, and mix. Within 15 minutes of this final dilution, concomitantly determine the absorbances of the test solution and the Standard solution at the wavelength of maximum absorbance at about 485 nm, with a suitable spectrophotometer, against the blank. The absorbance of the test solution is not greater than that of the Standard solution (0.05%).

### Residual methanol—

**Standard stock solution**—Transfer 200 mg of methanol to a tared 200-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix.

**Internal standard solution**—Transfer 200 mg of dehydrated alcohol to a tared 200-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard solutions A, B, C, and D**—Transfer 0.5, 1.0, 2.0, and 4.0 mL of Standard stock solution to separate 10-mL volumetric flasks, each containing 2 mL of water. Add 1.0 mL of Internal standard solution to each flask, dilute with water to volume, and mix to obtain Standard solutions A, B, C, and D having concentrations of 0.5, 1.0, 2.0, and 4.0 mg of methanol per L, respectively.

**Test solution**—Transfer about 1 g of Ioxilan to a tared 10-mL ampul, and add water to the ampul to a final weight of 10.45 g, taking care to avoid leaving water in the neck of the ampul. Seal the ampul, and immerse it in a water bath at 90° until the Ioxilan is dissolved. Mix, and allow to cool to room temperature. Mix again, open the ampul, and dilute the contents with a volume of water suitable to obtain a methanol concentration within the range of the standard curve (between 0.5 and 4 mg per L), allowing for the addition of 1.0 mL of Internal standard solution.

**Chromatographic system** (see System Suitability under Chromatography (621))—The gas chromatograph is equipped with a flame-ionization detector and a 10-m  $\times$  0.53-mm fused silica capillary column coated with support S2. The column temperature is programmed to increase from 45° to 80° at a rate of 5° per minute. The injection port temperature is maintained at 180°, and the detector temperature is maintained at 200°. Helium is used as the carrier gas at a flow rate of about 30 mL per minute. Chromatograph Standard solution D, and record the peak responses as directed for Procedure: the resolution, *R*, between the methanol and alcohol peaks is not less than 10; the tailing factor for the methanol peak is not more than 3.0;



and the relative standard deviation for replicate injections is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 1  $\mu$ L) of each *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. From a linear regression equation calculated from the ratios of the peak responses of methanol to those of alcohol for each of the *Standard solutions* versus the methanol concentrations, in mg per L, determine the methanol content in the *Test solution*: not more than 2.0% is found.

#### Limit of serinol impurity—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (91:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Transfer about 60 mg of USP Ioxilan RS to a 10-mL volumetric flask, add 1 mL of water, and heat at not more than 80° to dissolve. Cool to room temperature, dilute with acetonitrile to volume, and mix.

**Test solution**—Using about 60 mg of Ioxilan, proceed as directed for *Standard solution*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm  $\times$  4.6-mm stainless steel column that contains 5- $\mu$ m packing L18. The flow rate is about 2 mL per minute, and the column temperature is maintained at 30°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the retention time of the serinol peak is about 0.9 relative to that of the larger of the Ioxilan peaks; the resolution, *R*, between the larger of the Ioxilan peaks and the serinol peak is not less than 1.0; and the relative standard deviation of the response of the serinol peak for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, and allow the chromatogram to run for about 35 minutes. Measure the areas of the peak responses, and determine the percentage of serinol in the portion of Ioxilan taken: not more than 0.5% is found.

#### Chromatographic purity—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard solution**—Transfer about 60 mg of USP Ioxilan RS to a 10-mL volumetric flask, and add 1 mL of water. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] Dilute with acetonitrile to volume, and mix.

**Test solution**—Using about 60 mg of Ioxilan, proceed as directed for *Standard solution*.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm  $\times$  4.6-mm stainless steel column that contains 5- $\mu$ m packing L8. The column is maintained at 30°, and the flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the two largest impurity peaks eluting immediately after the second Ioxilan isomer peak is not less than 0.3.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Determine the percentage of each impurity (excluding the serinol impurity, which has a retention time of 0.9 relative to the larger Ioxilan isomer) in the portion of Ioxilan taken: not more than 0.5% of any individual impurity is found; and the total of all impurities is not more than 1.5%.

**Assay**—Transfer about 500 mg of Ioxilan, accurately weighed, to a 100-mL round-bottom flask. Add 1 g of powdered zinc and 40 mL of 1.25 N sodium hydroxide, connect

the flask to a reflux condenser that is cooled with water at 5° to 10°, add a few glass boiling beads, and gently reflux the mixture for 1 hour. Allow the flask to cool to room temperature, and rinse the condenser with 10 to 20 mL of water. Disconnect the flask from the condenser, add 5 mL of glacial acetic acid, mix, and allow the mixture to cool. Pass through general purpose fast-flowing filter paper mixture. Rinse the flask and the filter with 1 N acetic acid, and add the rinsings to the filtrate. Add 5 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.1 N silver nitrate VS, with continuous stirring, until the precipitate turns green. Each mL of 0.1 N silver nitrate is equivalent to 26.37 mg of  $C_{18}H_{24}I_3N_3O_8$ .

## Ioxilan Injection

» Ioxilan Injection is a sterile solution of Ioxilan in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of Ioxilan ( $C_{18}H_{24}I_3N_3O_8$ ) as organically bound iodine. It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. Ioxilan Injection contains no antimicrobial agents.

**Packaging and storage**—Preserve injection in single-dose containers of Type I glass, protected from light.

**Labeling**—Label containers of the Injection to direct the user to discard any unused portion. The label states that it is not to be used if it is discolored or contains a precipitate and states also that it is not for intrathecal use.

#### USP Reference standards <11>—

USP Endotoxin RS

USP Ioxilan RS

#### Identification—

**A:** Evaporate a volume of Injection, equivalent to about 500 mg of Ioxilan, to dryness. Char the residue so obtained in a suitable crucible: violet vapors are evolved (*presence of iodine*).

**B:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the major peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 0.2 USP Endotoxin Unit per 50 mg of iodine.

**Heavy metals, Method II** <231>: not more than 0.002%.

**pH** <791>: between 6.0 and 7.5.

**Free iodine**—Transfer an accurately measured volume of Injection, equivalent to about 1 g of Ioxilan, to a tared 25-mL volumetric flask, add 13 mL of water, and swirl to dissolve. Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene. Stopper the flask, and shake vigorously for 1 minute. Allow layers to separate: the toluene layer shows no red color. Reserve the contents of the flask for use as the test solution in the test for *Free iodide*.

**Free iodide**—Proceed as directed in the test for *Free iodide* under *Ioxilan*. The limit is 200  $\mu$ g of iodide per mL.

#### Residual methanol—

**Standard stock solution, Internal standard solution, Standard solutions A, B, C, D, and Chromatographic system**—Proceed as directed in the test for *Residual methanol* under *Ioxilan*.

**Test solution**—Transfer an accurately measured volume of Injection, equivalent to about 1 g of Ioxilan, to a 10-mL volumetric flask. Add 1.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the test for *Residual methanol* under *Ioxilan*: not more than 0.005% is found.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation A**—Transfer about 60 mg of USP Ioxilan RS, to a 10-mL volumetric flask, and add 1 mL of water. [NOTE—Heat gently, if necessary, to effect dissolution. Cool to room temperature before proceeding.] Dilute with acetonitrile to volume, and mix.

**Standard preparation B**—Prepare a 1 in 10 dilution of *Standard preparation A* in *Mobile phase*.

**Assay preparation A**—Transfer an accurately measured volume of *Injection*, equivalent to about 60 mg of Ioxilan, to a 10-mL volumetric flask, add 1 mL of water, and swirl to mix. Dilute with acetonitrile to volume, and mix.

**Assay preparation B**—Prepare a 1 in 10 dilution of *Assay preparation A* in *Mobile phase*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm × 4.6-mm stainless steel column that contains packing L8. The column is maintained at 30°, and the flow rate is about 1 mL per minute. Chromatograph *Standard preparation A*, and record the peak responses as directed under *Procedure*: the resolution, *R*, between the two largest impurity peaks eluting immediately after the second Ioxilan isomer peak is not less than 0.3. Chromatograph *Standard preparation B*, and record the peak responses as directed under *Procedure*: the resolution, *R*, between the two Ioxilan isomer peaks is not less than 2.2, the column efficiency, based on the larger Ioxilan isomer peak, is not less than 4000 theoretical plates, and the relative standard deviation of the sums of the responses of the two Ioxilan isomer peaks from replicate injections is not less than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of *Assay preparation A*, *Assay preparation B*, *Standard preparation A*, and *Standard preparation B* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Record the integrated results, summing the areas of the two Ioxilan peaks. From the chromatogram of *Assay preparation A* in comparison to that of *Standard preparation B*, determine the quantity of Ioxilan in the *Injection* taken. Determine the percentage of impurities (excluding the serinol impurity with a relative retention time of 0.9 relative to the larger Ioxilan isomer peak) by area percent, using the chromatogram of *Assay preparation A*. Not more than 0.5% of any individual impurity is found, and the total of all impurities is not more than 1.5%. [NOTE—The impurities eluting on the tail of the second Ioxilan isomer peak should be integrated by skimming the peaks.]

## Ipecac

» Ipecac consists of the dried rhizome and roots of *Cephaelis acuminata* Karsten, or of *Cephaelis ipecacuanha* (Brotero) A. Richard (Fam. Rubiaceae).

Ipecac yields not less than 2.0 percent of the total ether-soluble alkaloids of Ipecac. Its content of emetine (C<sub>29</sub>H<sub>40</sub>N<sub>2</sub>O<sub>4</sub>) and cephaeline (C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>) together is not less than 90.0 percent of the amount of the total ether-soluble alkaloids. The content of cephaeline varies from an

amount equal to, to an amount not more than 2.5 times, the content of emetine.

**USP Reference standards** (11)—

USP Emetine Hydrochloride RS

**Botanic characteristics**—A mixture of segments of the roots and rhizomes. The root segments are mostly curved and flexuous, occasionally branched, up to 15 cm in length and usually from 3 to 6.5 mm in diameter, but may be up to 9 mm in diameter, grayish, grayish brown, or reddish brown, the reddish brown type often having light-colored abrasions, transverse ridges about 0.5 to 1.0 mm wide that extend about halfway around the circumference of the root and fade at their tapering extremities into the general surface, with from 1 to 6 of these ridges per cm, and annulations sometimes seen at irregular intervals. The rhizomes are cylindrical, about 2 mm thick, finely longitudinally wrinkled, with a few elliptical scars. The odor is distinctive; the dust is sternutatory.

**Histology**—At the center of the root is a well-defined primary xylem but no pith. Surrounding this is a dense wood of secondary xylem crossed by medullary rays. These elements are all lignified. External to the wood is a narrow band of secondary phloem and a wide parenchymatous phelloderm surrounded by a narrow layer of cork a few cells thick. The secondary xylem consists of narrow, bordered-pitted tracheidal vessels and tracheids in combination with xylem parenchyma. The latter have simple pits and contain starch grains. Starch is present also in the medullary rays. The phloem occurs as small groups of sieve tissue embedded in parenchyma. The wide phelloderm consists of round-celled cellulose parenchyma filled with starch grains and a few idioblasts, each of which contains a bundle of acicular raphides of calcium oxalate crystals about 30 to 80 µm long. The starch grains are rarely single but usually occur as 2 to 4 and sometimes 8 in a clump. Individual grains measure up to 22 µm in diameter.

The rhizome differs from the root in having a ring of xylem around a large pith. The pericycle contains characteristic sclerenchymatous cells. Spiral vessels are found in the protoxylem. The pith is composed of pitted parenchyma, which is somewhat lignified.

**Overground stems**—The proportion of overground stems does not exceed 5%.

**Foreign organic matter** (561)—The proportion of foreign organic matter does not exceed 2.0%.

**Assay for total ether-soluble alkaloids**—[NOTE—It is important that the ether used in this assay shall have been shown by test to be free from peroxides within 24 hours prior to use.] The alkaloids may be extracted by either of the methods given in the following two paragraphs.

I—To 10 g of finely powdered Ipecac, in a suitable container, add 100 mL of ether, accurately measured at 25°, insert the stopper in the container tightly, shake the mixture thoroughly, and allow it to stand for 5 minutes. Then add 10 mL of 6 N ammonium hydroxide, close again tightly, shake it for 1 hour in a mechanical shaker or intermittently during 2 hours, and allow to stand overnight at a temperature not exceeding 25°. Again shake the mixture intermittently during 30 minutes, and allow the drug to settle at 25°. Transfer to a separator a 50.0-mL aliquot of the clear, supernatant, representing 5 g of Ipecac.

II—Place 5 g of the finely powdered Ipecac in a continuous-extraction thimble. Add enough ether to cover the powder, and allow to stand for 10 minutes with occasional stirring. Add 3 mL of ammonium hydroxide, mix, and allow to stand overnight. Cover the drug with a pledget of cotton, pack well, and extract with ether for 5 hours. Transfer the ether extract to a separator.

Extract the alkaloids from the ether with 2 N sulfuric acid, using at first 15 mL, or more, if necessary, to ensure an acid reaction, then successive 10-mL portions until extraction is complete, and filtering all extracts through the same filter

into a second separator. To the combined acid solutions add about an equal volume of ether, render the mixture distinctly alkaline with 6 N ammonium hydroxide (at least pH 10, by test paper), and extract with successive portions of ether until the last extract shows not more than a slight turbidity when treated as follows: Evaporate 1 mL of the last extraction, dissolve the residue in 0.5 mL of 0.5 N hydrochloric acid, and add 1 drop of mercuric iodide TS.

Filter each portion of the ether extract into a flask or beaker, and carefully evaporate the combined ether extracts on a steam bath almost to dryness. Add 5 mL of ether and 10.0 mL of 0.1 N sulfuric acid VS, and heat on a steam bath to dissolve the alkaloids and to remove all the ether. Cool, add 15 mL of water, then add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sulfuric acid is equivalent to 24.0 mg of the total ether-soluble alkaloids of ipecac, calculated as emetine ( $C_{29}H_{40}N_2O_4$ ).

#### Assay for emetine and cephaeline—

**Standard preparation**—Accurately weigh a suitable quantity of USP Emetine Hydrochloride RS, and dissolve in 0.5 N sulfuric acid. Dilute quantitatively and stepwise with the same dilute sulfuric acid to obtain a solution having a known concentration equivalent to about 50 µg of emetine per mL.

**Assay preparation**—Prepare a *Test Sample* as directed in *Methods of Analysis* under *Articles of Botanical Origin* (561). Transfer to a 150-mL beaker about 200 mg, accurately weighed, of the fine powder. Add 2 mL of dimethyl sulfoxide, mix with a flattened stirring rod to assure complete wetting of the powder, and allow to stand for about 30 minutes. Add 2 mL of water and about 1 g of sodium bicarbonate, and mix.

**Phosphate buffer**—Prepare approximately 0.5 M solutions of monobasic potassium phosphate (containing 5.1 g per 75 mL) and dibasic potassium phosphate (containing 2.2 g per 25 mL). Mix 3 volumes of 0.5 M monobasic potassium phosphate with 1 volume of 0.5 M dibasic potassium phosphate, and adjust by the addition of one or the other of the solutions to a pH of  $6.0 \pm 0.05$ . Dissolve 7.5 g of potassium chloride in 100 mL of the resulting solution.

**Citric acid buffer**—Prepare approximately 0.5 M solutions of sodium citrate (containing 6.5 g per 50 mL) and citric acid (containing 4.8 g per 50 mL). Mix equal volumes of these solutions, and adjust by addition of one or the other of the solutions to a pH of  $4.0 \pm 0.05$ .

**Chromatographic columns**—For each column, pack a pledget of fine glass wool in the base of a chromatographic tube (25- × 200-mm test tube to which is fused a 5-cm length of 7-mm tubing) with the aid of a tamping rod having a disk with a diameter about 1 mm less than that of the tube.

Prepare *Column I* as follows. To the *Assay preparation* add 6 g of purified siliceous earth, mix, transfer the mixture to the column, scrub the beaker with about 1 g of the purified siliceous earth, transfer this to the top of the column, and tamp. Prepare *Column II* using 3 g of the purified siliceous earth and 2 mL of *Phosphate buffer*; prepare *Column III* using 2 mL of *Citric acid buffer* and 3 g of the purified siliceous earth; and prepare *Column IV* using 2 mL of sodium hydroxide solution (1 in 50) and 3 g of the purified siliceous earth. Pack a pledget of glass wool on the top of each column.

**Procedure**—[NOTE—Use water-saturated solvents throughout this procedure. Rinse the tips of the chromatographic columns before discarding them.] Mount *Columns I* and *II* so that the effluent from *Column I* flows onto *Column II*. Pass three 50-mL portions of ether through the columns, and discard *Column I* and the eluate. Mount *Column III* below *Column II* and pass three 50-mL portions of a mixture of 1 volume of ether and 3 volumes of chloroform through the columns. Discard *Column II* and the eluate. Wash *Column III* with 25 mL of the ether-chloroform mixture, followed by 25 mL of a mixture of equal volumes of ether and isooctane,

and discard the washings. Wash *Column IV* with 20 mL of a 1 in 50 solution of triethylamine in the ether-isooctane mixture, and discard the washing. Mount *Column IV* below *Column III*, and place as a receiver under *Column IV* a 125-mL separator containing 15 mL of 4 N sulfuric acid. Pass through the columns 10 mL of a 1 in 5 solution of triethylamine in the ether-isooctane mixture, followed by three 10-mL portions of a 1 in 50 solution of triethylamine in the ether-isooctane mixture. Discard *Column III*, and pass through *Column IV* 20 mL of the 1 in 50 solution of triethylamine in the ether-isooctane mixture. Shake the separator, allow the phases to separate, and transfer the aqueous extract to a 50-mL volumetric flask. Extract with two additional 10-mL portions of 0.5 N sulfuric acid, combining the extracts in the volumetric flask. Add 0.5 N sulfuric acid to volume, and mix (*emetine solution*).

Elute *Column IV* with 75 mL of chloroform, collecting the eluate in a 250-mL separator containing 150 mL of ether. Discard *Column IV*. Extract with one 20-mL and then with two 10-mL portions of 0.5 N sulfuric acid, collecting the extracts in a 50-mL volumetric flask. Rinse the stem of the separator, add the acid to volume, and mix (*cephaeline solution*).

Concomitantly determine the absorbances of the *emetine solution*, the *cephaeline solution*, and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 283 nm and at 350 nm, with a suitable spectrophotometer, using 0.5 N sulfuric acid as the blank.

Calculate the quantity, in mg, of emetine in the portion of Ipecac taken by the formula:

$$0.05C(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

in which C is the concentration, in µg per mL, of emetine in the *Standard preparation*; and the parenthetical expressions are the differences in the absorbances of the solution of emetine from the *Assay preparation* (U) and the *Standard preparation* (S), respectively, at the wavelengths indicated by the subscripts.

Calculate the quantity, in mg, of cephaeline in the portion of Ipecac taken by the formula:

$$0.971(0.05C)(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

in which 0.971 is the ratio of the molecular weight of cephaeline to that of emetine; C is as defined above; and the parenthetical expressions are the differences in the absorbances of the solution of cephaeline from the *Assay preparation* (U) and the *Standard preparation* (S), respectively, at the wavelengths indicated by the subscripts.

### Powdered Ipecac

» Powdered Ipecac is Ipecac reduced to a fine or a very fine powder and adjusted to a potency of not less than 1.9 percent and not more than 2.1 percent of the total ether-soluble alkaloids of ipecac, by the addition of exhausted marc of ipecac or of other suitable inert diluent or by the addition of powdered ipecac of either a lower or a higher potency.

The content of emetine ( $C_{29}H_{40}N_2O_4$ ) and cephaeline ( $C_{28}H_{38}N_2O_4$ ) together is not less than 90.0 percent of the total amount of the ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount not more than 2.5 times, the content of emetine.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Emetine Hydrochloride RS

**Botanic characteristics**—Thin-walled, fairly small cork cells, the starch grains rarely simple and usually 2- to 8-compound, the single grains up to 22  $\mu\text{m}$  in diameter; raphides of calcium oxalate 30 to 80  $\mu\text{m}$  in length; tracheids and tracheidal vessels found in groups having very numerous, small, bordered pits; parenchyma of phelloderm filled with starch or acicular crystals of calcium oxalate, having cells thin-walled, oval with intercellular spaces; parenchyma of the xylem composed of small rectangular and longitudinally elongated cells with moderately thick walls and scattered bordered or simple pits; rhizome parenchyma cells larger than root parenchyma cells, with slightly thicker walls and lignified with fairly numerous simple pits; sclereids from the rhizome large, rectangular, with uneven walls and large, conspicuous pits.

**Assay for total ether-soluble alkaloids**—Proceed with Powdered Ipecac as directed in the *Assay for total ether-soluble alkaloids* under *Ipecac*.

**Assay for emetine and cephaeline—**

*Standard preparation, Phosphate buffer, Citric acid buffer, and Chromatographic columns*—Prepare as directed in the *Assay for emetine and cephaeline* under *Ipecac*.

*Assay preparation*—Transfer to a 150-mL beaker about 200 mg, accurately weighed, of Powdered Ipecac. Add 2 mL of dimethyl sulfoxide, mix with a flattened stirring rod to ensure complete wetting of the powder, and allow to stand for about 30 minutes. Add 2 mL of water and about 1 g of sodium bicarbonate, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*. Calculate the quantity, in mg, of emetine in the portion of Powdered Ipecac taken by the formula:

$$0.05C(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

in which the parenthetical expressions are the differences in the absorbances of the solution of emetine from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts; and *C* is as defined for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*. Calculate the quantity, in mg, of cephaeline in the portion of Powdered Ipecac taken by the formula:

$$0.971(0.05C)(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

in which 0.971 is the ratio of the molecular weight of cephaeline to that of emetine; the parenthetical expressions are the differences in the absorbances of the solution of cephaeline from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts; and *C* is as defined above.

## Ipecac Oral Solution

» Ipecac Oral Solution yields, from each 100 mL, not less than 123 mg and not more than 157 mg of the total ether-soluble alkaloids of ipecac.

The content of emetine ( $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_4$ ) and cephaeline ( $\text{C}_{28}\text{H}_{38}\text{N}_2\text{O}_4$ ) together is not less than 90.0 percent of the amount of the total ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount not more than 2.5 times, the content of emetine.

Powdered Ipecac . . . . .	70 g
Glycerin . . . . .	100 mL
Syrup, a sufficient quantity, to make	1000 mL

Exhaust the powdered Ipecac by percolation, using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum, macerating for 72 hours, and percolating slowly. Reduce the entire percolate to a volume of 70 mL by evaporation at a temperature not exceeding 60° and preferably in vacuum, and add 140 mL of water. Allow the mixture to stand overnight, filter, and wash the residue on the filter with water. Evaporate the filtrate and washings to 40 mL, and to this add 2.5 mL of hydrochloric acid and 20 mL of alcohol, mix, and filter. Wash the filter with a mixture of 30 volumes of alcohol, 3.5 volumes of hydrochloric acid, and 66.5 volumes of water, using a volume sufficient to produce 70 mL of the filtrate. Add 100 mL of Glycerin and enough Syrup to make the product measure 1000 mL, and mix.

**Packaging and storage**—Preserve in tight containers, preferably at a temperature not exceeding 25°. Containers intended for sale to the public without prescription contain not more than 30 mL of Oral Solution.

**USP Reference standards** (11)—

USP Emetine Hydrochloride RS

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Escherichia coli*.

**Alcohol content** (611): between 1.0% and 2.5% of  $\text{C}_2\text{H}_5\text{OH}$ .

**Assay for total ether-soluble alkaloids**—[NOTE—It is important that the ether used in this assay shall have been shown by test to be free from peroxides within 24 hours prior to use.] Transfer about 50 mL, accurately measured, of Oral Solution to a liquid-liquid automatic extractor, add water, if necessary, to reduce the viscosity, render the liquid distinctly alkaline with ammonium hydroxide, and extract with ether for at least 4 hours or until the extraction is complete. Use a water bath to boil the ether. Frequently disconnect the extractor from the condenser, and agitate the lower layer by raising and lowering the center tube or by other suitable manipulation. At the conclusion of the extraction period, transfer the ether extract to a separator, and rinse the extraction flask with 2 or more small volumes of ether, adding the rinsings to the separator. Complete the assay as directed in the *Assay for total ether-soluble alkaloids* under *Ipecac*, beginning with "Extract the alkaloids from the ether."

**Assay for emetine and cephaeline—**

*Standard preparation, Phosphate buffer, and Citric acid buffer*—Prepare as directed in the *Assay for emetine and cephaeline* under *Ipecac*.

*Assay preparation*—Pipet 10 mL of water into a 25-mL volumetric flask. With the aid of a 20-mL pipet, add Oral Solution to volume, taking care to prevent contact of the Oral Solution with the neck of the flask above the graduation line. Insert the stopper, and mix.

*Chromatographic columns*—Pack a pledget of fine glass wool in the base of a chromatographic tube (25-mm  $\times$  200-mm test tube to which is fused a 5-cm length of 7-mm tubing) with the aid of a tamping rod having a disk with a diameter about 1 mm less than that of the tube.

To prepare *Column I*, transfer 4.0 mL of the *Assay preparation* to a 150-mL beaker, add about 1 g of sodium bicarbonate, and mix. Then proceed as directed for *Chromatographic columns* in the *Assay for emetine and cephaeline* under *Ipecac*, beginning with "add 6 g of purified siliceous earth," and prepare *Columns II, III, and IV* as directed therein.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*.

Calculate the quantity, in mg, of emetine in each 100 mL of Oral Solution taken by the formula:

$$2.08C(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

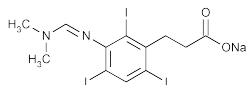
in which the parenthetic expressions are the differences in the absorbances of the solution of emetine from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts, and *C* is as defined in the *Procedure*.

Calculate the quantity, in mg, of cephaeline in each 100 mL of Oral Solution taken by the formula:

$$0.971(2.08C)(A_{283} - A_{350})_U / (A_{283} - A_{350})_S$$

in which 0.971 is the ratio of the molecular weight of cephaeline to that of emetine, the parenthetic expressions are the differences in the absorbances of the solution of cephaeline from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts, and *C* is as defined in the *Procedure*.

## Ipodate Sodium



$C_{12}H_{12}I_3N_2NaO_2$  619.94

Benzenepropanoic acid, 3-[[(dimethylamino)methylene]amino]-2,4,6-triiodo-, sodium salt.

Sodium 3-[[[(dimethylamino)methylene]amino]-2,4,6-triiodohydrocinnamate [1221-56-3].

» Ipodate Sodium contains not less than 97.5 percent and not more than 102.5 percent of  $C_{12}H_{12}I_3N_2NaO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Ipodate Sodium RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* methanol.

Absorptivities at 235 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** Heat about 500 mg in a porcelain crucible over a free flame: violet vapors of iodine are evolved.

**D:** It responds to the flame test for *Sodium* (191).

**Loss on drying** (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 0.5% of its weight.

**Iodide or iodine**—Dissolve about 200 mg in 10 mL of 6 N acetic acid, add 2 mL of 1 N sulfuric acid and 15 mL of chloroform, and shake vigorously. Allow the layers to separate: the chloroform layer shows not more than a faint violet color. Add 1 mL of 0.1 N potassium iodate, shake vigorously, and allow the layers to separate: the chloroform layer shows at most a slight trace of violet color.

**Heavy metals, Method II** (231): 0.003%.

**Assay**—Transfer about 300 mg of Ipodate Sodium, accurately weighed, to a 250-mL flask, add 30 mL of 1.25 N sodium hydroxide and 0.5 g of powdered zinc, and reflux the mixture for 60 minutes. Cool, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 3 mL of a mixture of 2 drops of nitric acid in 5 mL of water, then add 3 drops of eosin Y TS, and titrate with 0.05 N silver nitrate VS until the entire mixture changes to a permanent pink color. Each mL of 0.05 N silver nitrate is equivalent to 10.33 mg of  $C_{12}H_{12}I_3N_2NaO_2$ .

## Ipodate Sodium Capsules

» Ipodate Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{12}H_{12}I_3N_2NaO_2$ .

**Packaging and storage**—Preserve in tight containers.

**Identification**—

**A:** Transfer a portion of the contents of Capsules, equivalent to about 2 g of ipodate sodium, to a 250-mL separator, add 100 mL of water and 50 mL of solvent hexane, and shake. Transfer the aqueous layer to a beaker, add 5 mL of 3 N hydrochloric acid, and mix. Filter (retain the filtrate), and wash the precipitate with several portions of water. Dry the precipitate in vacuum at 60° for 4 hours. A 1 in 100,000 solution of the residue so obtained, in a 1 in 100 mixture of 2 N hydrochloric acid in methanol, exhibits an UV absorbance maximum at  $242 \pm 2$  nm.

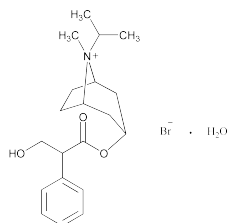
**B:** The residue obtained in *Identification* test A responds to *Identification* test C under *Ipodate Sodium*.

**C:** The filtrate obtained in *Identification* test A responds to the flame test for *Sodium* (191).

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Place a number of Capsules, equivalent to about 5 g of ipodate sodium, in a 400-mL beaker, add 200 mL of 1 N sodium hydroxide and 50 mL of solvent hexane, and stir by mechanical means until the capsules have completely disintegrated. Transfer the mixture to a 500-mL separator, wash the beaker with a total of 25 mL of 1 N sodium hydroxide in divided portions, and add the washings to the separator. Allow the layers to separate, and transfer the aqueous layer to a 500-mL volumetric flask. Wash the solvent hexane layer with two 50-mL portions of 1 N sodium hydroxide, add the washings to the volumetric flask, dilute with 1 N sodium hydroxide to volume, and mix. Pipet 25 mL of the solution, which may be milky in appearance, into a 250-mL conical flask, add 500 mg of powdered zinc, and proceed as directed in the *Assay* under *Ipodate Sodium*, beginning with "and reflux the mixture for 60 minutes."

## Ipratropium Bromide



$C_{20}H_{30}BrNO_3 \cdot H_2O$  430.38

8-Azoniabicyclo[3.2.1]octane-3-(3-hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methylethyl)-, bromide, monohydrate(*endo, syn*)-, (±)-  
(8*r*)-3 $\alpha$ -Hydroxy-8-isopropyl-1 $\alpha$ H,5 $\alpha$ H-tropanium bromide (±)-tropate monohydrate [66985-17-9].

» Ipratropium Bromide contains not less than 98.0 percent and not more than 102.0 percent of  $C_{20}H_{30}BrNO_3 \cdot H_2O$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, and store at room temperature.

### USP Reference standards (11)—

USP Ipratropium Bromide RS

USP Ipratropium Bromide Related Compound A RS  
(1*R*,3*r*,5*S*,8*r*)-3-Hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane, bromide.

$C_{11}H_{22}BrNO$  264.20

USP Ipratropium Bromide Related Compound B RS  
(1*R*,3*r*,5*S*,8*s*)-3-[[[(2*RS*)-3-Hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane, bromide.

$C_{20}H_{30}BrNO_3$  412.36

USP Ipratropium Bromide Related Compound C RS  
(2*RS*)-3-Hydroxy-2-phenylpropanoic acid.

$C_9H_{10}O_3$  166.17

### Identification—

**A:** Infrared Absorption (197M).

**B:** A solution (1 in 100) meets the requirements of the test for Bromide (191).

**C:** The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**pH** (791): between 5.0 and 7.0, in a solution (1 in 10).

**Water**, Method I (921): between 3.9% and 4.4%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, Method II (231): 0.001%.

### Limit of ipratropium related compound A—

**Adsorbent**—Use a suitable high-performance thin-layer chromatographic silica gel mixture.

**Test solution**—Dissolve about 100 mg of Ipratropium Bromide, accurately weighed, in 5 mL of methanol.

**Stock standard solution**—Dissolve an accurately weighed quantity of USP Ipratropium Bromide Related Compound A RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

**Standard solutions**—In separate flasks, dilute 0.5, 1.0, and 2.0 mL of the Stock standard solution with methanol to 5 mL

to obtain a set of standard solutions having known concentrations of approximately 0.005, 0.01, and 0.02 mg of ipratropium bromide per mL. These solutions correspond to 0.025% (Standard solution C), 0.05% (Standard solution B), and 0.1% (Standard solution A), respectively, relative to the Test solution.

**Application volume:** 1  $\mu$ L.

**Developing solvent system**—Prepare a mixture of methylene chloride, dehydrated alcohol, water, and formic acid (18:18:3:1).

**Procedure**—Proceed as directed for Thin-Layer Chromatography under Chromatography (621). Develop the plate for about 15 minutes, then remove from the tank and dry at 60° for 15 minutes. Spray the plate with the Dragendorff's TS, and allow to dry briefly. Spray the plate with sodium nitrite solution (5 in 100), and immediately cover with a glass plate. The  $R_f$  for ipratropium bromide related compound A and ipratropium bromide are about 0.15 and 0.36, respectively. Any spot in the chromatogram obtained from the Test solution, except for the principal spot, is not more intense than the spot in the chromatogram obtained from Standard solution A (0.1%).

### Related compounds—

**Phosphate solution, Buffer, Mobile phase, and Chromatographic system**—Proceed as directed in the Assay.

**System suitability solution**—Dissolve suitable quantities of USP Ipratropium Bromide RS and USP Ipratropium Bromide Related Compound B RS in Mobile phase to obtain a solution containing about 0.03 mg per mL and 0.01 mg per mL, respectively.

**Standard solution**—Dissolve an accurately weighed quantity of USP Ipratropium Bromide RS in Mobile phase and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.03 mg per mL.

**Test solution**—Transfer about 250 mg of Ipratropium Bromide, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

**Chromatographic system** (see Chromatography (621))—Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention times are listed in the accompanying table; the resolution,  $R$ , between ipratropium and ipratropium related compound B is not less than 4; the tailing factor of the ipratropium peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 5%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of related compounds in the portion of Ipratropium Bromide taken by the formula:

$$100(1/F)(C_s / C_T)(r_i / r_s)$$

in which  $F$  is the relative response factor of the related compound relative to ipratropium bromide;  $C_s$  is the concentration, in mg per mL, of USP Ipratropium Bromide RS in the Standard solution;  $C_T$  is the concentration, in mg per mL, of Ipratropium Bromide in the Test solution;  $r_i$  is the individual peak response of the individual related compound; and  $r_s$  is the peak response of ipratropium in the Standard solution. See the accompanying table for relative retention times, relative response factors, and acceptance criteria.

Related Compound	Relative Retention Time	Relative Response Factor	Limit (%)
Ipratropium related compound C <sup>1</sup>	0.7	3.8	0.10
Ipratropium bromide	1.0	1.0	—
Ipratropium related compound B [(8 <i>s</i> )-ipratropium bromide] <sup>2</sup>	1.3	1.0	0.10
<i>N</i> -isopropylnoratropinium bromide <sup>3</sup>	2.3	1.0	0.10
Apo-ipratropium bromide <sup>4</sup>	5.1	2.0	0.10
Any individual unknown impurity	—	—	0.10
Total impurities	—	—	0.25

<sup>1</sup>(2*RS*)-3-Hydroxy-2-phenylpropanoic acid.

<sup>2</sup>(1*R*,3*r*,5*S*,8*s*)-3-[[[(2*RS*)-3-Hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane, bromide.

<sup>3</sup>(1*R*,3*r*,5*S*)-8-(1-Methylethyl)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate.

<sup>4</sup>(1*R*,3*r*,5*S*,8*r*)-8-Methyl-8-(1-methylethyl)-3-[(2-phenylpropenyl)oxy]-8-azoniabicyclo[3.2.1]octane.

### Assay—

**Phosphate solution**—Transfer 8.9 g of dibasic sodium phosphate dihydrate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

**Buffer**—Transfer 14.3 g of monobasic sodium phosphate dihydrate and 2.0 g of tetrapropylammonium chloride to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix. Adjust with *Phosphate solution* to a pH of 5.5 ± 0.2. Pass through a nylon membrane filter having a porosity of 0.45 μm or finer.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer* and methanol (87:13). [NOTE—Do not use the *Mobile phase* after 36 hours.] Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve suitable quantities of USP Ipratropium Bromide RS and USP Ipratropium Related Compound C RS in *Mobile phase* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL and 0.1 mg per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ipratropium Bromide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer about 50 mg of Ipratropium Bromide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and 3.9-mm × 15-cm column that contains 4-μm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 30°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for ipratropium related compound C and 1.0 for ipratropium bromide; the resolution, *R*<sub>s</sub>, between ipratropium related compound C and ipratropium is not less than 4; the tailing factor of the ipratropium peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1%.

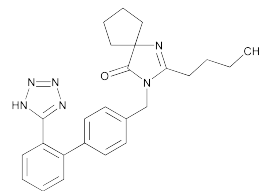
**Procedure**—Separately inject equal volumes (about 5 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in percent, of C<sub>20</sub>H<sub>30</sub>BrNO<sub>3</sub> · H<sub>2</sub>O in the portion of Ipratropium Bromide taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which *C*<sub>S</sub> is the concentration, in mg per mL, of USP Ipratropium Bromide RS in the *Standard preparation*; *C*<sub>T</sub> is the concentration, in mg per mL, of Ipratropium Bromide in the *Assay preparation*; and *r*<sub>U</sub> and *r*<sub>S</sub> are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Irbesartan



C<sub>25</sub>H<sub>28</sub>N<sub>6</sub>O 428.53

1,3-Diazaspiro[4.4]non-1-en-4-one, 2-butyl-3-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-. 2-Butyl-3-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one [138402-11-6].

» Irbesartan contains not less than 98.0 percent and not more than 102.0 percent of C<sub>25</sub>H<sub>28</sub>N<sub>6</sub>O, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, and store at a temperature below 30°.

### USP Reference standards (11)—

USP Irbesartan RS

USP Irbesartan Related Compound A RS

1-Pentanoylamino-cyclopentanecarboxylic acid [2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.

C<sub>25</sub>H<sub>30</sub>N<sub>6</sub>O<sub>2</sub> 446.54

### Identification—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water, Method I** (921): not more than 0.5%.

**Heavy metals, Method II** (231): 0.002%

### Limit of azide—

**Mobile phase**—Prepare a filtered and degassed 0.1 N sodium hydroxide solution (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 25 mg of sodium azide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 250 μL of this solution into a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.312 μg of sodium azide per mL.

**Test solution**—Transfer about 100 mg of Irbesartan, accurately weighed, to a 5-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductimetric detector [NOTE—A suitable background suppression unit may be used.] and a 4.0-mm × 25-cm column that contains packing L31. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak

responses as directed for *Procedure*: the signal-to-noise ratio for the azide peak is not less than 10.

**Procedure**—Separately inject equal volumes (about 200  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for azide. Calculate the amount of azide, in ppm, in the portion of Irbesartan taken by the formula:

$$1000(C_S / C_T)(42.02/65.01)(r_U / r_S)$$

in which  $C_S$  is the concentration, in  $\mu$ g per mL, of sodium azide in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Irbesartan in the *Test solution*;  $r_U$  is the peak area for azide obtained from the *Test solution*; and  $r_S$  is the peak area for azide obtained from the *Standard solution*: not more than 10 ppm of azide is found.

#### Related compounds—

**pH 3.2 Phosphate buffer and Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Prepare as directed for the *System suitability solution* in the *Assay*.

**Test solution**—Dissolve an accurately weighed quantity of Irbesartan in methanol to obtain a solution having a known concentration of about 1 mg per mL.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*. Chromatograph the *Standard solution* and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area for the irbesartan related compound A peak. Calculate the percentage of irbesartan related compound A in the portion of Irbesartan taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Irbesartan Related Compound A RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Irbesartan in the *Test solution*;  $r_U$  is the peak response for irbesartan related compound A obtained from the *Test solution*; and  $r_S$  is the peak response for irbesartan related compound A obtained from the *Standard solution*.

Calculate the percentage of other impurities in the portion of Irbesartan taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Irbesartan RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Irbesartan in the *Test solution*; and  $r_U$  and  $r_S$  are the peak responses for each of the other impurities and USP Irbesartan RS obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.2% of irbesartan related compound A is found; not more than 0.1% of any other impurity is found; and not more than 0.5% of total impurities is found.

#### Assay—

**pH 3.2 Phosphate buffer**—Mix 5.5 mL of phosphoric acid with about 950 mL of water, and adjust pH to 3.2 with triethylamine.

**Mobile phase**—Prepare a filtered and degassed mixture of pH 3.2 phosphate buffer and acetonitrile (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**System suitability solution**—Dissolve accurately weighed quantities of USP Irbesartan RS and USP Irbesartan Related Compound A RS in methanol to obtain a solution having a

known concentration of about 0.05 mg per mL of each USP Reference Standard.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Irbesartan RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer about 50 mg of Irbesartan, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.0-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for irbesartan related compound A and 1.0 for irbesartan; the resolution,  $R$ , between irbesartan and irbesartan related compound A is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of  $C_{25}H_{28}N_6O$  in the portion of Irbesartan taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Irbesartan RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Irbesartan Tablets

» Irbesartan Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of irbesartan ( $C_{25}H_{28}N_6O$ ).

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards <11>—

USP Irbesartan RS

USP Irbesartan Related Compound A RS

1-Pentanoylamino-cyclopentanecarboxylic acid [2'-(1H-tetrazol-5-yl)-byphenyl-4-ylmethyl]-amide.

$C_{25}H_{30}N_6O_2$  446.54

#### Identification—

**A: Infrared Absorption** <197K>—

**Test specimen:** Transfer 1 Tablet into a suitable vial. Add 10 mL of methanol, and sonicate for 10 minutes. Pass the solution through a glass microfiber membrane filter having a 0.45- $\mu$ m or finer porosity, and evaporate to dryness, using a stream of nitrogen. Mix approximately 1 mg of the residue with approximately 250 mg of potassium bromide, and mix well to obtain a homogenous mixture. The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a potassium bromide dispersion of a similar preparation using USP Irbesartan RS.

**B:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.



**Dissolution** <711>—

*Medium:* 0.1 N hydrochloric acid; 1000 mL.

*Apparatus 2:* 50 rpm.

*Time:* 20 minutes.

*Procedure*—Determine the amount of  $C_{25}H_{28}N_6O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 244 nm on portions of the solution under test, passed through a 0.45- $\mu$ m filter of acrylic copolymer on a nylon support,\* and suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Irbesartan RS in the same *Medium*. Calculate the amount of  $C_{25}H_{28}N_6O$  dissolved, in percentage, by the formula:

$$\frac{A_U \times C_S \times 1000 \times 100}{A_S \times L}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the solution under test and the Standard solution, respectively;  $C_S$  is the concentration, in mg per mL, of the Standard solution; 1000 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $L$  is the label claim, in mg, of irbesartan.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{25}H_{28}N_6O$  is dissolved in 20 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Related compounds**—

*Buffer solution, Mobile phase, System suitability preparation, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Use the *Standard preparation*, prepared as directed in the Assay.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Inject a volume of about 15  $\mu$ L of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 0.2% of irbesartan related compound A is found; not more than 0.2% of any individual impurity is found; and not more than 0.5% of total impurities is found.

**Assay**—

*Buffer solution*—Dilute about 5.5 mL of phosphoric acid in approximately 950 mL of water, and add triethylamine, slowly and dropwise, to adjust to a pH of 3.0. Further dilute this solution with water to a final volume of 1 L.

*Mobile phase*—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*System suitability preparation*—Dissolve accurately weighed quantities of USP Irbesartan RS and USP Irbesartan Related Compound A RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL of each of the USP Reference Standards.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Irbesartan RS in methanol to obtain a solution having a known concentration of about 0.15 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 5 Tablets. Transfer an accurately weighed portion of

the powder, equivalent to about 15 mg of irbesartan, to a 100-mL volumetric flask. Add methanol up to about three-fourths of the volume of the flask. Sonicate for 15 minutes, with stirring at 5-minute intervals. Dilute with methanol to volume, and pass a portion of this solution through a glass microfiber membrane filter having a 0.45- $\mu$ m or finer porosity.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between irbesartan and irbesartan related compound A is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for five replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 15  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of irbesartan ( $C_{25}H_{28}N_6O$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Irbesartan RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Irbesartan and Hydrochlorothiazide Tablets

**DEFINITION**

Irbesartan and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of irbesartan ( $C_{25}H_{28}N_6O$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

**IDENTIFICATION**

- A. INFRARED ABSORPTION** <197K>

**Sample solution:** Transfer the contents of 1 Tablet, previously crushed with a mortar and pestle, to a suitable vial. Add 5 mL of acetone, sonicate for 10 min, and pass through a membrane filter of 0.45- $\mu$ m pore size. Evaporate the filtrate to dryness.

- B.** The relative retention times of the major peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- PROCEDURE**

**Buffer:** Dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, add 2 mL of triethylamine, and adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ . Dilute further with water to 1 L.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (13:20:67)

**Acidified water:** Adjust with phosphoric acid (or sodium hydroxide, if necessary) to a pH of  $2.0 \pm 0.1$ .

**Extraction solution:** Methanol and *Acidified water* (7:3)

**Irbesartan standard stock solution:** Dissolve USP Irbesartan RS in a suitable volumetric flask in methanol (1/5 of the volume of the flask), and dilute with *Extraction solution* to prepare a 0.6 mg/mL solution. Sonicate for 2 min.

**Hydrochlorothiazide standard stock solution:** Dissolve USP Hydrochlorothiazide RS in methanol (1/20 of the volume of the flask), and dilute with *Extraction solution* to prepare a 0.1 mg/mL solution. Sonicate for 2 min.

\*A suitable filter is Acrodisc, manufactured by Gelman Sciences and distributed by Pall Corp. (www.pall.com).

**Irbesartan related compound A standard stock solution:** 0.1 mg/mL of USP Irbesartan Related Compound A RS in methanol. Sonicate for 2 min.

**Benzothiadiazine related compound A standard stock solution:** 0.05 mg/mL of USP Benzothiadiazine Related Compound A RS in methanol. Sonicate for 2 min.

**Standard solution:** 0.24 mg/mL of irbesartan and 0.02 mg/mL of hydrochlorothiazide from the *Irbesartan standard stock solution* and the *Hydrochlorothiazide standard stock solution* in the *Extraction solution*

**System suitability solution:** Prepare 0.05 mg/mL of USP Irbesartan RS, 0.005 mg/mL of USP Hydrochlorothiazide RS, 1.0 µg/mL of USP Irbesartan Related Compound A RS, and 3.0 µg/mL of USP Benzothiadiazine Related Compound A RS in the *Extraction solution* from the respective Standard stock solutions

**Sample stock solution:** 0.75 mg/mL of irbesartan from NLT 5 Tablets in a suitable volumetric flask. Add *Acidified water* up to 30% of the volume of the flask, and sonicate until the Tablets disintegrate. Add methanol to fill the flask up to 90% of the total volume. Sonicate for 5 min, and stir. Dilute with methanol to volume, and pass through a filter of 0.45-µm pore size.

**Sample solution:** 0.225 mg/mL of irbesartan in the *Extraction solution* from the *Sample stock solution*. [NOTE—The hydrochlorothiazide concentration may vary depending on the ratio of irbesartan to hydrochlorothiazide in the Tablet.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L10

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.7 between irbesartan and irbesartan related compound A; NLT 1.7 between hydrochlorothiazide and benzothiadiazine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 1.5% for both the irbesartan and hydrochlorothiazide peaks, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>25</sub>H<sub>28</sub>N<sub>6</sub>O and C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of irbesartan or hydrochlorothiazide from the *Sample solution*

$r_S$  = peak area of irbesartan or hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Irbesartan RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of irbesartan or hydrochlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** 0.1 N hydrochloric acid; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**pH 3.0 phosphate buffer:** 1.36 g/L of monobasic potassium phosphate in water. Adjust with 10% phosphoric acid to a pH of 3.0 ± 0.1. This solution is stable for 3 months.

**Mobile phase:** pH 3.0 phosphate buffer, methanol, and acetonitrile (45:35:20)

**Irbesartan standard stock solution:** Transfer 50 mg of USP Irbesartan RS to a 100-mL volumetric flask. Add 15 mL of methanol, and sonicate for 5 min. Dilute with *Medium* to volume. This solution is stable for 14 days when stored at 4°.

**Hydrochlorothiazide standard stock solution:** Transfer 20 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask. Add 5 mL of methanol, and sonicate for 5 min. Dilute with *Medium* to volume. This solution is stable for 14 days when stored at 4°.

**Standard solution:** Prepare on day of use dilutions of the *Irbesartan standard stock solution* and *Hydrochlorothiazide standard stock solution* in *Medium* as directed in the table below:

Label Claim of Irbesartan/ Hydrochlorothiazide (mg/Tablet)	Volume of the Irbesartan standard stock solution (mL)	Volume of the Hydrochlorothiazide standard stock solution (mL)	Final Volume (mL)
75/12.5	15	12.5	100
150/12.5	30	12.5	100
300/12.5	60	12.5	100
300/25	60	25.0	100

**System suitability solution:** Transfer 10 mg of USP Irbesartan Related Compound A RS to a 100-mL volumetric flask. Add 5 mL of methanol, and sonicate to dissolve. Dilute with *Medium* to volume. Transfer 10.0 mL of this solution, 5.0 mL of the *Irbesartan standard stock solution*, and 12.5 mL of the *Hydrochlorothiazide standard stock solution* to a 100-mL volumetric flask. Dilute with *Medium* to volume.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 272 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L10

**Column temperature:** 40°

**Flow rate:** 1.4 mL/min

**Injection volume:** 25 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between irbesartan and irbesartan related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for both the irbesartan and hydrochlorothiazide peaks, *Standard solution*

Calculate the percentage of irbesartan and hydrochlorothiazide dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response for irbesartan or hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response for irbesartan or hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of irbesartan or hydrochlorothiazide in the *Standard solution*

$L$  = label claim for irbesartan or hydrochlorothiazide (mg/Tablet)

$V$  = volume of *Medium* (mL), 1000

**Tolerances:** NLT 80% (Q) of the labeled amounts of irbesartan (C<sub>25</sub>H<sub>28</sub>N<sub>6</sub>O) and hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

**Buffer, Mobile phase, Acidified water, Extraction solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of irbesartan related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of irbesartan related compound A from the *Sample solution*  
 $r_S$  = peak response of irbesartan from the *Standard solution*

$C_S$  = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of irbesartan in the *Sample solution* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

$r_U$  = peak response of benzothiadiazine related compound A from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each other impurity in the *Sample solution*

$r_T$  = sum of the peak responses excluding hydrochlorothiazide and benzothiadiazine related compound A from the *Sample solution*

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.5% (sum of all the individual unknown impurities, irbesartan related compound A, and benzothiadiazine related compound A)

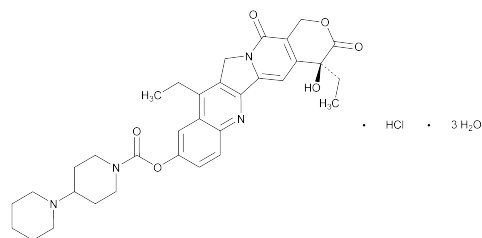
**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A	0.15	1.3	1.0
Hydrochlorothiazide	0.18	—	—
Irbesartan related compound A	0.86	1.0	0.3
Irbesartan	1.00	—	—
Any other individual, unidentified impurity	—	1.0	0.2

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Hydrochlorothiazide RS
  - USP Irbesartan RS
  - USP Irbesartan Related Compound A RS
  - 1-Pentanoylamino-cyclopentanecarboxylic acid [2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.  
C<sub>25</sub>H<sub>30</sub>N<sub>6</sub>O<sub>2</sub> 446.54
  - USP Benzothiadiazine Related Compound A RS
  - 4-Amino-6-chloro-1,3-benzenedisulfonamide.  
C<sub>6</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 285.73

## Irinotecan Hydrochloride



C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> · HCl · 3H<sub>2</sub>O

Anhydrous: 623.14

Trihydrate: 677.18

[1,4'-Bipiperidine]-1'-carboxylic acid, 4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ester, monohydrochloride, trihydrate, (S)-;  
 (+)-7-Ethyl-10-hydroxycamptothecin 10-[1,4'-bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate  
 [136572-09-3].

## DEFINITION

Irinotecan Hydrochloride contains NLT 98.0% and NMT 102.0% of C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> · HCl, calculated on the anhydrous basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to the irinotecan (S-enantiomer) peak in the *Identification solution*, as obtained in the test for *Limit of Irinotecan Hydrochloride Enantiomer*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A 2-mg/mL solution meets the requirements of the tests.

## ASSAY

### • PROCEDURE

**Solution A:** 2.8 g/L of monobasic sodium phosphate monohydrate and 1.8 g/L of 1-octanesulfonic acid sodium salt monohydrate in water

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (17:24:59)

**Diluent:** Use *Mobile phase* adjusted with diluted hydrochloric acid to a pH of 3.65 ± 0.15.

**Standard solution:** 1 mg/mL of USP Irinotecan Hydrochloride RS in *Diluent*

**Sample solution:** 1 mg/mL of Irinotecan Hydrochloride in *Diluent*

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 255 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 1.5 mL/min**Injection size:** 15 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of irinotecan hydrochloride ( $C_{33}H_{38}N_4O_6 \cdot HCl$ ) in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Irinotecan Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES**• **RESIDUE ON IGNITION** <281>: NMT 0.1%• **HEAVY METALS**, *Method II* <231>: NMT 10 ppm• **LIMIT OF IRINOTECAN HYDROCHLORIDE ENANTIOMER****Mobile phase:** Hexane, dehydrated alcohol, and diethylamine (250:250:1)**Diluent:** Dehydrated alcohol and diethylamine (250:1)**System suitability solution:** 0.1 mg/mL each of USP Irinotecan Hydrochloride RS and USP Irinotecan Related Compound D RS in *Diluent***Identification solution:** 1 mg/mL of USP Irinotecan Hydrochloride RS in *Diluent*. [NOTE—This solution is used for *Identification* test B.]**Standard solution:** 1.5 μg/mL of USP Irinotecan Related Compound D RS in *Diluent***Sensitivity solution:** 0.5 μg/mL of USP Irinotecan Related Compound D RS in *Diluent*, from the *Standard solution***Sample solution:** 1 mg/mL of Irinotecan Hydrochloride in *Diluent***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 370 nm**Column:** 4.6-mm × 25-cm; 10-μm packing L40**Flow rate:** 1.0 mL/min**Injection size:** 20 μL**System suitability****Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*[NOTE—The relative retention times for irinotecan related compound D (*R*-enantiomer) and irinotecan (*S*-enantiomer) are 0.7 and 1.00, respectively.]**Suitability requirements****Resolution:** NLT 2.5 between irinotecan related compound D and irinotecan, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Sensitivity:** The irinotecan related compound D peak should be visible, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of irinotecan hydrochloride *R*-enantiomer in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak area of irinotecan related compound D from the *Sample solution* $r_S$  = peak area of irinotecan related compound D from the *Standard solution* $C_S$  = concentration of USP Irinotecan Related Compound D RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria*****R*-enantiomer:** NMT 0.15%[NOTE—On the basis of the synthetic route, perform either *Organic Impurities Procedure 1* or *Organic Impurities Procedure 2*.]• **ORGANIC IMPURITIES PROCEDURE 1 (FOR MATERIAL LABELED AS PRODUCED BY A SYNTHETIC PROCESS)****Mobile phase, Diluent, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.**System suitability stock solution:** 0.01 mg/mL each of USP Irinotecan Related Compound B RS and USP Irinotecan Related Compound C RS in methanol**System suitability solution:** 1.0 μg/mL each of USP Irinotecan Related Compound B RS and USP Irinotecan Related Compound C RS in *Diluent*, from *System suitability stock solution***Standard solution:** 2.0 μg/mL of USP Irinotecan Hydrochloride RS in *Diluent***Sensitivity solution:** 0.5 μg/mL of USP Irinotecan Hydrochloride RS in *Diluent***System suitability****Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution***Suitability requirements****Resolution:** NLT 1.1 between irinotecan related compound B and irinotecan related compound C, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak area of each impurity from the *Sample solution* $r_S$  = peak area of irinotecan from the *Standard solution* $C_S$  = concentration of irinotecan hydrochloride in the *Standard solution* (mg/mL) $C_U$  = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria****Individual impurities:** See *Table 1*. [NOTE—Disregard any impurity peaks less than 0.05%.]**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Irinotecan related compound B	0.55	0.15
Irinotecan related compound C	0.60	0.10
Irinotecan hydrochloride	1.0	—

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	0.10
Total impurities	—	0.5

• **ORGANIC IMPURITIES PROCEDURE 2 (FOR MATERIAL LABELED AS PRODUCED BY A SEMI-SYNTHETIC PROCESS)**

**Solution A:** 2.72 g/L of monobasic potassium phosphate in water. Adjust with dilute phosphoric acid (1 in 20) to a pH of 3.5 ± 0.05.

**Solution B:** Acetonitrile and methanol (3:2)

**Mobile phase:** See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	30	70
45	30	70
50	80	20
55	80	20

**Diluent:** Acetonitrile, methanol, and Solution A (1:1:2)

**System suitability solution:** 0.1 mg/mL each of USP Irinotecan Hydrochloride RS and USP Irinotecan Related Compound A RS in Diluent

**Standard solution:** 1 µg/mL of USP Irinotecan Hydrochloride RS in Diluent

**Sample solution:** 1 mg/mL of Irinotecan Hydrochloride in Diluent

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 3.0 between irinotecan and irinotecan related compound A, System suitability solution

**Relative standard deviation:** NMT 2.0%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each individual impurity from the Sample solution

$r_S$  = peak area of irinotecan from the Standard solution

$C_S$  = concentration of USP Irinotecan Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = concentration of Irinotecan Hydrochloride in the Sample solution (mg/mL)

$F$  = relative response factor for each individual impurity (see Table 3)

**Acceptance criteria**

**Individual impurities:** See Table 3. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Desethyl irinotecan <sup>a</sup>	0.82	0.77	0.15
Irinotecan	1.00	—	—
Irinotecan related compound A <sup>b</sup>	1.15	1.4	0.15
11-Ethyl irinotecan <sup>c</sup>	1.27	0.63	0.15
Camptothecin <sup>d</sup>	1.35	1.4	0.15
Irinotecan related compound B <sup>e</sup>	1.50	1.3	0.15
7-Ethylcamptothecin <sup>f</sup>	1.76	1.2	0.15
7,11-Diethyl-10-hydroxycamptothecin <sup>g</sup>	2.05	0.65	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.50

<sup>a</sup> (S)-4-Ethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione-9-yl (1,4'-bipiperidine)-1'-carboxylate.

<sup>b</sup> (S)-4-Ethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.

<sup>c</sup> (S)-4,8,11-Triethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione-9-yl (1,4'-bipiperidine)-1'-carboxylate.

<sup>d</sup> (S)-4-Ethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.

<sup>e</sup> (S)-4,11-Diethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.

<sup>f</sup> (S)-4,11-Diethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.

<sup>g</sup> (S)-4,8,11-Triethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>:** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g.

- **WATER DETERMINATION, Method I <921>:** Between 7.0% and 9.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **LABELING:** If a test for Organic Impurities other than Procedure 1 is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS <11>**

USP Irinotecan Hydrochloride RS

USP Irinotecan Related Compound A RS

(S)-4-Ethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.  
C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>O<sub>5</sub> 364.35

USP Irinotecan Related Compound B RS

(S)-4,11-Diethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione.  
C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub> 392.40

USP Irinotecan Related Compound C RS

(S)-9-[(1,4'-Bipiperidine)-1'-carbonyloxy]-4-methyl-11-ethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline hydrochloride.  
C<sub>32</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub> · HCl 609.11

USP Irinotecan Related Compound D RS

(R)-9-[(1,4'-Bipiperidine)-1'-carbonyloxy]-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline hydrochloride, trihydrate.  
C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> · HCl · 3 H<sub>2</sub>O 677.18

**Add the following:****Iron, Carbonyl**

Fe 55.85  
[7439-89-6].

**DEFINITION**

Carbonyl Iron is elemental iron produced by chemical decomposition of purified iron pentacarbonyl. It is a powder, composed of spherical microparticles. It contains NLT 98.0% of iron (Fe), calculated on the as-is basis.

**IDENTIFICATION**

- **A.**  
**Analysis:** Dissolve a sample in a dilute mineral acid.  
**Acceptance criteria:** Hydrogen is evolved, and the resulting solutions give a positive test for *Identification Tests—General* (191), *Iron, Ferrous Salts*.
- **B.**  
**Analysis:** View a sample under a microscope having a magnifying power of 500 or greater.  
**Acceptance criteria:** It appears as spheres built up with concentric shells. Its particle size is 45–75 µm.

**ASSAY****• PROCEDURE**

**Sample:** 200 mg

**Blank:** Proceed as directed in the *Analysis*, omitting use of the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N ceric sulfate VS

**Endpoint detection:** Potentiometric

**Electrode system:** Platinum indicating electrode and a silver–silver chloride reference electrode (or an equivalent combination electrode)

**Analysis:** Transfer the *Sample* into a 300-mL Erlenmeyer flask. Add 50 mL of 2 N sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath to completely dissolve the *Sample*. [NOTE—The solution should be clear.] Remove the flask from the steam bath, and allow the solution to cool at room temperature with the stopper in place.

Add a stir bar and 50 mL of recently boiled and cooled water to the flask. Titrate the solution with the *Titrant* through the inflection point. Perform a blank determination.

Calculate the percentage of iron (Fe) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F\} / W \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual *Titrant* normality (mEq/mL)

$F$  = equivalency factor, 55.85 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** NLT 98.0% on the as-is basis

**IMPURITIES****• ACID-INSOLUBLE SUBSTANCES**

**Sample:** 1 g of Carbonyl Iron

**Analysis:** Dissolve the *Sample* in 25 mL of 2 N sulfuric acid, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible,

wash the residue with water until free from sulfate, dry at 105° for 1 h, cool to room temperature, and weigh.

**Acceptance criteria:** NMT 0.2%. The residue weighs NMT 2 mg.

**• ELEMENTAL IMPURITIES, Procedures (233)**

**Acceptance criteria**

**Arsenic:** NMT 3 µg/g

**Lead:** NMT 4 µg/g

**Mercury:** NMT 2 µg/g

**SPECIFIC TESTS**

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING, Test Sieves (786):** NLT 100% passes through a 200-mesh sieve and NLT 95% passes through a 325-mesh sieve.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲USP36

**Iron Dextran Injection**

» Iron Dextran Injection is a sterile, colloidal solution of ferric hydroxide in complex with partially hydrolyzed Dextran of low molecular weight, in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iron. It may contain not more than 0.5 percent of phenol as a preservative.

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**USP Reference standards (11)—**

USP Endotoxin RS

**Identification—**To 1 mL of Injection on a watch glass add 2 drops of ammonium hydroxide: no precipitate is formed. Add 2 mL of hydrochloric acid, mix, and add 2 mL of ammonium hydroxide: a brown precipitate is formed.

**Bacterial endotoxins (85)—**It contains not more than 0.50 USP Endotoxin Unit per mg of iron.

**Acute toxicity—**Select five mice each weighing between 18 and 25 g, maintained on an adequately balanced diet. Inject a dose of Injection, equivalent to 200 mg of iron per kg of body weight, into a tail vein at a rate not exceeding 0.1 mL per second. Keep the mice under observation for 48 hours after the injection. If none of the mice show outward symptoms of toxicity, the requirements of the test are met. If any of the mice die within the observation period, select four groups of ten mice each weighing between 18 and 25 g. Inject, intravenously, all mice of one group with one of the following doses of Injection: 375, 500, 750, or 1000 mg of iron per kg of body weight. Observe the mice for 7 days, and record the number of deaths in each group. If more than 16 mice die, calculate the LD<sub>50</sub> as directed under *Design and Analysis of Biological Assays* (111): the LD<sub>50</sub> is not less than 500 mg of iron per kg of body weight.

**Absorption from injection site—**Prepare a site over the semitendinosus muscle of one leg of each of two rabbits by clipping the fur and disinfecting the exposed skin. Inject each site with a dose of 0.4 mL per kg of body weight in the following manner. Place the needle in the distal end of the semitendinosus muscle at an angle such as to ensure that the full length of the needle is used, then pass it through the sartorius and vastus medialis muscles. House the rabbits separately. Seven days after the injection, sacrifice the rabbits and dissect the treated legs to examine the muscles: no heavy black deposit of unabsorbed iron compounds is observed, and the tissue is only lightly colored.

**pH** (791): between 4.5 and 7.0.

**Nonvolatile residue**—Using a “to contain” pipet, transfer 1.0 mL onto 3 to 5 g of sand spread in a shallow layer in a stainless steel dish, the dish and sand having been previously dried and weighed. Rinse the pipet, with several small portions of water, onto the sand. Evaporate on a steam bath to dryness, continue the drying in an oven at 105° for 15 hours, and weigh: the weight of the residue for Injection labeled to contain 50 mg of iron per mL is not less than 28.0% and not more than 32.0%, that for Injection labeled to contain 75 mg of iron per mL is not less than 35.0% and not more than 40.0%, and that for Injection labeled to contain 100 mg of iron per mL is not less than 37.0% and not more than 43.0%.

**Chloride content**—Using a “to contain” pipet, transfer 10.0 mL of Injection into a 150-mL beaker, rinsing the pipet into the beaker with several small portions of water. Add 50 mL of water and 2 mL of nitric acid, mix, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically with silver-glass electrodes. Each mL of 0.1 N silver nitrate consumed is equivalent to 3.545 mg of chloride (Cl). The chloride content of Injection labeled to contain 50 mg of iron per mL is not less than 0.48% and not more than 0.68%, and that of Injection labeled to contain either 75 mg or 100 mg of iron per mL is not less than 0.8% and not more than 1.1%.

**Limit of phenol**—Proceed as directed for *Phenol* under *Antimicrobial Agents*—**Content** (341): not more than 0.5% is found.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay for iron—

**Iron stock solution**—Transfer an accurately weighed quantity of about 350 mg of ferrous ammonium sulfate hexahydrate to a 1000-mL volumetric flask, add water to dissolve, dilute with water to volume, and mix to obtain a solution having a concentration of 50 µg of iron per mL.

**Calcium chloride solution**—Transfer 2.64 g of calcium chloride dihydrate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.

**Standard preparations**—To separate 100-mL volumetric flasks transfer 2.0, 4.0, 6.0, 8.0, and 10.0 mL, respectively, of *Iron stock solution*. Dilute each flask with *Calcium chloride solution* to volume, and mix to obtain *Standard preparations* having known concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 µg of iron per mL.

**Assay preparation**—Using a “to contain” pipet, transfer an accurately measured volume of Injection, equivalent to about 100 mg of iron, to a 200-mL volumetric flask. Dilute with *Calcium chloride solution* to volume, and mix. Pipet 2.0 mL of this solution into a 250-mL volumetric flask, dilute with *Calcium chloride solution* to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the iron emission line of 248.3-nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with an iron hollow-cathode lamp and an air-acetylene flame, using the *Calcium chloride solution* as the blank. Plot the absorbance of each *Standard preparation* versus concentration, in µg per mL, of iron, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of iron in the *Assay preparation*. Calculate the quantity, in mg, of iron in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of iron in the *Assay preparation*; and V is the volume of Injection taken.

## Iron Sorbitex Injection

» Iron Sorbitex Injection is a sterile solution of a complex of iron, Sorbitol, and Citric Acid that is stabilized with the aid of Dextrin and an excess of Sorbitol. It contains not less than 94.0 percent and not more than 104.0 percent of the labeled amount of iron.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate its expiration date, which is not more than 24 months after date of manufacture.

**USP Reference standards** (11)—

USP Endotoxin RS

#### Identification—

**A:** To 1 mL of Injection add 5 mL of water and 1 mL of ammonium hydroxide: no precipitate is formed.

**B:** To 1 mL of Injection add 0.1 mL of 3 N hydrochloric acid and 0.5 mL of potassium ferrocyanide TS: a dark blue precipitate is formed.

**C:** To 1 mL of Injection in a separator add 4 mL of water and 10 mL of hydrochloric acid, and extract with three 15-mL portions of isopropyl ether. Dilute the aqueous layer with water to 25 mL, and mix. To 0.5 mL of this solution add 10 mL of water, 2 mL of 2 N sulfuric acid, and 10 mg of potassium periodate, allow to stand for 10 minutes, and then add 10 mL of 0.1 N sodium arsenite. When the solution is colorless, add 10 mL of a 1 in 250 solution of phenylhydrazine hydrochloride in 0.5 N hydrochloric acid. Allow to stand for 10 minutes, add 1 mL of potassium ferricyanide solution (1 in 20), allow to stand for 15 minutes, and then add 3 mL of hydrochloric acid: a wine-red color is produced (*presence of sorbitol*).

**D:** Dilute 1 mL of Injection with 50 mL of water, and to 4 mL of this solution add 1 mL of 6 N sulfuric acid and 0.5 mL of phosphoric acid, mix, and allow to stand for about 5 minutes or until decolorized. Add 1 mL of potassium bromide solution (1 in 10) and 1 mL of potassium permanganate solution (1 in 20). After 10 minutes, add hydrogen peroxide TS, dropwise, to discharge the pink color. Transfer the solution to a small separator, shake with 20 mL of solvent hexane, discard the water layer, and wash the hexane layer with 20 mL of water. To the washed hexane solution add 5 mL of a 1 in 25 solution of thiourea in sodium borate solution (1 in 50), and shake the mixture: the aqueous layer that separates shows a yellow color (*presence of citric acid*).

**Specific gravity** (841): between 1.17 and 1.19 at 20°.

**Viscosity—Capillary Viscometer Methods** (911): between 8 and 13 centipoises, determined at 20° with a capillary tube viscometer.

**Bacterial endotoxins** (85)—It contains not more than 10.0 USP Endotoxin Units per mL.

**pH** (791): between 7.2 and 7.9.

**Limit of ferrous iron**—Using a “to contain” pipet, transfer 10 mL of Injection to a glass-stoppered, 125-mL flask, rinsing the pipet into the flask with several small portions of water. Add 5 mL of sulfuric acid, shake vigorously until decolorization is effected, add 2 drops of orthophenanthroline TS, and immediately titrate with 0.1 N ceric sulfate VS. Each mL of 0.1 N ceric sulfate is equivalent to 5.585 mg of ferrous iron. The ferrous iron content is not more than 8.5 mg per mL of Injection.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay for iron—**

**Standard preparation**—Transfer an accurately weighed portion of ferrous ammonium sulfate, equivalent to about 100 mg of iron (Fe), to a 300-mL Kjeldahl flask, and proceed as directed under *Assay preparation*, beginning with “Add 10 mL of nitric acid,” to obtain a *Standard preparation* having a known concentration of about 2 µg of iron per mL.

**Assay preparation**—Using a “to contain” pipet, transfer an accurately measured volume of Injection, equivalent to about 100 mg of iron, to a 300-mL Kjeldahl flask, rinsing the pipet into the flask with several small portions of water. Add 10 mL of nitric acid, 10 mL of sulfuric acid, and a few glass beads, and boil the solution gently until fumes of sulfur trioxide appear. Cool, add 3 mL of nitric acid, and heat gently again until fumes of sulfur trioxide appear. Continue the addition of nitric acid, followed by gentle boiling, until the solution is clear and light yellow or green in color, and then boil for an additional 30 minutes. Cool, cautiously add 100 mL of water, and boil gently until solution is complete. Cool, transfer the solution to a 500-mL volumetric flask, rinse the Kjeldahl flask with several small portions of water, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a second 500-mL volumetric flask, add 100 mL of water and 1 g of ascorbic acid, dilute with water to volume, and mix.

**Procedure**—Transfer 5.0-mL portions of the *Standard preparation*, of the *Assay preparation*, and of water to serve as the blank to separate, clean, dry test tubes, and to each add 3.0 mL of a 1 in 1500 solution of 2,2'-bipyridine in 0.6 N glacial acetic acid, mix, and allow to stand for 15 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 510 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of iron in each mL of the Injection taken by the formula:

$$(50C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and *Standard preparation*, respectively.

## Iron Sucrose Injection

» Iron Sucrose Injection is a sterile, colloidal solution of ferric hydroxide in complex with Sucrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iron. Sodium Hydroxide may be added to adjust the pH. It contains no antimicrobial agent, chelating agent, dextran, gluconate, or other added substances.

**Packaging and storage**—Preserve in single-dose containers of Type I glass. Store at controlled room temperature. Do not freeze.

**Labeling**—Label it to state that it is for intravenous use only. Label it to indicate that when administered by intravenous infusion, the Injection must be diluted with 0.9% Sodium Chloride Injection to a concentration of 0.5 to 2.0 mg of elemental iron per mL. Label it also to state the total osmolality of the solution expressed in mOsmol per L.

**USP Reference standards (11)—**

USP Endotoxin RS

USP Sucrose RS

**Identification—**

**A: Iron**—To 2.5 mL of Injection add 17.5 mL of water and 5 mL of hydrochloric acid, mix, and heat for 5 minutes in a boiling water bath. Cool, add dropwise 13.5 N ammonium hydroxide until no further precipitation of ferric hydroxide occurs, and filter. Wash the precipitate with water to remove excess ammonium hydroxide, dissolve the precipitate in a minimum volume of 2 N hydrochloric acid, and add sufficient water to make a volume of 20 mL. To 3 mL of the solution so obtained add 1 mL of 2 N hydrochloric acid and 1 mL of potassium thiocyanate TS: the resulting solution (*Solution 1*) is red. To 1 mL of *Solution 1* add 5 mL of amyl alcohol or ethyl ether, shake, and allow to stand: the organic layer is pink. To a separate 1-mL aliquot of *Solution 1* add 2 mL of mercuric chloride TS: the red color is discharged [iron (III) salts].

**B: Sucrose**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for sucrose*.

**C: Molecular weight determination—**

**Mobile phase**—Dissolve 7.12 g of dibasic sodium phosphate dihydrate, 5.52 g of monobasic sodium phosphate, and 0.40 g of sodium azide in 2 L of water.

**System suitability solution**—Dissolve 200 mg of high molecular weight dextran and 100 mg of glucose in 20 mL of *Mobile phase*.

**Standard solutions**—Transfer about 20 mg of each polysaccharide molecular weight standard (5,000–400,000 Da), accurately weighed, to separate 5-mL volumetric flasks. Add 4 mL of *Mobile phase* to each flask, and allow each aliquot to stand at or below 25° for a minimum of 12 hours. After the agglomerate particles of each *Standard solution* have swelled to their fullest extent, gently swirl each *Standard solution* until dissolved. [NOTE—The chromatograms of freshly prepared *Standard solutions* regularly show a small, unidentified secondary peak following the main peak. Discard the *Standard solutions* if the secondary peak reaches half the height of the main peak.]

**Test solution**—Transfer 5.0 mL of Injection to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector maintained at a constant temperature of 45° and two 7.8-mm × 30-cm columns set up in series that contain packing L39 with pore sizes of 1000Å and 120Å, respectively. The column temperatures are maintained at 45 ± 2° and the flow rate is about 0.5 mL per minute. Chromatograph the *System suitability solution*, and measure the peak areas as directed for *Procedure*: the resolution,  $R$ , between dextran and glucose is not less than 4.0. Chromatograph the *Standard solutions*, and measure the peak areas as directed for *Procedure*. Using a suitable program, plot the retention times of the *Standard solutions* and their molecular weights to generate a third order (cubic) calibration curve. The correlation coefficient obtained is not less than 0.98.

**Procedure**—Separately inject equal volumes (about 25 µL) of each *Standard solution*, the *System suitability solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the retention times and peak areas. The molecular weight of the complex is calculated from the calibration curve. The molecular weight distribution curve of the sample is sliced into fractions. Calculate the weight-average molecular weight,  $M_w$ , as follows:

$$\Sigma(A_T M_T) / \Sigma A_T$$

and the number-average molecular weight,  $M_n$ , as follows:

$$\Sigma(A_T) / \Sigma(A_T / M_T)$$

in which  $A_T$  is the area of each fraction of the sample distribution; and  $M_T$  is the corresponding mean molecular weight



of each fraction as determined from its retention time on the calibration curve. The molecular weight distribution curve obtained for the Injection conforms to the following parameters:

$$M_W = 34,000\text{--}60,000 \text{ Da,}$$

$$M_N = \text{not less than } 24,000 \text{ Da, and}$$

$$M_W / M_N = \text{not more than } 1.7.$$

**Specific gravity** (841): not less than 1.135 and not more than 1.165 at 20°.

**Bacterial endotoxins** (85): not more than 3.7 USP Endotoxin Units per mg of iron contained in the Injection.

**Alkalinity**—Transfer 5 mL of Injection to a suitable vessel, and titrate with 0.1 N hydrochloric acid VS with constant stirring to a pH of 7.4. Record the volume of 0.1 N hydrochloric acid VS consumed, and calculate the alkalinity of the Injection as the volume of acid, in mL, consumed per mL of Injection. Not less than 0.5 mL and not more than 0.8 mL of 0.1 N hydrochloric acid VS is consumed per mL of Injection.

**pH** (791): between 10.5 and 11.1 at 20°.

**Osmolarity** (785): not less than 1150 mOsmol per L and not more than 1350 mOsmol per L for the Injection. The solution for test is prepared by diluting the Injection 1 in 10.

**Absence of low-molecular weight Fe(II) and Fe(III) complexes**—In the polarograms obtained in the test for *Limit of iron (II)*, no additional peaks are found.

**Turbidity**—Transfer 0.5 g of Injection to a 150-mL beaker, add 100 mL of water, and with constant stirring adjust with 0.1 N hydrochloric acid VS to a pH of about 6.0. Remove the pH electrode from the solution. Adjust a light source such that the beam hits the beaker at a parallel angle about 2 cm below the surface of the liquid. The light must shine through to the surface, and the solution must not have any turbidity. Measurement must be carried out in as dark a room as possible. Slowly add 0.1 N hydrochloric acid VS, dropwise, until a slight but lasting turbidity develops. Record the pH of the solution as the turbidity point of the Injection: not less than 4.4 and not more than 5.3.

**Particulate matter** (788)—Prepare a solution of Injection (1 in 40) using water that has been passed through a filter having a 1.2- $\mu$ m or finer porosity: meets the requirements for *Light Obscuration Particle Count Test* for small-volume injections.

#### Limit of iron (II)—

**Supplementary electrolyte solution**—Dissolve 15.0 g of sodium acetate in 100 mL of water, and adjust with 0.1 N acetic acid to a pH of 7.0.

**Procedure**—Transfer a suitable amount of *Supplementary electrolyte solution* to a polarographic cell equipped with a mercury drop electrode. With the electrode submerged in the liquid, bubble nitrogen through the liquid for 5 minutes. Avoiding any undue exposure to air, immediately transfer a volume of Injection, accurately measured, equivalent to a concentration of about 20 to 120  $\mu$ g of elemental iron per mL, to the polarographic cell. [NOTE—The sample must be analyzed immediately upon opening the container.] Record the polarogram from 0 mV and  $-1700$  mV. The iron (III)/iron (II) peak is detected at  $-750 \pm 50$  mV and the iron (II)/iron (0) peak is detected at  $-1400 \pm 50$  mV. Measure the iron (II)/iron (III) peak responses obtained from the polarogram, and perform a blank determination. Calculate the iron (II) content, in % w/v, in the volume of Injection taken by the formula:

$$[1 - (2/R)] \times \text{Fe}$$

in which *R* is the peak response ratio of iron (II) to iron (III); and Fe is the total iron concentration, in % w/v, of the Injection. Not more than 0.4% (w/v) of iron (II) is found.

**Content of chloride**—Transfer about 12 g of Injection, accurately weighed, into a 50-mL beaker. Add 40 mL of water,

0.3 mL of 65% nitric acid, and, while stirring, titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically with silver–glass electrodes. Calculate the chloride content, in mg, of Injection taken. Each mL of 0.01 N silver nitrate consumed is equal to 0.3545 mg of chloride (Cl). The chloride content of the Injection is not less than 0.012% and not more than 0.025%.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay for sucrose—

**Mobile phase**—Prepare a mixture of acetonitrile and water (79:21).

**Standard preparations**—Dissolve an accurately weighed quantity of USP Sucrose RS in water, and quantitatively dilute with water to obtain solutions having known concentrations of about 13, 16, 18, 21, and 23 mg of sucrose per mL.

**Assay preparation**—Transfer about 1.875 g of Injection, accurately weighed, into a 25-mL flask, add 1.25 mL of water, and mix. Add 1.25 mL of a monobasic sodium phosphate solution, prepared by dissolving 30 g in 50 mL, and mix. Allow the resulting solution to stand for 10 minutes to precipitate the colloidal ferric hydroxide. Dilute with water to volume, and mix. Centrifuge this solution at 3000 rpm for 15 minutes. Pass the resulting solution through a filter, discarding the first 2 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a column compartment and a refractive index detector each maintained at a controlled temperature between 20° and 25° ( $\pm 2^\circ$ ) and a 4-mm  $\times$  25-cm column that contains packing L8. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparations*, and measure the peak areas as directed for *Procedure*. The correlation coefficient obtained from the linear regression of the *Standard preparations* is not less than 0.998. [NOTE—The retention time for sucrose is about 8 minutes.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of each *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Plot the peak area for each *Standard preparation* versus concentration, in mg per mL, of sucrose, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in mg per mL, of sucrose in the *Assay preparation*. Calculate the quantity, in mg, of sucrose in each mL of Injection taken by the formula:

$$CDG/W$$

in which *C* is the concentration, in mg per mL, of sucrose in the *Assay preparation*; *D* is the dilution volume of the *Assay preparation*; *G* is the density, in g per mL, of Injection taken; and *W* is the weight, in g, of Injection taken. It contains not less than 260 mg and not more than 340 mg of sucrose per mL.

#### Assay for iron—

**Iron stock solution**—Transfer about 350 mg of ferrous ammonium sulfate, accurately weighed, to a 1000-mL volumetric flask, add water to dissolve, dilute with water to volume, and mix to obtain a solution having a concentration of about 50  $\mu$ g of iron per mL.

**Calcium chloride solution**—Transfer 2.64 g of calcium chloride to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.

**Standard preparations**—To separate 50-mL volumetric flasks transfer 2.0, 4.0, 6.0, 8.0, and 10.0 mL of *Iron stock solution*. Dilute each flask with *Calcium chloride solution* to volume, and mix to obtain *Standard preparations* having known concentrations of about 2.0, 4.0, 6.0, 8.0, and 10.0  $\mu$ g of iron per mL.

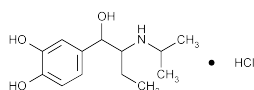
**Assay preparation**—Using a “to contain” pipette, transfer 2.0 mL of Injection to a 100-mL volumetric flask. Rinse the pipette several times with *Calcium chloride solution*. Add 5 mL of hydrochloric acid, and swirl until the solution turns yellow. After the solution has cooled to room temperature, dilute with *Calcium chloride solution* to volume, and mix. Pipet 2.0 mL of this solution to a 100-mL volumetric flask, dilute with *Calcium chloride solution* to volume, and mix to obtain a solution with a theoretical concentration of about 8.0 µg of iron per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the iron emission line at 248.3 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* (851)) equipped with an iron hollow-cathode lamp and air-acetylene flame, using *Calcium chloride solution* as a blank. Plot the absorbances for each *Standard preparation* versus concentration, in µg per mL, of iron and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of iron in the *Assay preparation*. Calculate the quantity, in mg, of iron in each mL of the Injection taken by the formula:

$$5C/V$$

in which C is the concentration, in µg per mL, of iron in the *Assay preparation*; and V is the volume of Injection taken.

## Isoetharine Hydrochloride



$C_{13}H_{21}NO_3 \cdot HCl$  275.77

1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino]butyl]-, hydrochloride.

3,4-Dihydroxy- $\alpha$ -[1-(isopropylamino)propyl]benzyl alcohol hydrochloride [2576-92-3].

» Isoetharine Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of  $C_{13}H_{21}NO_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Isoetharine Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** A solution (1 in 100) responds to the tests for *Chloride* (191).

**pH** (791): between 4.0 and 5.6, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 100° for 4 hours: it loses not more than 1.0% of its weight.

**Aromatic ketones**—Its absorptivity (see *Spectrophotometry and Light-scattering* (851)) at 312 nm, determined in a solution in 0.01 N hydrochloric acid containing 2.0 mg per mL, is not more than 0.20.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoetharine Hydrochloride RS in freshly prepared sodium bisulfite solution (3 in 1000) to obtain a solution having a concentration of about 5 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix to obtain a

solution having a known concentration of about 500 µg per mL.

**Assay preparation**—Transfer about 125 mg of Isoetharine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The mobile phase is 0.17 N acetic acid, having a flow rate of about 2.2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%.

**Procedure**—Using a microsyringe or sampling valve, chromatograph 10 µL of the *Standard preparation*, and adjust the specimen size and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Chromatograph equal volumes of the *Standard preparation* and the *Assay preparation*, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_{13}H_{21}NO_3 \cdot HCl$  in the portion of Isoetharine Hydrochloride taken by the formula:

$$0.25C(h_u / h_s)$$

in which C is the concentration, in µg per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation*; and  $h_u$  and  $h_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoetharine Inhalation Solution

» Isoetharine Inhalation Solution is a sterile solution of Isoetharine Hydrochloride in Purified Water. It may contain Sodium Chloride. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of isoetharine hydrochloride ( $C_{13}H_{21}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

**Labeling**—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—

USP Isoetharine Hydrochloride RS

**Color and clarity**—

**Standard solution**—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Visually examine a portion of the Inhalation Solution (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

**Identification**—Dilute the Inhalation Solution with water to obtain a solution containing about 2.5 mg of isoetharine hydrochloride per mL. Apply 10-µL portions of this solution and a solution of USP Isoetharine Hydrochloride RS containing 2.5 mg per mL to a thin-layer chromatographic plate

coated with silica gel mixture. Develop the plate in a mixture consisting of *n*-butyl alcohol, water, and formic acid (70:20:10) to a height of 12 to 14 cm above the point of application. Remove the plate, and evaporate the solvents with the aid of warm, circulating air. Examine under short-wavelength UV light. Spray the plate with Folin–Ciocalteu phenol TS, and then expose to ammonia vapor until the isoetharine spots develop an intense blue color: the  $R_f$  value and color of the principal spot obtained from the solution under test correspond to those obtained from the *Standard solution*.

**Sterility** <71>: meets the requirements.

**pH** <791>: between 2.5 and 5.5.

**Assay—**

*Standard preparation*—Prepare as directed in the Assay under *Isoetharine Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 50 mg of isoetharine hydrochloride, to a 100-mL volumetric flask, dilute with 0.17 N acetic acid solution to volume, and mix.

*Chromatographic system*—Proceed as directed in the Assay under *Isoetharine Hydrochloride*.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Isoetharine Hydrochloride*. Calculate the quantity, in mg, of  $C_{13}H_{21}NO_3 \cdot HCl$  in each mL of the Inhalation Solution taken by the formula:

$$0.1(C/V)(h_U / h_S)$$

in which  $V$  is the volume, in mL, of Inhalation Solution taken; and  $C$ ,  $h_U$ , and  $h_S$  are as defined therein.

## Isoetharine Mesylate

$C_{13}H_{21}NO_3 \cdot CH_3O_3S$  335.42  
1,2-Benzenediol, 4-[1-hydroxy-2-  
[(1-methylethyl)amino]butyl]-, methanesulfonate (salt).  
3,4-Dihydroxy- $\alpha$ -[1-(isopropylamino)propyl]benzyl alcohol  
methanesulfonate (salt) [7279-75-6].

» Isoetharine Mesylate contains not less than 97.0 percent and not more than 102.0 percent of  $C_{13}H_{21}NO_3 \cdot CH_3O_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Isoetharine Hydrochloride RS

**Identification—**

**A:** It responds to the *Thin-layer Chromatographic Identification Test* <201>, the test solution and the Standard solution of USP Isoetharine Hydrochloride RS being prepared at a concentration of 2.5 mg per mL in methanol, the solvent mixture being *n*-butanol, water, and formic acid (64:25:11), and the spots being located by spraying with sodium hydroxide solution (1 in 10).

**B:** Mix about 50 mg with about 200 mg of powdered sodium hydroxide, transfer the mixture to a small test tube, heat in a small flame to fusion, and continue the heating for a few minutes longer. Cool, add about 0.5 mL of water, then add a moderate excess of hydrochloric acid, and warm: starch iodate paper placed over the mouth of the test tube turns blue.

**Melting range** <741>: between 162° and 168°.

**pH** <791>: between 4.5 and 5.5, in a solution (1 in 100).

**Loss on drying** <731>—Dry it at 80° under vacuum at a pressure of not more than 5 mm of mercury for 4 hours: it loses not more than 1.0% of its weight.

**Limit of keto precursor**—Its absorptivity (see *Spectrophotometry and Light-scattering* <851>) at 312 nm, determined in a solution in 0.01 N hydrochloric acid containing 2.0 mg per mL, is not more than 0.20.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of 0.1 M sodium sulfate in 0.8% acetic acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Transfer about 60 mg of USP Isoetharine Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add 4.0 mL of alcohol, and mix. Add 3 drops of 1 N hydrochloric acid, dilute with water to volume, and mix.

*Assay preparation*—Transfer about 75 mg of Isoetharine Mesylate, accurately weighed, to a 25-mL volumetric flask, add 4.0 mL of alcohol, and mix. Add 3 drops of 1 N hydrochloric acid, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L9. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of  $C_{13}H_{21}NO_3 \cdot CH_3O_3S$  in the portion of Isoetharine Mesylate taken by the formula:

$$0.025C(335.42 / 275.77)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation*; 335.42 and 275.77 are the molecular weights of isoetharine mesylate and isoetharine hydrochloride, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoetharine Mesylate Inhalation Aerosol

» Isoetharine Mesylate Inhalation Aerosol is a solution of Isoetharine Mesylate in Alcohol in an inert propellant base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoetharine mesylate ( $C_{13}H_{21}NO_3 \cdot CH_3O_3S$ ).

**Packaging and storage**—Preserve in small, nonreactive, light-resistant, aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

**USP Reference standards** <11>—

USP Isoetharine Hydrochloride RS

**Identification—**

**A:** Expel a quantity of Inhalation Aerosol, equivalent to about 12 mg of isoetharine mesylate, into 2 mL of methanol, dilute with methanol to 5 mL, and mix: this solution responds to *Identification test A* under *Isoetharine Inhalation Solution*.

**B:** Expel a quantity of Inhalation Aerosol, equivalent to about 12 mg of isoetharine mesylate, into a test tube, evaporate on a steam bath just to dryness, and add 50 mg of powdered sodium hydroxide. Heat in a small flame to fusion, and continue heating for a few seconds longer. Cool, add about 0.5 mL of water, then add a moderate excess of

3 N hydrochloric acid: starch iodate paper placed over the mouth of the test tube turns blue.

**Alcohol content** (611)—Weigh accurately a filled Inhalation Aerosol container, and record the weight. Invert the container, and place the outlet tip against the bottom of a 50-mL beaker containing 5 mL of water. Slowly actuate the valve 10 times. Raise the unit above the contents of the beaker, and wash the outlet with 1 mL of water. Collect the washings in the beaker. Dip the outlet stem in alcohol, shake to remove the solvent completely, air-dry the valve, and again weigh the Inhalation Aerosol container. Record the weight of the expelled specimen. Transfer the contents of the beaker, with the aid of 4 mL of water, to a glass-stoppered graduated cylinder. Determine the alcohol content of the test solution thus prepared by the gas-liquid chromatographic procedure, 2 mL of dilute isopropyl alcohol (15 in 100) being used as the internal standard. Calculate the alcohol content of the Inhalation Aerosol taken by the formula:

$$SV / W$$

in which *S* is the percentage (w/v) of alcohol in the test solution; *V* is the total volume, in mL, of the test solution; and *W* is the weight, in g, of the expelled specimen taken: between 25.9% and 35.0% (w/w) of C<sub>2</sub>H<sub>5</sub>OH is found.

**Dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

**PROCEDURE FOR DOSE UNIFORMITY—**

*Ferro-citrate solution and Buffer solution*—Prepare as directed under *Epinephrine Assay* (391).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoetharine Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 1000), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 34 µg per mL.

*Test preparation*—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with two 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 5 minutes and centrifuge. Use the clear supernatant as directed in the *Procedure*.

*Procedure*—Into three separate 25-mL volumetric flasks transfer the *Test preparation*, 10.0 mL of the *Standard preparation*, and 10.0 mL of a freshly prepared sodium bisulfite solution (1 in 1000) to provide the blank. To each flask add 0.5 mL of *Ferro-citrate solution* followed by 5 mL of *Buffer solution*. Dilute with the sodium bisulfite solution (1 in 1000) to volume, mix, and allow the color to develop for 10 minutes. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation* relative to the blank at the wavelength of maximum absorbance at about 530 nm. Calculate the quantity, in µg, of C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub> · CH<sub>4</sub>O<sub>3</sub>S contained in the minimum dose taken by the formula:

$$10CN(335.42 / 275.77)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation*; *N* is the number of sprays discharged to obtain the minimum recommended dose; 335.42 and 275.77 are the molecular weights of isoetharine mesylate and isoetharine hydrochloride, respectively; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay—**

*Mobile phase and Standard preparation*—Proceed as directed in the *Assay* under *Isoetharine Mesylate*.

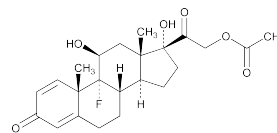
*Assay preparation*—Weigh 1 Inhalation Aerosol container with contents. Invert the container, and place the outlet tip against the bottom of a 50-mL beaker containing 2.5 mL of 0.01 N hydrochloric acid. Slowly actuate the valve about 90 times (the weight of the assay specimen is approximately 5 g). Raise the unit above the contents of the beaker, and wash the outlet with a few mL of water. Collect the washings in the beaker. Dip the outlet stem in alcohol, shake to remove the solvent completely, air-dry the valve, and then again weigh the Inhalation Aerosol container. Record the weight of the expelled specimen. Transfer the contents of the beaker to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Isoetharine Mesylate*. Calculate the percentage of C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub> · CH<sub>4</sub>O<sub>3</sub>S in the portion of Inhalation Aerosol taken by the formula:

$$0.01(335.42 / 275.77)(C / W)(r_U / r_S)$$

in which *W* is the weight, in g, of Inhalation Aerosol taken; and the other terms are as defined therein.

## Isoflupredone Acetate



C<sub>23</sub>H<sub>29</sub>FO<sub>6</sub> 420.47

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β)-.

9-Fluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-acetate [338-98-7].

» Isoflupredone Acetate contains not less than 97.0 percent and not more than 103.0 percent of C<sub>23</sub>H<sub>29</sub>FO<sub>6</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Isoflupredone Acetate RS

USP Prednisolone Acetate RS

**Identification, Infrared Absorption** (197M).

**Assortivity—**

*Test preparation:* 25 mg in 2000 mL of alcohol.

*Procedure*—Proceed as directed under *Spectrophotometry and Light-Scattering* (851), and measure the absorbance at 240 nm: the absorptivity is between 35.0 and 38.0.

**Specific rotation** (781S): between +110° and +120°.

*Test solution:* 10 mg per mL, in dioxane.

**Bacterial endotoxins** (85)—Where the label states that Isoflupredone Acetate is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 125 USP Endotoxin Units per mg of isoflupredone acetate.

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%.

**Chromatographic purity—**

**Solution A**—Prepare a mixture of water, methanol, acetonitrile, and glacial acetic acid (500:350:150:3), and degas.

**Solution B**—Prepare a mixture of acetonitrile, methanol, and water (550:500:3), and degas.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve accurately weighed quantities of USP Isoflupredone Acetate RS and USP Prednisolone Acetate RS in *Solution A* to obtain a solution having known concentrations of about 0.03 mg of each per mL. Sonicate, if necessary, to dissolve.

**Test solution**—Dissolve an accurately weighed quantity of Isoflupredone Acetate in *Solution A* to obtain a solution having a concentration of about 0.3 mg per mL. Sonicate, if necessary, to dissolve. Use this solution within 16 hours.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Protect the column from temperature fluctuations. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–32.5	100	0	isocratic
32.5–47.5	100→0	0→100	linear gradient
47.5–50.5	0	100	isocratic
50.5–51.5	0→100	100→0	linear gradient
51.5–61.5	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the retention time for isoflupredone acetate is between 21 and 26 minutes; the relative retention times are about 1.1 for prednisolone acetate and 1.0 for isoflupredone acetate; the resolution,  $R$ , between isoflupredone acetate and prednisolone acetate is not less than 1.2; and the column efficiency determined from isoflupredone is not less than 6000 theoretical plates.

**Procedure**—Inject a volume (about 50  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Isoflupredone Acetate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found, excluding those that are present in amounts less than 0.05%.

**Other requirements**—Where the label states that it is sterile, it meets the requirements for *Sterility Tests* (71) when tested as directed for *Direct Transfer Method* under *Test Procedures*.

**Assay—**

**Mobile phase**—Prepare a mixture of *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Use water-saturated chloroform.

**Internal standard solution**—Dissolve an accurately weighed quantity of fluoxymesterone in *Diluent* to obtain a solution having a known concentration of about 0.9 mg per mL.

**Standard preparation**—Dissolve about 4 mg of USP Isoflupredone Acetate RS, accurately weighed, in 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent*.

**Assay preparation**—Transfer about 4 mg of Isoflupredone Acetate, accurately weighed, to a suitable container. Dissolve in 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent*, centrifuge, and use the clear chloroform portion.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 0.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the relative retention times are about 1.0 for isoflupredone acetate and 1.2 for fluoxymesterone; the resolution,  $R$ , between isoflupredone acetate and fluoxymesterone is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 12  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{23}H_{29}FO_6$  in the portion of Isoflupredone Acetate taken by the formula:

$$W_s(R_U / R_s)$$

in which  $W_s$  is the weight, in mg, of USP Isoflupredone Acetate RS taken to prepare the *Standard preparation*; and  $R_U$  and  $R_s$  are the peak area ratios of isoflupredone acetate to fluoxymesterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoflupredone Acetate Injectable Suspension

» Isoflupredone Acetate Injectable Suspension contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoflupredone acetate ( $C_{23}H_{29}FO_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Isoflupredone Acetate RS

USP Prednisolone Acetate RS

**Identification, Infrared Absorption** (197M).

**Test specimen**—Transfer about 25 mg of Injectable Suspension to a centrifuge tube, add 20 mL of water, and shake well. Centrifuge, and discard the liquid layer. Repeat this washing step with three additional 20-mL portions of water. Dry the material so obtained at 105° for 3 hours.

**Bacterial endotoxins** (85)—It contains not more than 125 USP Endotoxin Units per mg of isoflupredone acetate.

**Sterility** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Mobile phase, Diluent, Internal standard solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Isoflupredone Acetate*.

*Assay preparation*—Transfer an accurately measured volume of Injectable Suspension, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent*, and swirl to dissolve.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Isoflupredone Acetate*. Calculate the quantity, in mg, of isoflupredone acetate ( $C_{23}H_{29}FO_6$ ) in each mL of Injectable Suspension taken by the formula:

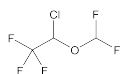
$$(W_S / V)(R_U / R_S)$$

in which *V* is the volume, in mL, of Injectable Suspension taken to prepare the *Assay preparation*; and the other terms are as defined therein.

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**Isoflurane**


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$C_3H_2ClF_5O$  184.49  
 Ethane, 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-;  
 1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether  
 [26675-46-7].

**DEFINITION**

Isoflurane contains NLT 99.9% of isoflurane ( $C_3H_2ClF_5O$ ).

**IDENTIFICATION**

- A.** The IR absorption spectrum of it obtained using a gas cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoflurane RS.

**ASSAY****PROCEDURE**

**Analysis:** Using the results from *Organic Impurities*, calculate the percentage of isoflurane ( $C_3H_2ClF_5O$ ) in the volume of Isoflurane taken by subtracting the sum of percentages for all impurities found from 100.0%.

**Acceptance criteria:** NLT 99.9%

**IMPURITIES****CHLORIDE**

**Sample solution:** Pipet 10 mL into a suitable vessel containing 60 mL of isopropyl alcohol and 4 drops of dilute nitric acid (1:1), and stir to dissolve.

**Analysis:** Titrate potentiometrically with 0.0020 N silver nitrate.

**Acceptance criteria:** NMT 2.11 mL is required (NMT 10 ppm).

**LIMIT OF FLUORIDE**

Use plasticware throughout this test.

**Buffer:** Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2-L volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix: the pH of this solution is between 5.0 and 5.5. [NOTE—This solution may be used for 6 weeks if stored at room temperature.]

**Standard stock solution:** Transfer 55 mg of USP Sodium Fluoride RS, previously dried at 150° for 4 h, to a

25-mL volumetric flask. Add 5 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 10,000), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ion. Store in a tightly closed plastic container. [NOTE—This solution may be used for 2 weeks if stored in a refrigerator.]

**Standard solutions:** Dilute portions of the *Standard stock solution* with water to obtain 100-mL volumes of stock solutions having concentrations of 2.0, 6.0, 10.0, and 20.0 µg/mL of fluoride. Transfer 25.0 mL of each of these stock solutions to separate 50-mL volumetric flasks, dilute with *Buffer* to volume, and mix to obtain *Standard solutions* having concentrations of 1.0, 3.0, 5.0, and 10.0 µg/mL of fluoride.

**Sample solution:** Shake 50.0 mL of Isoflurane with 50.0 mL of water for 5 min, and allow the liquids to separate completely. Transfer 25.0 mL of the water layer to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix.

**Analysis**

(See pH <791>.)

Concomitantly measure the potentials in mV, of the *Standard solutions* and the *Sample solution* with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride ion electrode and a glass-sleeved calomel reference electrode. When taking measurements, immerse the electrodes in the solution under test, which has been transferred to a 150-mL beaker containing a polytetrafluoroethylene-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the fluoride ion electrode.

A satisfactory response is achieved if the difference in potential between the potentials obtained with the *Standard solutions* having fluoride concentrations of 1.0 and 10.0 µg/mL is in the range of 50–60 mV. Plot the logarithm of the fluoride ion concentrations, in µg/mL, of the *Standard solutions* versus potential, in mV. From the measured potential of the *Sample solution* and the standard response line determine the concentration, in µg/mL, of fluoride in the *Sample solution*.

**Acceptance criteria:** NMT 5 µg/mL [NMT 0.001% (w/v)]

**NONVOLATILE RESIDUE**

**Analysis:** Transfer 10.0 mL to a suitable weighed evaporating dish, evaporate with the aid of a current of air to dryness, and dry the residue at 50° for 2 h.

**Acceptance criteria:** The weight of the residue does not exceed 2.0 mg.

**ORGANIC IMPURITIES**

[NOTE—The *Internal standard solution* and the *Standard solution* are prepared using the same Isoflurane that is under test. If multiple lots or samples of Isoflurane are under test, one sample may be selected for the *Internal standard solution* and the *Standard solution*. An appropriate blank correction should be made when determining the percentages of impurities in the other lots or samples.]

**Internal standard solution:** 10 mg/mL of normal butyl acetate in Isoflurane

**Standard solution:** To 95 mL of Isoflurane in a 100-mL volumetric flask add 10.0 µL of USP Isoflurane Related Compound A RS, 7.0 µL of USP Isoflurane Related Compound B RS, 10.0 µL of acetone, and 250 µL of *Internal standard solution*. Dilute with Isoflurane to volume, and mix. It contains 0.01% of isoflurane related compound A, 0.007% of isoflurane related compound B, and 0.01% of acetone.

**Sample solution:** To 20.0 mL of Isoflurane add 50.0 µL of *Internal standard solution*. It contains about 0.0025% (w/v) of normal butyl acetate.

**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 2.4-mm × 3.7-m nickel or stainless steel; packed with 10% phase G31 and 15% phase G18 on 60- to 80-mesh sodium hydroxide-washed support S1C**Temperatures****Detector:** 200°**Injection port:** 150°**Column:** See *Table 1*.**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
65	—	65	7
65	4	110	22

**Carrier gas:** Helium**Flow rate:** 25 mL/min**Injection volume:** 3 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5 for the normal butyl acetate peak**Relative standard deviation:** NMT 2.0% for the ratio of the response of the acetone peak to the response of the normal butyl acetate peak**Analysis****Samples:** *Standard solution* and *Sample solution*

Separately calculate the percentages of acetone, isoflurane related compound A, and isoflurane related compound B in the portion of Isoflurane taken:

$$\text{Result} = [R_U / (R_S - R_U)] \times P$$

 $R_U$  = peak response ratio of the relevant analyte to the internal standard from the *Sample solution* $R_S$  = peak response ratio of the relevant analyte to the internal standard from the *Standard solution* $P$  = percentage of the relevant analyte in the *Standard solution*

Calculate the percentage of any other individual impurity:

$$\text{Result} = [R_U / (R_S - R_U)] \times P$$

 $R_U$  = peak response ratio of any individual impurity to the internal standard from the *Sample solution* $R_S$  = peak response ratio of isoflurane related compound B to the internal standard from the *Standard solution* $P$  = percentage of isoflurane related compound B in the *Standard solution***Acceptance criteria:** See *Table 2*.**Table 2**

Name	Acceptance Criteria, NMT (%)
Acetone	0.01
Isoflurane related compound A	0.01
Isoflurane related compound B	0.007
Any other individual impurity	0.003

**SPECIFIC TESTS**• **REFRACTIVE INDEX** (831): 1.2990–1.3005 at 20°• **WATER DETERMINATION, Method I** (921): NMT 0.10%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.• **USP REFERENCE STANDARDS** (11)

USP Isoflurane RS

USP Isoflurane Related Compound A RS

1-Chloro-2,2,2-trifluoroethylchlorodifluoromethyl ether.

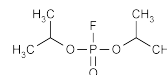
C<sub>3</sub>HCl<sub>2</sub>F<sub>5</sub>O 219.49

USP Isoflurane Related Compound B RS

2,2,2-Trifluoroethyldifluoromethyl ether.

C<sub>3</sub>H<sub>3</sub>F<sub>5</sub>O 150.05

USP Sodium Fluoride RS

**Isoflurophate**C<sub>6</sub>H<sub>14</sub>FO<sub>3</sub>P 184.15

Phosphorofluoridic acid, bis(1-methylethyl) ester.

Diisopropyl phosphorofluoridate [55-91-4].

» Isoflurophate contains not less than 95.0 per cent of C<sub>6</sub>H<sub>14</sub>FO<sub>3</sub>P.**Caution**—Handle Isoflurophate with exceptional care since it is very toxic. Wear full-face breathing apparatus and gloves. Open the container only in a hood.**Packaging and storage**—Preserve in glass, fuse-sealed containers, or in other suitable sealed containers, in a cool place.**Labeling**—Label it to indicate that in the handling of Isoflurophate in open containers, the eyes, nose, and mouth are to be protected with a suitable mask, and contact with the skin is to be avoided.**Identification**—**A:** Under a hood with a good draft place a few drops of Isoflurophate in a small platinum crucible, quickly add 2 mL of sulfuric acid, and immediately cover the crucible with a small, clear watch glass. Allow to stand for 10 minutes, then heat on a steam bath for 5 to 10 minutes: the side of the watch glass exposed to the mixture is visibly etched.**B:** Slowly heat under the hood the crucible and contents from Identification test A until copious white fumes are evolved. Cool, place the crucible in a beaker, and add a sufficient quantity of water nearly to cover the crucible. After a few minutes remove the crucible from the beaker with the aid of a glass rod, add 3 mL of nitric acid, and boil for a few minutes. Cool, cautiously add 6 N ammonium hydroxide with stirring until a slight odor of ammonia persists, then add 1 mL of nitric acid. Filter the liquid if not clear, warm to about 40°, and add about 10 mL of ammonium molybdate TS: a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.**Acidity**—**Mixed indicator**—Mix 3 volumes of a 1 in 1000 solution of bromocresol green in alcohol and 1 volume of a 1 in 500 solution of methyl red in alcohol.**0.1 N Sodium hydroxide in dehydrated alcohol**—Dissolve about 0.4 g of sodium hydroxide in 100 mL of dehydrated alcohol, and when solution is complete, standardize the alcoholic solution as follows. Pipet 25.0 mL of 0.1 N hydrochloric acid VS into a suitable container. Dilute with 50 mL

of water, add 2 drops of *Mixed indicator*, and titrate with the alcoholic sodium hydroxide solution to the first appearance of a green color. Calculate the normality. [NOTE—Store in tightly stoppered bottles.]

**Test preparation**—Prepare as directed in the Assay, except to draw 1.0 mL instead of 0.5 mL of Isoflurophate into the bulb.

**Procedure**—Carefully place the bulb containing the *Test preparation* in a 250-mL flask containing 20 mL of dehydrated alcohol. Break the bulb as directed in the *Procedure* under *Ionic fluorine*. Rinse the glass tube with 30 mL of dehydrated alcohol, and titrate immediately with 0.1 N Sodium hydroxide in dehydrated alcohol, using the *Mixed indicator*, to the appearance of the first green color. Each mL of 0.1 N Sodium hydroxide in dehydrated alcohol is equivalent to 100.8 µg of hydrogen ion (acidity). The limit is 0.01%.

#### **Ionic fluorine—**

**Sodium methoxide solution**—Dissolve 10 g of sodium methoxide in dehydrated alcohol to make 500 mL, and mix.

**Buffer solution**—Dissolve 9.55 g of monochloroacetic acid and 2 g of sodium hydroxide in water to make 100 mL. If necessary, adjust by the addition, of either reagent to obtain a solution having a pH of 3.0.

**Standard fluoride solution**—Dissolve 2.2105 g of sodium fluoride in water to make 1000.0 mL. Each mL is equivalent to 1.00 mg of fluoride ion.

**Thorium nitrate solution**—Dissolve 9 g of thorium nitrate in water to make 1000.0 mL, and mix. Standardize as directed under *Standard curve*.

**Standard curve**—Into each of four 180-mL beakers pipet 50.0 mL of *Sodium methoxide solution* and 0.25, 0.50, 1.0, and 2.0 mL, respectively, of *Standard fluoride solution*. Treat each beaker in the same manner, as follows. Add 2 drops of phenolphthalein TS, and render just acid with 6 N hydrochloric acid. Add 1.0 mL of sodium alizarinsulfonate solution (1 in 2000), and add 6 N hydrochloric acid dropwise until the pink color is discharged. Dilute with water to 100 mL, and add *Buffer solution* (approximately 4 mL) until the pH is 3.1. Titrate with *Thorium nitrate solution*, while stirring constantly and rapidly, to a permanent pink color. During the titration, maintain the solution at a pH between 2.9 and 3.1 by adding small volumes of *Buffer solution*, if necessary, but not more than a total of 10 mL. Plot the mg of fluoride ion versus the mL of *Thorium nitrate solution* consumed.

**Test preparation**—Prepare as directed in the Assay, except to draw 1.0 mL instead of 0.5 mL of Isoflurophate into the bulb.

**Procedure**—Place 50.0 mL of *Sodium methoxide solution* in a 180-mL beaker, and add 2 drops of phenolphthalein TS. Acidify, dropwise, with 6 N hydrochloric acid. Add 1.0 mL of sodium alizarinsulfonate solution (1 in 2000), then add 6 N hydrochloric acid dropwise until the pink color disappears. Add 0.5 N sodium hydroxide until a faint pink color appears, then add 0.05 N hydrochloric acid until the pink color just disappears. Add 4 mL of the *Buffer solution*. Carefully place the *Test preparation* in the beaker with the stem of the bulb inserted in a suitable length of glass tubing. Break the bulb by pressing down on the glass tubing, making sure that the bulb is beneath the surface of the liquid and that the bottom of the beaker is properly supported so that it will not break when the bulb is broken. Wash down the glass tube with water, and dilute with water to 100 mL. Titrate immediately with the *Thorium nitrate solution*. Determine the mg of ionic fluorine present in the *Test preparation* directly from the thorium nitrate standardization curve. Not more than 0.15% of ionic fluorine is found.

#### **Assay—**

**Solvent**—Use dry carbon disulfide, chromatographic grade.

**Internal standard solution**—Pipet 1.0 mL of chromatographic grade cyclohexanone into a 100-mL volumetric

flask, dilute with *Solvent* to volume, and mix. Pipet 3.0 mL of the resulting solution into a 100-mL volumetric flask, dilute with *Solvent* to volume, and mix. Each mL of the *Internal standard solution* contains 0.30 µL of cyclohexanone.

**Standard preparation**—Dissolve a suitable quantity of Isoflurophate, previously subjected to the Assay, in peanut oil, and dilute quantitatively and stepwise with peanut oil to obtain a solution having a known concentration of about 0.8 mg of isoflurophate per g of solution. Transfer about 1.2 g of this Isoflurophate solution in peanut oil, accurately weighed, to a 10-mL volumetric flask, pipet 1.0 mL of *Internal standard solution* into the flask, dilute with *Solvent* to volume, and mix.

**Assay preparation**—Tare an unsealed, thin-walled glass bulb with a thin, long stem, having a capacity of 1 to 2 mL. Under a hood, open the Isoflurophate container and place it in a firmly based container in a suitable vacuum-filtration flask. Insert the stem of the bulb under the surface of the liquid, and insert the stopper in the filtration flask. Allow about 0.5 mL of liquid to be drawn up into the bulb, and release the vacuum. Remove the bulb from the container, wipe the stem clean, fire-seal it without loss of any glass, cool, and again weigh.

Place the glass bulb in a 125-mL conical flask, add about 70 mL of *Solvent*, and break the bulb with the aid of a glass rod by pressing down on the glass tubing over the neck of the bulb. Take care to assure that the bulb is beneath the surface of the liquid in the flask and that the bottom of the flask is properly supported so that it will not break when the bulb is broken. Remove the rod, transfer the solution to a 100-mL volumetric flask, dilute with *Solvent* to volume, and mix (*Solution A*). Pipet 2.0 mL of *Solution A* into a 10-mL volumetric flask, dilute with *Solvent* to volume, and mix (*Solution B*). Pipet 1.0 mL of *Solution B* into another 10-mL volumetric flask, pipet 1.0 mL of *Internal standard solution* into the flask, dilute with *Solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—Under typical conditions, the gas chromatograph is equipped with a flame-ionization detector, and contains a 1.8-m × 4-mm glass column packed with 5% phase G33 on 80- to 100-mesh support S1AB, utilizing either a glass-lined sample introduction system or on-column injection. The column is maintained isothermally at a temperature between 75° and 80°, and the injector port and detector block are maintained at 200° and 250°, respectively; dry, oxygen-free helium is used as the carrier gas at a flow rate adjusted to obtain a cyclohexanone peak about 6 minutes after sample introduction.

**Procedure**—Inject 6 µL of the *Standard preparation* into a suitable gas chromatograph, and record the chromatogram. Measure the areas under the first (cyclohexanone) and second (isoflurophate) peaks, and record the values as  $A_D$  and  $A_S$ , respectively. Calculate the factor  $F$  taken by the formula:

$$(A_D / A_S)(W_S / 10)(C / 1000)$$

in which  $W_S$  is the weight, in mg, of Isoflurophate solution in peanut oil in the *Standard preparation*, and  $C$  is the weight, in mg, of isoflurophate per g of Isoflurophate solution in peanut oil. Similarly inject 6 µL of the *Assay preparation*, and record the chromatogram. Measure the areas under the first (cyclohexanone) and second (isoflurophate) peaks, and record the values as  $a_D$  and  $a_U$ , respectively. Calculate the percentage of  $C_6H_{14}FO_3P$  in the portion of Isoflurophate taken by the formula:

$$(F(a_U / a_D)(100 / W_U)(5000)$$

in which  $W_U$  is the weight, in mg, of Isoflurophate in the *Assay preparation*; and  $F$  is the factor as determined above.



## Isoflurophate Ophthalmic Ointment

» Isoflurophate Ophthalmic Ointment contains not less than 0.0225 percent and not more than 0.0275 percent of  $C_6H_{14}FO_3P$ , in a suitable anhydrous ointment base. It is sterile.

**Packaging and storage**—Preserve in collapsible ophthalmic ointment tubes.

**Labeling**—Label it to indicate the expiration date, which is not later than 2 years after date of manufacture.

**Identification**—Place about 100 mg of Ointment in one eye of each of 3 rabbits, and examine the eyes 18 to 20 hours later: the average diameter of the pupils of the treated eyes is not less than 2 mm smaller than the average diameter of the pupils of the untreated eyes.

**Irritation**—The conjunctivas of the eyes treated as directed in the *Identification* test, as compared with those of the untreated eyes, after 1 hour, show not more than a slight reddening, which practically disappears in 4 hours.

**Sterility** (71): meets the requirements.

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921)—Dissolve about 10 g, accurately weighed, in a mixture of 25 mL each of methanol and toluene. Not more than 0.03% is found.

**Metal particles**—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* (751).

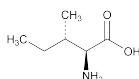
**Assay**—

*Solvent, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Isoflurophate*.

*Assay preparation*—Transfer about 3.5 g of Ophthalmic Ointment, accurately weighed, to a 50-mL centrifuge tube. Add 9 mL of *Solvent* and 1.0 mL of *Internal standard solution*, shake, and centrifuge. The bottom layer is the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Isoflurophate*.

## Isoleucine



$C_6H_{13}NO_2$   
L-Isoleucine [73-32-5].

131.17

### DEFINITION

Isoleucine contains NLT 98.5% and NMT 101.5% of L-isoleucine ( $C_6H_{13}NO_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Sample:** 130 mg of Isoleucine

**Blank:** Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of isoleucine ( $C_6H_{13}NO_2$ ) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times N \times F / W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 131.2 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

- **CHLORIDE AND SULFATE, Chloride** (221)

**Standard solution:** 0.50 mL of 0.020 N hydrochloric acid

**Sample:** 0.73 g of Isoleucine

**Acceptance criteria:** NMT 0.05%

- **CHLORIDE AND SULFATE, Sulfate** (221)

**Standard solution:** 0.10 mL of 0.020 N sulfuric acid

**Sample:** 0.33 g of Isoleucine

**Acceptance criteria:** NMT 0.03%

- **IRON** (241): NMT 30 ppm

- **HEAVY METALS, Method I** (231): NMT 15 ppm

### RELATED COMPOUNDS

**System suitability solution:** 0.4 mg/mL each of USP L-Isoleucine RS and USP L-Valine RS in 0.1 N hydrochloric acid

**Standard solution:** 0.05 mg/mL of USP L-Isoleucine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

**Sample solution:** 10 mg/mL of Isoleucine in 0.1 N hydrochloric acid

### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

### System suitability

**Suitability requirements:** The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

### Analysis

**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

**Acceptance criteria:** Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

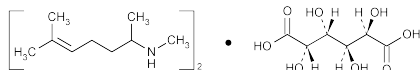
Individual impurities: NMT 0.5%  
Total impurities: NMT 2.0%

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S)  
Sample solution: 40 mg/mL in 6 N hydrochloric acid  
Acceptance criteria: +38.9° to +41.8°
- **pH** (791)  
Sample solution: 10 mg/mL of solution  
Acceptance criteria: 5.5–7.0
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP L-Isoleucine RS  
USP L-Valine RS

**Isometheptene Mucate**

(C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub> 492.65  
Isometheptene, galactarate (2:1) (salt).  
6-Methylamino-2-methylheptene, tetrahydroxyadipic acid  
(2:1) (salt) [7492-31-1].

» Isometheptene Mucate contains not less than 99.0 percent and not more than 103.0 percent of (C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
USP Isometheptene Mucate RS

**Identification**, *Infrared Absorption* (197K).

**pH** (791): between 6.0 and 7.5, in a solution (1 in 20).

**Loss on drying** (731)—Dry it at 60° for 18 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Assay**—Transfer about 500 mg of Isometheptene Mucate, accurately weighed, to a 125-mL conical flask. Add 25 mL of water, and swirl to dissolve. Add 45.0 mL of 0.1 N bromine VS, attach a 30-mL separator to the conical flask, and evacuate the system. Stir with a magnetic stirrer for 1 hour. Equilibrate the system to atmospheric pressure. Add 5 mL of hydrochloric acid to the separator, and allow 4 mL of it to enter the conical flask. Add 10 mL of potassium iodide TS to the separator, and allow the contents to pass into the conical flask. Immediately titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank titration (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.32 mg of (C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>.

**Isometheptene Mucate, Dichloralphenazone, and Acetaminophen Capsules**

» Isometheptene Mucate, Dichloralphenazone, and Acetaminophen Capsules contain not less than 85.0 percent and not more than 110.0 percent of the labeled amounts of isometheptene mucate [(C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>] and dichloralphenazone (C<sub>15</sub>H<sub>18</sub>Cl<sub>6</sub>N<sub>2</sub>O<sub>5</sub>), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Dichloralphenazone RS

USP Isometheptene Mucate RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation* obtained as directed in the *Assay*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 60 minutes.

Determine the amounts of acetaminophen, dichloralphenazone, and isometheptene mucate dissolved using the following method.

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay*.

*Standard preparation*—Prepare a solution in water containing about 0.0011 A mg of USP Acetaminophen RS, 0.0011 A/ mg of USP Dichloralphenazone RS, and 0.0011 A/ mg of USP Isometheptene Mucate RS per mL, A being the labeled amount, in mg, of acetaminophen per Capsule, / being the ratio of the labeled amount, in mg, of dichloralphenazone to the labeled amount, in mg, of acetaminophen per Capsule, and /' being the ratio of the labeled amount, in mg, of isometheptene mucate to the labeled amount, in mg, of acetaminophen per Capsule, and filter.

*Test preparation*—Filter about 20 mL of the solution under test through a glass fiber filter, discarding the first 15 mL of the filtrate.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* except to inject 100 µL, instead of 10 µL. Calculate the quantity, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), dichloralphenazone (C<sub>15</sub>H<sub>18</sub>Cl<sub>6</sub>N<sub>2</sub>O<sub>5</sub>), and isometheptene mucate [(C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>] dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of the corresponding analyte peaks obtained from the *Test preparation* and the *Standard preparation*, respectively.

*Tolerances*—Not less than 65% (Q) of the labeled amounts of isometheptene mucate [(C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>], dichloralphenazone (C<sub>15</sub>H<sub>18</sub>Cl<sub>6</sub>N<sub>2</sub>O<sub>5</sub>), and acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) are dissolved after 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

**Mobile phase**—Prepare a mixture of 0.07 M monobasic potassium phosphate, acetonitrile, 0.007 M sodium 1-decanesulfonate, and diethylamine (750:250:25:15). Adjust with phosphoric acid to a pH of 3.5. Filter and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Chromatographic test solution**—Empty the contents of 1 Capsule into a 100-mL volumetric flask, add about 80 mL of water, and swirl to dissolve. Dilute with water to volume, and mix. Pass a portion of this solution through a glass-fiber filter, discarding the first 5 mL of the filtrate. [NOTE—Prepare this solution and chromatograph it as directed under *Chromatographic system* before preparing the *Standard preparation* and the *Assay preparation*.]

**Standard preparation**—Prepare a solution having known concentrations of about 3.25 mg of USP Acetaminophen RS, 3.25/ mg of USP Dichloralphenazone RS, and 3.25/ mg of USP Isometheptene Mucate RS per mL, / being the ratio of the labeled amount, in mg, of dichloralphenazone to the labeled amount, in mg, of acetaminophen per Capsule, and /' being the ratio of the labeled amount, in mg, of isometheptene mucate to the labeled amount, in mg, of acetaminophen per Capsule.

**Assay preparation**—Transfer 20 Capsules, accurately counted, to a 2000-mL volumetric flask, add about 1900 mL of water, and heat on a steam bath until the Capsules disintegrate. While still warm, shake by mechanical means for 15 minutes, sonicate for 15 minutes, and allow to cool to ambient temperature. Dilute with water to volume, and mix. Pass a portion of this mixture through a glass-fiber filter, discarding the first 5 mL of the filtrate.

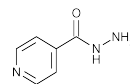
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a variable wavelength detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1 mL per minute. Chromatograph 10 μL of the *Chromatographic test solution*, and record the chromatogram to confirm that all three analytes are detected and recorded acceptable levels of sensitivity. The wavelength and sensitivity settings are at 280 nm and 3.0 absorbance units full-scale until the acetaminophen peak has been recorded, then at 243 nm and 0.5 absorbance units full-scale until the dichloralphenazone peak has been recorded, and then at 190 nm and 0.5 absorbance units full-scale until the isometheptene mucate peak has been recorded. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections determined from each analyte peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), dichloralphenazone (C<sub>15</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>), and isometheptene mucate [(C<sub>9</sub>H<sub>19</sub>N)<sub>2</sub> · C<sub>6</sub>H<sub>10</sub>O<sub>8</sub>] in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the responses of the corresponding analyte peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoniazid



C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O 137.14

4-Pyridinecarboxylic acid, hydrazide.

Isonicotinic acid hydrazide [54-85-3].

» Isoniazid contains not less than 98.0 percent and not more than 102.0 percent of C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Isoniazid RS

**Identification—**

**A: Infrared Absorption** (197K).

**B:** Transfer about 50 mg of it to a 500-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of 0.1 N hydrochloric acid, dilute with water to volume, and mix to obtain a 1 in 100,000 solution: the UV absorption spectrum of the solution so obtained exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Isoniazid RS, concomitantly measured.

**Melting range** (741): between 170° and 173°.

**pH** (791): between 6.0 and 7.5, in a solution (1 in 10).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

**Assay—**

**Mobile phase**—Dissolve 4.4 g of docusate sodium in 600 mL of methanol, add 400 mL of water, adjust with 2 N sulfuric acid to a pH of 2.5, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoniazid RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.32 mg per mL.

**Assay preparation**—Transfer about 16 mg of Isoniazid, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the isoniazid peak is not less than 1800 theoretical plates; the tailing factor for the isoniazid peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of  $C_6H_7N_3O$  in the portion of Isoniazid taken by the formula:

$$50C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Isoniazid RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of isoniazid obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoniazid Injection

» Isoniazid Injection is a sterile solution of Isoniazid in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_7N_3O$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Its package label states that if crystallization has occurred, the Injection should be warmed to redissolve the crystals prior to use.

**USP Reference standards** (11)—

USP Isoniazid RS

USP Endotoxin RS

**Identification**—

**A:** The retention time exhibited by isoniazid in the chromatogram of the *Assay preparation* corresponds to that of isoniazid in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**B:** A volume of Injection, equivalent to about 50 mg of isoniazid, responds to *Identification test B* under *Isoniazid*.

**Bacterial endotoxins** (85)—It contains not more than 0.3 USP Endotoxin Unit per mg of isoniazid.

**pH** (791): between 6.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Isoniazid*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of isoniazid, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Transfer 8.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Isoniazid*. Calculate the quantity, in mg, of  $C_6H_7N_3O$  in each mL of the Injection taken by the formula:

$$312.5(C / V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Isoniazid RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the peak responses of isoniazid obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoniazid Oral Solution

» Isoniazid Oral Solution contains, in each 100 mL, not less than 0.93 g and not more than 1.10 g of isoniazid ( $C_6H_7N_3O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Isoniazid RS

**Identification**—A volume of Oral Solution equivalent to about 50 mg of isoniazid meets the requirements of *Identification test B* under *Isoniazid*.

**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg of isoniazid, to a 100-mL beaker. Add 50 mL of a mixture of 1 part of potassium bromide in 10 parts of dilute hydrochloric acid (1 in 6), and proceed as directed under *Nitrite Titration* (451), beginning with "cool to 15°." Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of isoniazid ( $C_6H_7N_3O$ ).

## Isoniazid Tablets

» Isoniazid Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoniazid ( $C_6H_7N_3O$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Isoniazid RS

**Identification**—

**A:** The retention time of the isoniazid peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer a portion of finely powdered Tablets, equivalent to about 50 mg of isoniazid, to a 500-mL volumetric flask. Add water to volume, mix, and filter a portion of the mixture. Proceed as directed in *Identification test B* under *Isoniazid*, beginning with "Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask."

**Dissolution** (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_6H_7N_3O$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 263 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Isoniazid RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_6H_7N_3O$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Transfer 1 finely powdered Tablet to a 500-mL volumetric flask with the aid of 200 mL of water. Shake by mechanical means for 30 minutes, add water to volume, and mix. Filter, and discard the first 20 mL of the filtrate. Dilute a portion of the filtrate quantitatively and stepwise, if necessary, with a 3 in 100 mixture of 0.1 N hydrochloric acid and water to obtain a solution containing about 10 µg per mL. Dissolve an accurately weighed quantity of USP Isoniazid RS in a volume of water corresponding to that used to dissolve a similar amount of isoniazid from the Tablet, and dilute quantitatively and stepwise, if necessary, with a 3 in 100 mixture of 0.1 N hydrochloric acid and water to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorb-

ance at about 263 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isoniazid ( $C_6H_7N_3O$ ) in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of isoniazid in the Tablet;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Isoniazid RS in the Standard solution;  $D$  is the concentration, in  $\mu\text{g}$  per mL, of isoniazid in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

#### Assay—

**Buffer solution**—Prepare a 0.1 M monobasic potassium phosphate solution, adjust with 10 N sodium hydroxide to a pH of 6.9, add sufficient triethanolamine to obtain a solution having a known concentration of 0.2 mM of triethanolamine, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoniazid RS in *Mobile phase*, and dilute quantitatively, and stepwise, if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.32 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 32 mg of isoniazid, to a 100-mL volumetric flask, add 40 mL of *Mobile phase*, and sonicate for 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and centrifuge for 5 minutes.

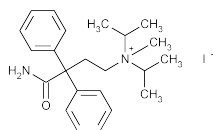
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 2.35; the column efficiency is not less than 1800 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isoniazid ( $C_6H_7N_3O$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Isoniazid RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isopropamide Iodide



$C_{23}H_{33}IN_2O$  480.43

Benzenepropanaminium,  $\gamma$ -(aminocarbonyl)- $N$ -methyl- $N$ , $N$ -bis(1-methylethyl)- $\gamma$ -phenyl-, iodide.

(3-Carbamoyl-3,3-diphenylpropyl)diisopropylmethylammonium iodide [71-81-8].

» Isopropamide Iodide, dried in vacuum at 60° for 2 hours, contains not less than 98.0 percent and not more than 101.0 percent of  $C_{23}H_{33}IN_2O$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards <11>—

USP Isopropamide Iodide RS

#### Identification—

**A: Infrared Absorption** <197K>.

**B:** To 5 mL of a solution (1 in 1000) add 5 mL of sodium carbonate solution (1 in 100), 0.5 mL of bromophenol blue TS, and 10 mL of chloroform, and shake for several minutes: the chloroform layer becomes an intense blue in color.

**C:** A solution (1 in 1000) responds to the tests for *Iodide* <191>.

**Loss on drying** <731>—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** <281>: not more than 0.5%, after ignition at  $550 \pm 25^\circ$  for 4 hours.

**Heavy metals, Method II** <231>: 0.002%.

#### Ordinary impurities <466>—

**Test solution:** methanol.

**Standard solution:** methanol.

**Eluant:** a mixture of methanol, glacial acetic acid, and water (8:1:1).

**Visualization:** 2.

**Assay**—Dissolve about 750 mg of Isopropamide Iodide, previously dried and accurately weighed, in 60 mL of glacial acetic acid, add 15 mL of mercuric acetate TS and crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 48.04 mg of  $C_{23}H_{33}IN_2O$ .

## Isopropamide Iodide Tablets

» Isopropamide Iodide Tablets contain an amount of isopropamide iodide ( $C_{23}H_{33}IN_2O$ ) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of isopropamide ( $C_{23}H_{33}N_2O$ ).

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards <11>—

USP Isopropamide Iodide RS

**Identification**—Triturate a portion of powdered Tablets, equivalent to about 10 mg of isopropamide, with 10 mL of water, and filter: the filtrate responds to *Identification* tests B and C under *Isopropamide Iodide*.

#### Dissolution <711>—

**Medium:** water; 500 mL.

**Apparatus 2:** 100 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of isopropamide ( $C_{23}H_{33}N_2O$ ) dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isopropamide Iodide RS in the same medium.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{23}H_{33}N_2O$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Crush and transfer 1 Tablet to a 100-mL volumetric flask with the aid of about 50 mL of water, and shake by mechanical means for 30 minutes. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution and a Standard solution of USP Isopropamide Iodide RS in the same medium having a known concentration of about 70  $\mu\text{g}$  per mL, in 5-cm cells at 280 nm and at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isopropamide ( $C_{23}H_{33}N_2O$ ) in the Tablet taken by the formula:

$$(353.53 / 480.43)(TC / D)[(A_{U258} - A_{U280}) / (A_{S258} - A_{S280})]$$

in which 353.53 and 480.43 are the molecular weights of isopropamide and isopropamide iodide, respectively;  $T$  is the labeled quantity, in mg, of isopropamide in the Tablet;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Isopropamide Iodide RS in the Standard solution;  $D$  is the concentration, in  $\mu\text{g}$  per mL, of isopropamide in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the test solution and the Standard solution, respectively, at the wavelengths indicated by the subscripts.

#### Assay—

**Ion-exchange column**—Insert a small pledget of glass wool in the bottom of a 6-mm  $\times$  240-mm glass tube fitted with a stopcock, fill the tube with water, and add an aqueous slurry of a suitable anion-exchange resin, chloride form (soaked in water for not less than 24 hours prior to use), until a height of about 200 mm is reached. Wash the column with 50 mL of water, and use without delay.

**Standard preparation**—Transfer about 135 mg of USP Isopropamide Iodide RS, accurately weighed, to a 250-mL volumetric flask, and dissolve in 150 mL of water. Add 5 mL of aluminum chloride solution (1 in 10) and 2 mL of ammonium hydroxide, then dilute with water to volume, mix, and filter, discarding the first 15 mL of the filtrate. Use the subsequent filtrate as directed for *Procedure*.

**Assay preparation**—Weigh and finely powder not less than 25 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of isopropamide, to a 250-mL volumetric flask, add 150 mL of water, and shake by mechanical means for 60 minutes. Add 5 mL of aluminum chloride solution (1 in 10) and 2 mL of ammonium hydroxide, dilute with water to volume, mix, and filter, discarding the first 15 mL of the filtrate. Use the subsequent filtrate as directed for *Procedure*.

**Procedure**—Pipet 50.0 mL each of the *Standard preparation* and the *Assay preparation*, respectively, onto separate ion-exchange columns, and collect the eluates in separate 200-mL volumetric flasks. Regulate the flow of effluent so that it does not exceed 40 drops per minute, and when the liquid level reaches the top of each column, add successively two 5-mL portions and one 10-mL portion of water to each column, allowing each portion just to enter the column before adding the next portion of water. After the eluates have been collected, dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 5-cm cells at 280 nm and at the maximum at about 258 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in

mg, of isopropamide ( $C_{23}H_{33}N_2O$ ) in the portion of Tablets taken by the formula:

$$(353.53 / 480.43)(0.25C)[(A_{U258} - A_{U280}) / (A_{S258} - A_{S280})]$$

in which 353.53 and 480.43 are the molecular weights of isopropamide and isopropamide iodide, respectively;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Isopropamide Iodide RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, at the wavelengths indicated by the subscripts.

## Isopropyl Alcohol



$C_3H_8O$

2-Propanol;

Isopropyl alcohol [67-63-0].

60.10

#### DEFINITION

Isopropyl Alcohol contains NLT 99.0% of isopropyl alcohol ( $C_3H_8O$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197F)

#### ASSAY

- **PROCEDURE**

**Sample:** Isopropyl Alcohol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 6.4-mm (OD)  $\times$  1.8-m stainless steel packed with 10% liquid phase G20 on support S1A

**Column temperature:** 55°

**Carrier gas:** Helium

**Flow rate:** 45 mL/min

**Injection volume:** 5  $\mu\text{L}$

**Analysis**

**Sample:** *Sample*

[NOTE—The relative retention times of some of the possible components, when present, are listed in *Table 1*.]

**Table 1**

Name	Relative Retention Time
Air	0.09
Diethyl ether	0.14
Diisopropyl ether	0.17
Acetone	0.37
Isopropyl alcohol	1.00
2-Butanol	1.64
<i>n</i> -Propyl alcohol	1.86
Water	3.14

Calculate the percentage of isopropyl alcohol ( $C_3H_8O$ ) in the *Sample* taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak response of isopropyl alcohol  
 $r_T$  = sum of all the peak responses

Acceptance criteria: NLT 99.0%

#### SPECIFIC TESTS

- **SPECIFIC GRAVITY** <841>: 0.783–0.787
- **REFRACTIVE INDEX** <831>: 1.376–1.378 at 20°
- **LIMIT OF NONVOLATILE RESIDUE**

Sample: 50 mL

**Analysis:** Evaporate the *Sample* in a tared porcelain dish on a steam bath to dryness, and heat at 105° for 1 h.

**Acceptance criteria:** NMT 2.5 mg (0.005%)

- **ACIDITY**

**Sample solution:** To 50 mL of Isopropyl Alcohol add 100 mL of carbon dioxide-free water.

**Analysis:** To the *Sample solution* add 2 drops of phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 s.

**Acceptance criteria:** NMT 0.70 mL of 0.020 N sodium hydroxide is required for neutralization.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, remote from heat.
- **USP REFERENCE STANDARDS** <11>  
USP 2-Propanol RS

### Azeotropic Isopropyl Alcohol

#### DEFINITION

Azeotropic Isopropyl Alcohol contains NLT 91.0% and NMT 93.0% of isopropyl alcohol, by volume, the remainder consisting of water.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION:** The IR absorption spectrum of a thin film of it exhibits a strong broad band at 3.0  $\mu\text{m}$ ; a strong region of absorption between 3.35 and 3.5  $\mu\text{m}$ , with its highest peak at 3.36  $\mu\text{m}$ , and others at 3.41 and 3.47  $\mu\text{m}$ ; many weak peaks between 3.6 and 6.0  $\mu\text{m}$ , among the most noticeable being those at 3.68, 3.77, 3.97, 4.17, and 5.26  $\mu\text{m}$ ; a broad band at 6.2  $\mu\text{m}$ ; a strong region of absorption between 6.7 and 7.8  $\mu\text{m}$ , the most prominent features being the peaks at 6.80, 7.09, 7.25 (the highest), 7.46, and 7.63  $\mu\text{m}$ ; a strong region of absorption between 8.5 and 9.2  $\mu\text{m}$ , peaking at 8.6, 8.85, and 9.0  $\mu\text{m}$ ; and strong peaks at 10.5 and 12.3  $\mu\text{m}$ .

#### IMPURITIES

- **LIMIT OF NONVOLATILE RESIDUE**

Sample: 50 mL

**Analysis:** Evaporate the *Sample* in a tared porcelain dish on a steam bath to dryness, and heat at 105° for 1 h.

**Acceptance criteria:** The weight of the residue does not exceed 2.5 mg (0.005%).

- **VOLATILE IMPURITIES**

**System suitability solution:** USP 2-Propanol System Suitability RS

**Sample solution:** Azeotropic Isopropyl Alcohol (Neat) Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 0.25-mm  $\times$  60-m, coated with a 1.4- $\mu\text{m}$  film of phase G43

**Temperature**

**Injector:** 150°

**Detector:** 200°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	0	35	5
35	1	45	2
45	10	100	1

**Carrier gas:** Helium

**Linear velocity:** 35 cm/s

**Injection size:** 1  $\mu\text{L}$

**Split ratio:** 10:1

**Run time:** 30 min

**System suitability**

**Sample:** *System suitability solution*

[NOTE—Approximate relative retention times for ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol are 0.6, 0.7, 1.0, 1.1, 1.3, and 1.5, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between acetone and isopropyl alcohol

**Signal-to-noise ratio:** NLT 10 for any of the following peaks: ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol

**Tailing factor:** NMT 2.0 for the isopropyl alcohol peak

**Relative standard deviation:** NMT 2.0% for the isopropyl alcohol peak

**Analysis**

**Samples:** *Sample solution*

Calculate the ratio of isopropyl alcohol ( $\text{C}_3\text{H}_8\text{O}$ ) in the portion of Azeotropic Isopropyl Alcohol taken:

$$\text{Result} = (r_i/r_T)$$

$r_i$  = peak area for isopropyl alcohol

$r_T$  = sum of all of the peak areas excluding the peak for water

**Acceptance criteria:** NLT 0.99

#### SPECIFIC TESTS

- **SPECIFIC GRAVITY** <841>: 0.815–0.810, indicating 91.0%–93.0% of isopropyl alcohol ( $\text{C}_3\text{H}_8\text{O}$ ) by volume
- **REFRACTIVE INDEX** <831>: 1.376–1.378 at 20°
- **ACIDITY**

**Sample:** 50 mL

**Analysis:** Place the *Sample* in a suitable flask, and add 100 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 s.

**Acceptance criteria:** NMT 0.70 mL of 0.020 N sodium hydroxide is required for neutralization.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, remote from heat.
- **USP REFERENCE STANDARDS** <11>  
USP 2-Propanol System Suitability RS  
It contains 0.1% of each of the following: ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol.

## Isopropyl Rubbing Alcohol

» Isopropyl Rubbing Alcohol contains not less than 68.0 percent and not more than 72.0 percent of isopropyl alcohol, by volume, the remainder consisting of water, with or without suitable stabilizers, perfume oils, and color additives certified by the FDA for use in drugs.

**Packaging and storage**—Preserve in tight containers, remote from heat.

**Labeling**—Label it to indicate that it is flammable.

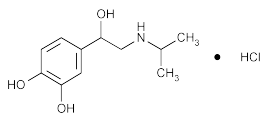
**Specific gravity** (841): between 0.872 and 0.883 at 20°.

**Acidity**—Transfer 50 mL to a suitable flask, and add about 75 mL of carbon dioxide-free water. Titrate potentiometrically to a pH of 8.5: not more than 1.0 mL of 0.020 N sodium hydroxide is required for neutralization.

**Limit of nonvolatile residue**—Evaporate 50 mL to dryness in a tared porcelain dish on a steam bath, and dry at 105° for 1 hour: the weight of the residue does not exceed 5 mg (0.01%).

**Assay**—Transfer 50.0 mL of Isopropyl Rubbing Alcohol to a 250-mL distilling flask, and add 100 mL of water. Arrange the flask for distillation, distil, and collect 95 mL of distillate in a 100-mL volumetric flask. Dilute to volume with water, mix, and determine the specific gravity of the distillate at 25° (see *Specific Gravity* (841)). The specific gravity is between 0.955 and 0.950, corresponding to between 68.0% and 72.0% of isopropyl alcohol in the specimen taken.

## Isoproterenol Hydrochloride



$C_{11}H_{17}NO_3 \cdot HCl$  247.72

1,2-Benzenediol, 4-[1-hydroxy-2-

[(1-methylethyl)amino]ethyl]-, hydrochloride.

3,4-Dihydroxy- $\alpha$ -[(isopropylamino)methyl]benzyl alcohol hydrochloride [51-30-9].

» Isoproterenol Hydrochloride contains not less than 97.0 percent and not more than 101.5 percent of  $C_{11}H_{17}NO_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Isoproterenol Hydrochloride RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 50  $\mu$ g per mL.

*Medium:* water.

**Melting range** (741): between 165° and 170°.

**Loss on drying** (731)—Dry about 1 g, accurately weighed, in vacuum over phosphorus pentoxide for 4 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Sulfate** (221)—A 0.10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).

**Limit of isoproterenone**—Its absorptivity (see *Spectrophotometry and Light-scattering* (851)) at 310 nm, determined in a solution containing 2 mg per mL, is not more than 0.2.

**Chloride content**—Dissolve about 500 mg, accurately weighed, in 5 mL of water. Add 5 mL of glacial acetic acid and 40 mL of methanol. Add eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 13.9% and 14.6% of Cl is found, calculated on the dried basis.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in freshly prepared sodium bisulfite solution (3 in 1000) to obtain a solution having a concentration of about 2.5 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix to obtain a solution having a known concentration of about 250  $\mu$ g per mL.

**Assay preparation**—Transfer about 125 mg of Isoproterenol Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 30-cm  $\times$  4-mm stainless steel column that contains packing L1. The mobile phase is 0.17 N acetic acid having a flow rate of about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%.

**Procedure**—Using a microsyringe or sampling valve, chromatograph 10  $\mu$ L of the *Standard preparation*, and adjust the specimen size and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Chromatograph equal volumes of the *Standard preparation* and the *Assay preparation*, and measure the peak responses. Calculate the quantity, in mg, of  $C_{11}H_{17}NO_3 \cdot HCl$  in the portion of Isoproterenol Hydrochloride taken by the formula:

$$0.5C(h_U / h_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; and  $h_U$  and  $h_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoproterenol Inhalation Solution

» Isoproterenol Inhalation Solution is a sterile solution of Isoproterenol Hydrochloride in Purified Water. It may contain Sodium Chloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

**Labeling**—Label it to indicate that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.



**USP Reference standards** (11)—  
USP Isoproterenol Hydrochloride RS**Color and clarity**—

**Standard solution**—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Visually examine a portion of the Inhalation Solution (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

**Sterility** (71): meets the requirements.

**pH** (791): between 2.5 and 5.5.

**Assay**—

**Standard preparation**—Prepare as directed in the *Assay* under *Isoproterenol Hydrochloride*.

**Assay preparation**—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 25 mg of isoproterenol hydrochloride, to a 100-mL volumetric flask, dilute with 0.17 N acetic acid solution to volume, and mix.

**Chromatographic system**—Proceed as directed in the *Assay* under *Isoproterenol Hydrochloride*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Isoproterenol Hydrochloride*. Calculate the quantity, in mg, of  $C_{11}H_{17}NO_3 \cdot HCl$  in each mL of the Inhalation Solution taken by the formula:

$$0.1(C/V)(h_u / h_s)$$

in which *V* is the volume, in mL, of Inhalation Solution taken; and *C*, *h<sub>u</sub>*, and *h<sub>s</sub>* are as defined therein.

**Isoproterenol Hydrochloride Inhalation Aerosol**

» Isoproterenol Hydrochloride Inhalation Aerosol is a solution of Isoproterenol Hydrochloride in Alcohol in an inert propellant base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

**USP Reference standards** (11)—  
USP Isoproterenol Hydrochloride RS**Identification**—

**A:** Place 10 mL of water in a small beaker, and deliver 10 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the stem tip against the bottom of the beaker. Filter, place 5 mL of the filtrate in a test tube, and retain the remainder of the solution for *Identification test B*. Add 1 drop of 0.2 N sulfuric acid and 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a salmon-pink color is produced.

**B:** Dilute the remainder of the filtrate obtained in *Identification test A* with an equal volume of water. Add a few

drops of 6 N ammonium hydroxide, filter, acidify the filtrate with nitric acid, and divide into two equal portions. To each portion add a few drops of silver nitrate TS: white precipitates are formed. To one portion add a slight excess of nitric acid: the white precipitate remains. To the other portion add a slight excess of 6 N ammonium hydroxide, and shake: the precipitate dissolves.

**Alcohol content** (611)—Weigh accurately a filled Inhalation Aerosol container, and record the weight. Place the container in a dry ice-alcohol bath, and cool for 60 minutes. Remove the container from the bath, and carefully remove the valve with wire cutters, taking precautions to save all pieces of the valve and cap. With the aid of three 5-mL portions of water, transfer the contents of the container to a beaker previously chilled in the bath. Dry the rinsed empty container and all of its parts in an oven at 105° for 2 hours, cool, and weigh. Calculate the weight of the container contents. Add a few boiling chips to the beaker, and carefully stir to help evaporate the propellant. After the bulk of the propellant has evaporated, transfer the contents of the beaker, with the aid of several mL of water, to a glass-stoppered graduated cylinder, measure the volume, and determine the alcohol content of the *Test solution* thus prepared by the gas-liquid chromatographic procedure, methyl ethyl ketone being used as the internal standard. Calculate the alcohol content of the Inhalation Aerosol taken by the formula:

$$SV / W$$

in which *S* is the percentage of alcohol (w/v) in the *Test solution*; *V* is the volume, in mL, of the *Test solution*; and *W* is the weight, in g, of the container contents: between 28.5% and 38.5% (w/w) of  $C_2H_5OH$  is found.

**Dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

**PROCEDURE FOR DOSE UNIFORMITY**—

**Ferro-citrate solution and Buffer solution**—Prepare as directed under *Epinephrine Assay* (391).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 6 µg per mL.

**Test preparation**—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as directed for *Procedure*.

**Procedure**—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* followed by 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg, of  $C_{11}H_{17}NO_3 \cdot HCl$  contained in the minimum dose taken by the formula:

$$20CN (A_u / A_s)$$

in which *C* is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; *N* is the number of sprays discharged to obtain the minimum dose; and *A<sub>u</sub>* and *A<sub>s</sub>* are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay—**

*Ferro-citrate Solution, Buffer Solution, and Standard preparation*—Prepare as directed under *Unit spray content*.

*Assay preparation*—Fit the Inhalation Aerosol container with its inhalation actuator, and prime the unit by firing it 10 times. Remove the actuator, and accurately weigh the container with its remaining contents. Invert the container, place the valve stem tip against the bottom of a 100-mL beaker containing 20 mL of chloroform, and deliver under the surface of the chloroform a number of sprays equivalent to about 500 µg of isoproterenol hydrochloride. Raise the unit above the contents of the beaker, and wash the valve stem with about 2 mL of chloroform. Collect the washings in the beaker. Allow the valve stem to dry, and again accurately weigh the Inhalation Aerosol container with its remaining contents. Record the weight of the expelled specimen. Transfer the contents of the beaker to a centrifuge tube with the aid of two 3-mL portions of chloroform, add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500), and shake vigorously for 1 minute. Centrifuge, and use the clear supernatant as directed for *Procedure*.

*Procedure*—Into three separate test tubes, pipet 5 mL each of the *Assay preparation*, *Standard preparation*, and water to provide the blank, respectively. To each tube add 100 µL of *Ferro-citrate Solution*, followed by 1.0 mL of *Buffer Solution*, and mix. Determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the percentage (w/w) of  $C_{11}H_{17}NO_3 \cdot HCl$  in the portion of Inhalation Aerosol taken by the formula:

$$0.001(C/W)(A_U/A_S)$$

in which *C* is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; *W* is the weight, in g, of Inhalation Aerosol taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoproterenol Hydrochloride Injection

» Isoproterenol Hydrochloride Injection is a sterile solution of Isoproterenol Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

**Labeling**—Label it to indicate that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Isoproterenol Hydrochloride RS

**Color and clarity**—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial endotoxins** (85)—It contains not more than 1250.0 USP Endotoxin Units per mg of isoproterenol hydrochloride.

**pH** (791): between 2.5 and 4.5.

**Particulate matter** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay—**

*Mobile phase*—Dissolve 1.76 g of sodium 1-heptanesulfonate in 800 mL of water. Add 200 mL of methanol, and adjust with 1 M phosphoric acid to a pH of  $3.0 \pm 0.1$ . Pass the solution through a membrane filter having a 1-µm or finer porosity.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in freshly prepared sodium bisulfite solution (1 in 1000) to obtain a solution having a known concentration of about 20 µg per mL.

*Resolution solution*—Prepare a solution of epinephrine bitartrate in freshly prepared *Mobile phase* containing 1.0% of sodium bisulfite, having a concentration of about 200 µg per mL. Mix 2.0 mL of this solution and 18.0 mL of the *Standard preparation*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with freshly prepared sodium bisulfite solution (1 in 1000) to obtain a solution having a concentration of about 20 µg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%. Chromatograph the *Resolution solution*: the relative retention times are about 0.55 for epinephrine and 1.0 for isoproterenol; the resolution, *R*, for epinephrine and isoproterenol is not less than 3.5; and the tailing factors for the epinephrine and isoproterenol peaks are not more than 2.5.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation*, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$C(L/D)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; *L* is the labeled quantity, in µg per mL, of isoproterenol hydrochloride in the Injection; *D* is the concentration, in µg per mL, of isoproterenol hydrochloride in the *Assay preparation*, on the basis of the labeled quantity in each mL and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoproterenol Hydrochloride Tablets

» Isoproterenol Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{11}H_{17}NO_3 \cdot HCl$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Isoproterenol Hydrochloride RS

**Identification**—Powder a number of Tablets, equivalent to about 50 mg of isoproterenol hydrochloride, digest with 15 mL of hot dehydrated alcohol for 20 minutes, cool, filter, and evaporate the filtrate on a steam bath to dryness: the residue responds to the *Identification* tests under *Isoproterenol Hydrochloride*.

**Dissolution** <711>—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{11}H_{17}NO_3 \cdot HCl$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isoproterenol Hydrochloride RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{11}H_{17}NO_3 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

*Procedure for content uniformity*—Crush 1 Tablet, and transfer it with the aid of 25 mL of water to a 50-mL volumetric flask. Shake gently until no more dissolves, add water to volume, and mix. Filter through a dry filter into a dry flask, rejecting the first 20 mL of the filtrate. Transfer a portion of the filtrate, equivalent to about 2.5 mg of isoproterenol hydrochloride and accurately measured, to a 50-mL volumetric flask, dilute with water to volume, and mix. Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly and without delay, determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 279 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{11}H_{17}NO_3 \cdot HCl$  in the Tablet taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the Standard solution; V is the volume, in mL, of the filtrate taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—

*Mobile phase*—Adjust 0.1 M sodium sulfate with phosphoric acid to a pH of 3.0. Mix 90 parts of this solution with 10 parts of methanol.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in 0.1 N sulfuric acid, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of isoproterenol hydrochloride, and transfer with the aid of 50 mL of 0.1 N sulfuric acid to a 100-mL volumetric flask. Shake gently until no more dissolves, dilute with the same solvent to volume, and mix. Filter through a dry filter into a dry flask, rejecting the first 20 mL of the filtrate.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into

the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention time is about 3.5 minutes for isoproterenol. Calculate the quantity, in mg, of  $C_{11}H_{17}NO_3 \cdot HCl$  in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoproterenol Hydrochloride and Phenylephrine Bitartrate Inhalation Aerosol

» Isoproterenol Hydrochloride and Phenylephrine Bitartrate Inhalation Aerosol is a suspension of microfine Isoproterenol Hydrochloride and Phenylephrine Bitartrate in suitable propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ) and phenylephrine bitartrate ( $C_9H_{13}NO_2 \cdot C_4H_6O_6$ ).

**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

**USP Reference standards** <11>—

USP Isoproterenol Hydrochloride RS  
USP Phenylephrine Hydrochloride RS

**Identification**—

**A:** Place 10 mL of water in a small beaker, and deliver 20 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To 5 mL of the solution add 1 drop of dilute sulfuric acid (1 in 200), add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

**B:** To the balance of the solution obtained in *Identification* test A add 3 mL of *Mercuric sulfate solution*, prepared as directed in the test for *Dose uniformity over the entire contents*. Heat on a steam bath for 5 minutes, cool, and add 3 mL of sodium nitrite solution (1 in 80): a deep red color is produced.

**Dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* <601>.

**PROCEDURE FOR DOSE UNIFORMITY**—

*Ferro-citrate solution and Buffer solution*—Prepare as directed under *Epinephrine Assay* <391>.

*Mercuric sulfate solution*—While stirring a mixture of 15 g of yellow mercuric oxide and 80 mL of water, slowly add 20 mL of sulfuric acid, and stir until completely dissolved.

*Isoproterenol hydrochloride standard preparation*—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in dilute sulfuric acid (1 in 1000), and dilute quantitatively and stepwise with the same dilute sulfuric acid as necessary to obtain a solution having a known concentration of about 8 µg per mL.

*Phenylephrine hydrochloride standard preparation*—Dissolve an accurately weighed quantity of USP Phenylephrine Hydrochloride RS in dilute sulfuric acid (1 in 1000), and dilute quantitatively and stepwise with the same dilute sulfuric

acid as necessary to obtain a solution having a known concentration of about 12 µg per mL.

**Test preparation for isoproterenol hydrochloride**—Discharge the minimum recommended dose into the sampling apparatus, and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of dilute sulfuric acid (1 in 1000), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 20 minutes. Use the clear supernatant as directed in the *Procedure for isoproterenol hydrochloride*.

**Test preparation for phenylephrine bitartrate**—Proceed as directed for *Test preparation for isoproterenol hydrochloride* and use the clear supernatant as directed in the *Procedure for phenylephrine bitartrate*.

**Procedure for isoproterenol hydrochloride**—Into three separate flasks transfer the *Test preparation for isoproterenol hydrochloride*, 20.0 mL of the *Isoproterenol hydrochloride standard preparation*, and 20.0 mL of dilute sulfuric acid (1 in 1000) to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the *Test preparation* and the *Standard preparation* against the blank at the wavelength of maximum absorbance at about 530 nm, in 5-cm cells, with a suitable spectrophotometer. Calculate the quantity, in µg, of  $C_{11}H_{17}NO_3 \cdot HCl$  contained in the minimum dose taken by the formula:

$$20CN(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation*; N is the number of sprays discharged to obtain the minimum dose; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Procedure for phenylephrine bitartrate**—Into three separate flasks transfer the *Test preparation for phenylephrine bitartrate*, 20.0 mL of the *Phenylephrine hydrochloride standard preparation*, and 20.0 mL of dilute sulfuric acid (1 in 1000) to provide the blank. To each flask add 3.0 mL of *Mercuric sulfate solution*, and mix. Immerse in a water bath maintained between 35° and 40° for 10 minutes, remove, and allow to stand at room temperature for 30 minutes. Add 0.25 mL of a sodium nitrite solution (1 in 80), mix, and allow to stand, with occasional swirling, for 30 minutes. Concomitantly determine the absorbances of the *Test preparation* and the *Standard preparation* against the blank at the wavelength of maximum absorbance at about 495 nm, in 5-cm cells, with a suitable spectrophotometer. Calculate the quantity, in µg, of  $C_9H_{13}NO_2 \cdot C_4H_6O_6$  in the minimum dose taken by the formula:

$$(317.30 / 203.67)(20CN)(A_U / A_S)$$

in which 317.30 and 203.67 are the molecular weights of phenylephrine bitartrate and phenylephrine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation*; N is the number of sprays discharged to obtain the minimum dose; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Particle size**—Prime the valve of the Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450× magnification. Focus on the particles of 25 fields of view near the center of the sample pattern, and note the size of

the great majority of individual particles: they are less than 5 µm in diameter. Record the size of all individual crystalline particles (not agglomerates) more than 10 µm in length measured along the longest axis: not more than 10 such particles are observed.

#### Assay—

**Ferro-citrate solution, Buffer solution, and Mercuric sulfate solution**—Prepare as directed in the test for *Dose uniformity over the entire contents*.

**Isoproterenol hydrochloride standard preparation**—Transfer about 50 mg of USP Isoproterenol Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. The concentration of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation* is about 100 µg per mL.

**Phenylephrine hydrochloride standard preparation**—Transfer about 50 mg of USP Phenylephrine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. The concentration of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation* is about 100 µg per mL.

**Assay preparation**—[NOTE—Valve actuation during sampling is to be performed in a manner that will deliver freely the spray into the solvent in the bottom of the sampling beaker, but with minimal lateral pressure on the valve stem to avoid possible side-of-stem leakage. Any devices and techniques designed to accomplish these objectives may be used (e.g., actuating the valve by pressing the tip into an indentation in the bottom of the beaker or using a special adapter to spray at right angles to the valve held at the bottom of the beaker).] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of the Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform. Raise the Aerosol above the surface of the chloroform, and shake it gently preparatory to delivering another spray similarly under the surface of the chloroform. Deliver a total of 6 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the sample in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 6 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of dilute sulfuric acid (1 in 1000). Insert the stopper, shake vigorously for 1 minute, centrifuge for 20 minutes, and use the clear supernatant.

**Procedure for isoproterenol hydrochloride**—Transfer 3.0 mL each of the *Isoproterenol hydrochloride standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 0.10 mL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ) in each mL of the Aerosol taken by the formula:

$$(0.01Cd / W)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation*; d is the density, in g per mL, of Aerosol taken; W is the weight, in g, of the sample taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Isoproterenol hydrochloride standard preparation*, respectively. [The density, d, is determined as follows. Weigh a known volume (v) of the Aerosol in a suitable 5-mL gas-tight syringe equipped with a linear valve.

Calibrate the volume of the syringe by filling to the 5-mL mark with dichlorotetrafluoroethane withdrawn from a plastic-coated glass vial sealed with a neoprene multiple-dose rubber stopper and an aluminum seal, using 1.456 g per mL as the density of the calibrating liquid. Maintain the dichlorotetrafluoroethane, the Aerosol sample, and the syringe (protected from becoming wet) at 25° in a water bath. Obtain the sample, equivalent to the same volume as that obtained during the sampling procedure, from the Aerosol by means of a sampling device consisting of a replaceable rubber septum engaged in the plate threads at one end of a threaded fitting, the opposite end of which contains a sharpened tube capable of puncturing the aerosol container, and a rubber gasket around the tube to prevent leakage of the container contents after puncture.\* Calculate the density taken by the formula:

$$w/v$$

in which *w* is the weight of the volume, *v*, of Aerosol taken.]

**Procedure for phenylephrine bitartrate**—Transfer 5.0 mL each of the *Phenylephrine hydrochloride standard preparation*, the *Assay preparation*, and a blank consisting of dilute sulfuric acid (1 in 1000), to separate test tubes. To each tube add 3.0 mL of *Mercuric sulfate solution*, and mix. Immerse in a water bath maintained between 35° and 40° for 10 minutes, remove, and allow to stand at room temperature for 30 minutes. Add 0.25 mL of sodium nitrite solution (1 in 80), mix, and allow to stand, with occasional swirling, for 30 minutes. Concomitantly determine the absorbances of the solutions against the blank in 1-cm cells at the wavelength of maximum absorbance at about 495 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of phenylephrine bitartrate ( $C_9H_{13}NO_2 \cdot C_4H_6O_6$ ) in each mL of the Aerosol taken by the formula:

$$(317.30 / 203.67)(0.01Cd / W)(A_U / A_S)$$

in which 317.30 and 203.67 are the molecular weights of phenylephrine bitartrate and phenylephrine hydrochloride, respectively; *C* is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation*; *d* is the density, in g per mL, of Aerosol taken; *W* is the weight, in g, of the sample taken; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solutions from the *Assay preparation* and the *Phenylephrine hydrochloride standard preparation*, respectively.

## Isoproterenol Sulfate

( $C_{11}H_{17}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · 2H<sub>2</sub>O 556.62

1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]-, sulfate (2:1) (salt), dihydrate.

3,4-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol sulfate (2:1) (salt) dihydrate [6700-39-6].

Anhydrous 520.60 [299-95-6].

» Isoproterenol Sulfate contains not less than 97.0 percent and not more than 103.0 percent of ( $C_{11}H_{17}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Isoproterenol Hydrochloride RS

\* A suitable sampling system is available from Alltech Associates, 2051 Waukegan Rd., Deerfield, IL 60015.

## Identification—

**A: Ultraviolet Absorption** (197U)—

**Solution:** 50 µg per mL.

**Medium:** 0.1 N hydrochloric acid.

**B:** To a solution of 10 mg in 5 mL of water add 1 drop of ferric chloride TS: an intense green color is produced, and it becomes olive-green on standing.

**C:** To a solution of 10 mg in 1 mL of water add 1 drop of phosphotungstic acid TS: a white precipitate is formed immediately and it becomes brown on standing (*distinction from epinephrine, which forms no precipitate*).

**D:** Dilute 1.0 mL of a solution (1 in 1000) with water to 10 mL, add 0.1 mL of dilute hydrochloric acid (1 in 120), then add 1.0 mL of 0.10 N iodine. Allow to stand for 5 minutes, and add 2.0 mL of 0.10 N sodium thiosulfate: a salmon pink color is produced (*distinction from norepinephrine, which, at the same pH, about 3, produces no color or at most only a slight pink color*).

**E:** It responds to the tests for *Sulfate* (191).

**Water, Method I** (921): not more than 7.0%.

**Residue on ignition** (281): not more than 0.2%.

**Chloride** (221)—A 0.10-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.14%).

**Limit of isoproterenone**—It meets the requirements of the test for *Isoproterenone* under *Isoproterenol Hydrochloride*.

## Assay—

**Standard preparation**—Prepare as directed in the *Assay under Isoproterenol Hydrochloride*.

**Assay preparation**—Transfer about 125 mg of Isoproterenol Sulfate, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

**Chromatographic system**—Proceed as directed for *Procedure* in the *Assay under Isoproterenol Hydrochloride*. Calculate the quantity, in mg, of ( $C_{11}H_{17}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> in the portion of Isoproterenol Sulfate taken by the formula:

$$(260.30 / 247.72)(0.5C)(h_U / h_S)$$

in which 260.30 is one-half of the molecular weight of anhydrous isoproterenol sulfate, 247.72 is the molecular weight of isoproterenol hydrochloride; and *C*, *h<sub>U</sub>*, and *h<sub>S</sub>* are as defined therein.

## Isoproterenol Sulfate Inhalation Aerosol

» Isoproterenol Sulfate Inhalation Aerosol is a suspension of microfine Isoproterenol Sulfate in fluorochlorohydrocarbon propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoproterenol sulfate [( $C_{11}H_{17}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>].

**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

**USP Reference standards** (11)—  
USP Isoproterenol Hydrochloride RS

## Identification—

**A:** Place 10 mL of water in a small beaker, and deliver 10 sprays from the Aerosol under the surface of the water, ac-

tuating the valve by pressing the tip against the bottom of the beaker. Filter, and to 5 mL of the filtrate add 1 drop of dilute hydrochloric acid (1 in 120). Add 0.50 mL of 0.10 N iodine, allow to stand for 5 minutes, and add 1.0 mL of 0.10 N sodium thiosulfate: a red-brown color is produced.

**B:** A portion of the filtrate obtained in *Identification* test A responds to the tests for *Sulfate* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Dose uniformity over the entire contents:** meets the requirements for *Metered-Dose Inhalers* under *Aerosols*, *Nasal Sprays*, *Metered-Dose Inhalers*, and *Dry Powder Inhalers* (601).

#### PROCEDURE FOR DOSE UNIFORMITY—

*Ferro-citrate solution and Buffer solution*—Prepare as directed under *Epinephrine Assay* (391).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 4 µg per mL.

*Test preparation*—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

*Procedure*—Into three separate flasks transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg, of  $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$  contained in the minimum dose taken by the formula:

$$(260.30 / 247.72)(20CN)(A_U / A_S)$$

in which 260.30 is one-half of the molecular weight of isoproterenol sulfate (anhydrous), and 247.72 is the molecular weight of isoproterenol hydrochloride; C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

**Particle size**—Prime the valve of the Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of the spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450 × magnification. Focus on the particles of 25 fields of view near the center of the test specimen pattern, and note the size of the great majority of individual particles: they are less than 5 µm in diameter. Record the number and size of all individual crystalline particles (not agglomerates) more than 10 µm in length measured along the longest axis: not more than 10 such particles are observed.

#### Assay—

*Ferro-citrate solution and Buffer solution*—Prepare as directed under *Epinephrine Assay* (391).

*Standard preparation*—Transfer about 50 mg of USP Isoproterenol Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add a freshly prepared solution of sodium bisulfite (1 in 500) to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with the sodium bisulfite solution to volume, and mix. The concentration of USP Isoproterenol Hydrochloride RS in the *Standard preparation* is about 50 µg per mL.

*Assay preparation*—[NOTE—A suitable sampling beaker is one having a small indentation formed in its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the sample delivery.] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of the Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the chloroform, and shake it gently preparatory to delivering another spray similarly under the surface of the chloroform. Deliver a total of 5 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the sample in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 5 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500). Insert the stopper, shake vigorously for 1 minute, centrifuge for 5 minutes, and use the clear supernatant as directed in the *Procedure*.

*Procedure*—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$  in each mL of the Aerosol taken by the formula:

$$(260.30 / 247.72)(0.01Cd / W)(A_U / A_S)$$

in which 260.30 is one-half of the molecular weight of isoproterenol sulfate (anhydrous); 247.72 is the molecular weight of isoproterenol hydrochloride; C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; d is the density, in g per mL, of the Aerosol; W is the weight, in g, of the portion of Inhalation Aerosol taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. [The density, d, is determined as follows. Weigh a known volume (v) of the Inhalation Aerosol in a suitable, gas-tight, 5-mL syringe equipped with a linear valve. Calibrate the volume of the syringe by filling to the 5-mL mark with dichlorotetrafluoroethane withdrawn from a plastic-coated glass vial sealed with a neoprene multiple-dose rubber stopper and an aluminum seal, using 1.456 g per mL as the density of the calibrating liquid. Maintain the dichlorotetrafluoroethane, the Aerosol assay specimen, and the syringe (protected from becoming wet) in a water bath at 25°. Obtain the specimen equivalent to the same volume as that obtained during the sampling procedure, from the Aerosol by means of a sampling device consisting of a replaceable rubber septum engaged in the plate threads at one end of a threaded fitting, the opposite end of which contains a sharpened tube capable of puncturing the aerosol container, and a rubber gasket around the tube to prevent leakage of the container contents after puncture.\* Record the weight of the volume (v) of the Aerosol as w, and calculate the density by the formula  $w/v$ .]

\*A suitable sampling system is available from Alltech Associates, 2051 Waukegan Rd., Deerfield, IL 60015.

## Isoproterenol Sulfate Inhalation Solution

» Isoproterenol Sulfate Inhalation Solution is a sterile solution of Isoproterenol Sulfate in Purified Water. It may contain Sodium Chloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol sulfate  $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ .

**Packaging and storage**—Store in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

**Labeling**—Label it to indicate that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—  
USP Isoproterenol Hydrochloride RS

**Color and clarity**—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

**Identification**—It meets the requirements for *Identification* tests *C*, *D*, and *E* under *Isoproterenol Sulfate*.

**Sterility** (71): meets the requirements.

**Assay**—

*Standard preparation*—Prepare as directed in the *Assay* under *Isoproterenol Hydrochloride*.

*Assay preparation*—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 25 mg of isoproterenol sulfate, to a 100-mL volumetric flask, add 50.0 mL of 0.30 N acetic acid, dilute with water to volume, and mix.

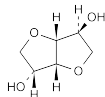
*Chromatographic system*—Proceed as directed in the *Assay* under *Isoproterenol Hydrochloride*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Isoproterenol Hydrochloride*. Calculate the quantity, in mg, of  $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$  in each mL of the Inhalation Solution taken by the formula:

$$(260.30/247.72)(0.1)(C/V)(h_u / h_s)$$

in which 260.30 is one-half of the molecular weight of anhydrous isoproterenol sulfate; 247.72 is the molecular weight of isoproterenol hydrochloride; *V* is the volume, in mL, of Inhalation Solution taken; and *C*, *h<sub>u</sub>*, and *h<sub>s</sub>* are as defined therein.

## Isosorbide Concentrate



$C_6H_{10}O_4$  146.14  
D-Glucitol, 1,4:3,6-dianhydro-  
1,4:3,6-Dianhydro-D-glucitol [652-67-5].

» Isosorbide Concentrate is an aqueous solution containing, in each 100 g, not less than 70.0 g and not more than 80.0 g of  $C_6H_{10}O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—The label states that this article is not intended for direct administration to humans or animals.

**USP Reference standards** (11)—  
USP Isosorbide RS

**Identification**—[NOTE—Isosorbide is hygroscopic. Take precautions to protect isolated isosorbide crystals from atmospheric moisture.]

**A:** Dry a portion of it in an evaporating dish over phosphorus pentoxide at 70° and at a pressure of 50 mm of mercury for 48 hours, changing the phosphorus pentoxide after 24 hours. Scratch the bottom of the dish with a glass rod or seed with a crystal of isosorbide, if necessary, to initiate crystallization: the crystals so obtained melt between 60° and 63° when tested by the procedure for *Class I* substances (see *Melting Range or Temperature* (741)).

**B:** The IR absorption spectrum of a potassium bromide dispersion of the crystals obtained as directed in *Identification* test *A* exhibits maxima and minima only at the same wavelengths as that of a similar preparation of USP Isosorbide RS.

**Specific rotation** (781S): between +44.5° and +47.0°.

*Test solution:* 80 mg of isosorbide per mL, in water.

**Water**, *Method I* (921): between 24.0% and 26.0%.

**Residue on ignition** (281): not more than 0.01%.

**Heavy metals**, *Method II* (231): not more than 5 ppm, calculated on the anhydrous basis.

**Periodate consumption**—Dilute about 15 g, accurately weighed, with 25 mL of water, and add 50.0 mL of a solution prepared by dissolving 5.4 g of periodic acid in 100 mL of water and adding 1900 mL of glacial acetic acid. Allow to stand for 1 hour. Add 20 mL of potassium iodide TS, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the brown color. Add 3 mL of starch TS, and complete the titration. Perform a blank determination, and note the difference in volumes required. If the volume required for the specimen is less than 0.8 of that required for the blank, repeat the procedure with a smaller specimen. The difference in volume corresponds to not more than 0.20 mL of 0.1 N sodium thiosulfate for each g of Concentrate taken.

**Acid value**—Dilute about 15 g, accurately weighed, with 50 mL of water, and titrate with 0.02 N potassium hydroxide VS to a phenolphthalein endpoint. Perform a blank determination, and make any necessary correction. Calculate the acid value taken by the formula:

$$56.11(AN / W)$$

in which *A* is the number of mL of potassium hydroxide VS consumed; *N* is its normality; and *W* is the weight, in g, of Concentrate taken. The limit is 0.5, calculated on the anhydrous basis.

**Methyl ethyl ketone**—

*Internal standard solution*—Prepare a solution in water containing about 1 mg per mL of methyl isobutyl ketone.

*Standard preparation*—Prepare a solution in water containing an accurately known concentration of methyl ethyl ketone equivalent to about 1 mg per mL. Pipet 5 mL of this solution into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, add water to volume, and mix.

*Test preparation*—Pipet 5 mL of *Internal standard solution* into a 100-mL volumetric flask, add Concentrate to volume, and mix.

*Support*—Place about 90 g of unsilanized support S1A in a crystallizing dish, and cover it with chloroform. Stir the mixture thoroughly, and carefully remove the supernatant chloroform with an aspirator. Spread the moist support on a clean surface, and allow it to air-dry. Place the dried support in the crystallizing dish, and cover it with 0.5 N alcoholic potassium hydroxide TS. Allow it to stand for one-half hour with occasional stirring. Carefully pour off the supernatant

alcoholic potassium hydroxide solution, and wash the moist support with water until the washing is neutral to phenolphthalein indicator. Spread the moist support on a clean surface, and allow it to air-dry.

**Chromatographic system**—The gas chromatograph is equipped with a flame-ionization detector and a 0.6-m × 2-mm column packed with 25% liquid phase G16 on unsilanized acid- and base-washed *Support* which has been washed with chloroform, and conditioned as directed (see *Chromatography* (621)). The column is maintained at 70°, and nitrogen is used as the carrier gas at a flow rate of about 30 mL per minute. In a suitable chromatogram, the relative standard deviation of five replicate injections is not more than 3.0%, and the resolution is not less than 2.0.

**Procedure**—Inject about 3 µL of the *Standard preparation* into the gas chromatograph, record the chromatogram, and measure the peak response of each component. [NOTE—Clean the syringe after each injection with pentane. Do not use acetone.] Similarly inject about 3 µL of the *Test preparation*, record the chromatogram, and measure the peak response of each component. Calculate the quantity, in mg, of methyl ethyl ketone in each mL of the Concentrate taken by the formula:

$$(1 / 0.95)C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of methyl ethyl ketone in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of the response of the methyl ethyl ketone to the response of the internal standard obtained from the *Test preparation* and the *Standard preparation*, respectively. The limit is 0.05 mg per mL.

#### Assay—

**Internal standard solution**—Dissolve a suitable quantity of triethylene glycol in water to obtain a solution containing about 15 mg per mL.

**Standard solution**—Prepare a solution of USP Isosorbide RS in water containing an accurately known concentration equivalent to about 25 mg of  $C_6H_{10}O_4$  per mL.

**Standard preparations**—Pipet 2-, 3-, 4-, and 5-mL quantities of *Standard solution* into separate 50-mL volumetric flasks, add 5.0 mL of *Internal standard solution* to each, add water to volume, and mix.

**Assay preparation**—Transfer about 200 mg of Concentrate, accurately weighed, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, add water to volume, and mix.

**Chromatographic system**—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 0.6-m glass column packed with support S9. The column is maintained at 230°, and nitrogen is used as the carrier gas. The retention time of the isosorbide peak is about 1.5, relative to that of triethylene glycol.

**System suitability and standard curve**—Inject 1-µL portions of each *Standard preparation*, and record each peak response. Plot the ratio of the peak response of isosorbide to that of triethylene glycol versus the concentration, in mg per mL, of isosorbide in the respective *Standard preparation*. The analytical system is suitable for conducting the assay if the correlation coefficient for the Standard curve is greater than 0.980, the resolution,  $R$ , is not less than 1.5, and neither tailing factor exceeds 2.0.

**Procedure**—Inject a 1-µL portion of the *Assay preparation*, record the peak responses for the two major peaks, calculate the ratio of the peak responses, and determine the concentration, C, in mg per mL, of isosorbide in the *Assay preparation* by reference to the *Standard curve*. Calculate the quan-

tity, in mg, of  $C_6H_{10}O_4$  in the Concentrate taken by the formula:

$$100C.$$

## Isosorbide Oral Solution

» Isosorbide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isosorbide ( $C_6H_{10}O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Isosorbide RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatograms of the *Standard preparations*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.2 and 3.8.

#### Assay—

**Internal standard solution, Standard solution, Standard preparations, Chromatographic system, and System suitability and standard curve**—Proceed as directed in the *Assay* under *Isosorbide Concentrate*.

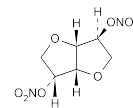
**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 450 mg of isosorbide, to a 250-mL volumetric flask, add 25.0 mL of *Internal standard solution*, then add water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Isosorbide Concentrate*. Calculate the quantity, in mg, of isosorbide ( $C_6H_{10}O_4$ ) in each mL of the Oral Solution taken by the formula:

$$250(C/V)$$

in which C is the concentration, in mg per mL, of isosorbide in the *Assay preparation* found by reference to the *Standard curve*; and V is the volume, in mL, of Oral Solution taken.

## Diluted Isosorbide Dinitrate



$C_6H_8N_2O_8$  236.14

D-Glucitol, 1,4:3,6-dianhydro-, dinitrate.

1,4:3,6-Dianhydro-D-glucitol dinitrate [87-33-2].

» Diluted Isosorbide Dinitrate is a dry mixture of isosorbide dinitrate ( $C_6H_8N_2O_8$ ) with Lactose, Mannitol, or suitable inert excipients to permit safe handling. It may contain up to 1.0 percent of a suitable stabilizer, such as Ammonium Phos-



phate. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_6H_8N_2O_8$ . It usually contains approximately 25 percent of isosorbide dinitrate.

**Caution**—Exercise proper precautions in handling undiluted isosorbide dinitrate, which is a powerful explosive and can be exploded by percussion or excessive heat. Only exceedingly small amounts should be isolated.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Diluted Isosorbide Dinitrate RS

**Identification**—Transfer to a medium-porosity, sintered-glass filtering crucible a quantity of it, equivalent to about 50 mg of isosorbide dinitrate, and pass three 5-mL portions of acetone through it. Evaporate the combined extracts at a temperature not exceeding 35°, with the aid of a gentle current of air, and dry the residue in vacuum over calcium chloride at room temperature for 16 hours: the IR absorption spectrum of a 1 in 40 solution of the residue so obtained, in chloroform, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS.

**Loss on drying** (731)—Dry it in vacuum over calcium chloride at room temperature for 16 hours: it loses not more than 1.0% of its weight.

**Heavy metals, Method II** (231): 0.001%.

**Assay**—

**Buffer solution**—Dissolve 15.4 g of ammonium acetate in water, add 11.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix to obtain a solution having a pH of about 4.7.

**Mobile phase**—Mix 350 mL of water, 100 mL of *Buffer solution*, and 550 mL of methanol. Cool to room temperature, dilute with water to 1000 mL, mix, degas, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer a quantity of diluted nitroglycerin to a suitable volumetric flask, add about 60% of the flask volume of methanol, sonicate for 5 minutes, and shake for 30 minutes. Dilute with methanol to volume to obtain a solution having a concentration of about 3 mg of nitroglycerin per mL, and mix. Allow any undissolved material to settle, filter, and store the filtrate in an airtight container.

**Standard preparation**—Transfer about 125 mg of recently mixed USP Diluted Isosorbide Dinitrate RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase*, shake for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 25-mL volumetric flask, and add 4.0 mL of *Internal standard solution* and 4 mL of dilute *Buffer solution* (1 in 10). Cool to room temperature, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.25 mg of isosorbide dinitrate per mL, based on the quantity of USP Diluted Isosorbide Dinitrate RS weighed and the labeled content of isosorbide dinitrate. Pass a portion of this solution through a 0.45- $\mu$ m filter.

**Assay preparation**—Transfer an accurately weighed quantity of recently mixed Diluted Isosorbide Dinitrate, equivalent to about 30 mg of isosorbide dinitrate, to a 50-mL volumetric flask. Proceed as directed for *Standard preparation*, beginning with “add about 30 mL of *Mobile phase*.”

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as di-

rected for *Procedure*: the resolution,  $R$ , between isosorbide dinitrate and nitroglycerin is not less than 2.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2%. [NOTE—The relative retention times are about 0.75 for isosorbide dinitrate and 1.0 for nitroglycerin. The relative retention times for isosorbide mononitrates, if present, are about 0.38.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_6H_8N_2O_8$  in the portion of Diluted Isosorbide Dinitrate taken by the formula:

$$125C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Dinitrate Extended-Release Capsules

» Isosorbide Dinitrate Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_8N_2O_8$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Diluted Isosorbide Dinitrate RS

**Identification**—The finely powdered contents of the Capsules respond to the *Identification* test under *Isosorbide Dinitrate Tablets*. If separation of interferences is required, transfer a quantity of the finely powdered contents of the Capsules, equivalent to about 20 mg of isosorbide dinitrate, to a glass-stoppered centrifuge tube, add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, add 15 mL of solvent hexane, and shake again. Centrifuge the mixture, and transfer the upper phase to a beaker. Place in a freezer, at a temperature of about  $-14^\circ$ , the beaker and a short-stem funnel fitted with a cotton plug that previously has been chloroform-washed and dried. After 30 minutes, filter the solution while still in the freezer. Evaporate the solvent, and dry the residue in vacuum over calcium chloride for 16 hours: the IR absorption spectrum of the residue so obtained, dissolved in 0.4 mL of chloroform and determined with the use of matched 0.1-mm cells, shows all of the significant absorption bands present in the spectrum obtained for a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS. The major peaks are at about 1650  $cm^{-1}$ , 1284  $cm^{-1}$  and 1275  $cm^{-1}$  (a doublet), 1106  $cm^{-1}$ , and 844  $cm^{-1}$ .

**Dissolution** (711)—Proceed as directed for *Method B* in *Delayed-Release Dosage Forms* in *Procedure, Apparatus 1 and Apparatus 2*, except to operate the apparatus in the acid medium for 1 hour instead of 2 hours and to use *Acceptance Table 2* in *Extended-Release Dosage Forms* in *Interpretation*.

*Apparatus 2*: 50 rpm.

*Times*: 2, 4, and 8 hours.

Determine the amount of  $C_6H_8N_2O_8$  dissolved employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of 0.05 M monobasic potassium phosphate and acetonitrile

(52:48). Make adjustments, if necessary (see *System Suitability* under *Chromatography* <621>).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 224-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same medium, and record the chromatograms as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of a filtered portion of the solution under test, and record the chromatograms. Determine the amount of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub> dissolved in comparison with a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same medium and similarly chromatographed.

**Tolerances**—The percentages of the labeled amount of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub> dissolved at the times specified conform to *Acceptance Table 2*. [NOTE—The test times given are cumulative, beginning with the 1 hour in the acid medium.]

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 40% and 75%
8	not less than 75%

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the Assay under *Diluted Isosorbide Dinitrate*.

**Assay preparation**—Weigh and finely powder the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase*, and shake the mixture by hand immediately, to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution*, cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Diluted Isosorbide Dinitrate*. Calculate the quantity, in mg, of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub> in the portion of Capsules taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Dinitrate Tablets

» Isosorbide Dinitrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub>.

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards <11>—

USP Diluted Isosorbide Dinitrate RS

**Identification**—Transfer a suitable quantity of finely powdered Tablets to a glass-stoppered centrifuge tube. Add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, then add 15 mL of solvent hexane, and again shake. Centrifuge the mixture, and transfer the upper phase to a beaker. Evaporate the solvent, and dry the residue in vacuum over anhydrous calcium chloride at room temperature for 16 hours: the IR absorption spectrum of a suitable solution in chloroform of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS.

#### Dissolution <711>—

*Medium*: water; 1000 mL.

*Apparatus 2*: 75 rpm.

*Time*: 45 minutes.

**Mobile phase**—Prepare a suitable filtered and degassed mixture of pH 3.0, 0.1 M ammonium sulfate and methanol (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>), using sulfuric acid for any necessary pH adjustment.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 5-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the Standard solution, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%, and the tailing factor is not more than 1.5.

**Procedure**—Separately inject equal volumes (about 20 µL) of the Standard solution and a filtered aliquot of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub> dissolved in comparison with a Standard solution having a known concentration of USP Diluted Isosorbide Dinitrate RS, similarly prepared and chromatographed.

**Tolerances**—Not less than 70% (Q) of the labeled amount of C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub> is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

#### Assay—

**Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system**—Prepare as directed in the Assay under *Diluted Isosorbide Dinitrate*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase*, and shake the mixture by hand immediately, to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod. Shake for 30 minutes. Add 8.0 mL of *Internal standard solution*, cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a disposable ion-exchange filter.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Diluted Isosorbide Dinitrate*. Calculate the quantity, in

mg, of  $C_6H_8N_2O_8$  in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Dinitrate Chewable Tablets

» Isosorbide Dinitrate Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_8N_2O_8$ .

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards <11>—

USP Diluted Isosorbide Dinitrate RS

**Identification**—Chewable Tablets respond to the *Identification* test under *Isosorbide Dinitrate Tablets*. Where separation of interferences is required, use the technique given under the *Identification* test for *Isosorbide Dinitrate Extended-release Capsules*.

**Uniformity of dosage units** <905>: meet the requirements.

### Assay—

*Buffer solution*, *Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Diluted Isosorbide Dinitrate*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase*, and shake the mixture by hand immediately, to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution*, cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Diluted Isosorbide Dinitrate*. Calculate the quantity, in mg, of  $C_6H_8N_2O_8$  in the portion of Chewable Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Dinitrate Extended-Release Tablets

» Isosorbide Dinitrate Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_8N_2O_8$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

### USP Reference standards <11>—

USP Diluted Isosorbide Dinitrate RS

**Identification**—Tablets respond to the *Identification* test under *Isosorbide Dinitrate Tablets*. Where separation of interferences is required, use the technique given under the *Identification* test for *Isosorbide Dinitrate Extended-Release Capsules*.

### Dissolution <711>—

TEST 1—

*Medium*: water; 500 mL.

*Apparatus 2*: 50 rpm.

*Times*: 1, 2, 4, and 6 hours.

Determine the amount of  $C_6H_8N_2O_8$  dissolved, using the following method.

*pH 3.0 Buffer solution*—Add 6.6 g of ammonium sulfate, accurately weighed, to 500 mL of water. Adjust with 1 N sulfuric acid to a pH of 3.0.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *pH 3.0 Buffer solution* (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a UV wavelength detector and a 5-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same *Medium*, and record the chromatograms as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of a filtered portion of the solution under test, and record the chromatograms. Determine the amount of  $C_6H_8N_2O_8$  dissolved in comparison with a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same *Medium*, similarly chromatographed.

*Tolerances*—The percentages of the labeled amount of  $C_6H_8N_2O_8$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 15% and 30%
2	between 50% and 70%
4	between 65% and 85%
6	not less than 75%

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

*Medium*: pH 1.2 simulated gastric fluid (without pepsin) for the first hour, 900 mL; pH 7.5 simulated intestinal fluid (without enzymes) for the subsequent hours, 900 mL.

*Apparatus 2*: 50 rpm, with helix sinkers.

*Times*: 1, 3, 6, and 12 hours.

Determine the amount of isosorbide dinitrate ( $C_6H_9NO_6$ ) dissolved by employing the following method.

*Buffer solution and Mobile phase*—Prepare as directed in the Assay under *Diluted Isosorbide Dinitrate*.

*Standard solution*—Prepare two solutions, one in each *Medium*. Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about 40 µg per mL.

*Test solution*—Pass 5 mL of the solution under test through a suitable 10-µm filter. Replace the *Medium* withdrawn at the 3- and 6-hour timepoints.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed in the Assay under *Diluted Isosorbide Dinitrate*. Chromatograph the *Standard solution*, and record the chromatogram as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the cumulative percentage of isosorbide dinitrate dissolved at each collection point, corrected for the quantities removed at previous collection points (not applicable for the first hour), as follows:

$$C_U = r_U \times \frac{C_S}{r_S}$$

Percentage released at first hour (see *Formula 1*).  
Percentage released at third hour (see *Formula 2*).  
Percentage released at sixth hour (see *Formula 3*).  
Percentage released at twelfth hour (see *Formula 4*).  
in which  $r_U$  and  $r_S$  are the peak responses for the *Test solution* and the *Standard solution*, respectively;  $C_U$  is the sample concentration, in µg per mL, at the indicated collection time point;  $C_S$  is the concentration, in µg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 1000 is the conversion factor from µg to mg; 100 is the conversion factor to percentage;  $LC$  is the Tablet label claim, in mg; and 5 is the volume, in mL, of sample withdrawn and of the *Medium* replaced.

*Tolerances*—The percentages of the labeled amount of  $C_6H_8N_2O_8$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 5% and 25%
3	between 30% and 50%
6	between 50% and 80%
12	not less than 75%

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

*Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the Assay under *Diluted Isosorbide Dinitrate*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase*, and shake the mixture by hand immediately to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution*, cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Diluted Isosorbide Dinitrate*. Calculate the quantity, in mg, of  $C_6H_8N_2O_8$  in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

$$\% \text{released} = C_1 \times \frac{900 \times 100}{1000 \times LC}$$

Formula 1

$$\% \text{released} = \left[ C_3 \times \frac{900 \times 100}{1000 \times LC} \right] + \% \text{released at 1st hour}$$

Formula 2

$$\% \text{released} = \frac{900 \times 100}{1000 \times LC} \times \left[ C_6 + \left( \frac{5 \times C_3}{900} \right) \right] + \% \text{released at 1st hour}$$

Formula 3

$$\% \text{released} = \frac{900 \times 100}{1000 \times LC} \times \left[ C_{12} + \left( \frac{5}{900} \times (C_3 + C_6) \right) \right] + \% \text{released at 1st hour}$$

Formula 4

## Isosorbide Dinitrate Sublingual Tablets

» Isosorbide Dinitrate Sublingual Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_8N_2O_8$ .

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Diluted Isosorbide Dinitrate RS

**Identification**—Tablets respond to the *Identification* test under *Isosorbide Dinitrate Tablets*.

**Disintegration** (701): 2 minutes, determined as directed for *Sublingual Tablets*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 20 minutes.

*Mobile phase*—Prepare a suitable degassed and filtered mixture of pH 3.0, 0.1 M ammonium sulfate and methanol (50:50).

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 5-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and a filtered aliquot of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of  $C_6H_8N_2O_8$  dissolved in comparison with a *Standard solution* having a known concentration of USP Diluted Isosorbide Dinitrate RS, similarly prepared and chromatographed.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_6H_8N_2O_8$  is dissolved in 20 minutes.

**Uniformity of dosage units** (905): meet the requirements.

### Assay—

*Buffer solution*, *Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the Assay under *Diluted Isosorbide Dinitrate*.

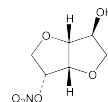
*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase*, and shake for 30 minutes. Add 8.0 mL of *Internal standard solution*, cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a 0.45- $\mu$ m filter.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Diluted Isosorbide Dinitrate*. Calculate the quantity, in mg, of  $C_6H_8N_2O_8$  in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Diluted Isosorbide Mononitrate



$C_6H_9NO_6$  191.14

D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate.

1,4:3,6-Dianhydro-D-glucitol 5-nitrate [16051-77-7].

» Diluted Isosorbide Mononitrate is a dry mixture of isosorbide mononitrate ( $C_6H_9NO_6$ ) with lactose or other suitable excipients to permit safe han-

dling. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of isosorbide mononitrate ( $C_6H_9NO_6$ ).

**Caution**—Exercise proper precautions in handling undiluted isosorbide mononitrate, which is a powerful explosive and can be exploded by percussion or excessive heat. Only exceedingly small amounts should be isolated.

**Packaging and storage**—Preserve in tight containers. Store at a temperature between 20° and 30°.

**USP Reference standards** (11)—

USP Isosorbide RS

[NOTE—The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.]

USP Diluted Isosorbide Dinitrate RS

USP Diluted Isosorbide Mononitrate RS

USP Diluted Isosorbide Mononitrate Related Compound A RS

1,4:3,5-Dianhydro-D-glucitol 2-nitrate.

$C_6H_9NO_6$  191.14

**Identification**—

**A:** Shake a quantity of it, equivalent to about 25 mg of isosorbide mononitrate, with 10 mL of acetone for 5 minutes. Filter, evaporate to dryness at a temperature below 40°, and dry the residue in a vacuum over phosphorus pentoxide for 16 hours: the IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Mononitrate RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Heavy metals, Method I** (231): not more than 10 µg per g.

**Related compounds**—

TEST 1—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Standard solution 1**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.0125 mg of isosorbide per mL.

**Standard solution 2**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.025 mg of isosorbide per mL.

**Standard solution 3**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.05 mg of isosorbide per mL.

**Test solution**—Transfer a portion of Diluted Isosorbide Mononitrate, equivalent to about 200 mg of isosorbide mononitrate, accurately weighed, to a suitable container, add 20.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Use the supernatant.

**Application volume:** 20 µL.

**Developing solvent system:** a mixture of absolute alcohol and toluene (8:2).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After developing, dry the plate with warm air for about 10 minutes, dip the plate in a solution prepared by dissolving 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL

of water (prepared fresh for each plate), and heat at 105° for 5 minutes. Any spot in the chromatogram obtained from the *Test solution* and corresponding to the  $R_f$  value of the spots obtained from the *Standard solutions* is not more intense than the spot in the chromatogram obtained from *Standard solution 3*: not more than 0.5% of any individual impurity is found. If the spot in the chromatogram obtained from the *Test solution* is nearly as intense as the spot obtained from *Standard solution 3*, further dilute the *Test solution* with absolute alcohol (1:1), repeat the test, and compare the intensity of the isosorbide spot in the diluted *Test solution* with the intensity of the spots obtained from the *Standard solutions*, correcting the percentage level for the additional dilution of the *Test solution*.

TEST 2—

**Mobile phase, Resolution solution, and Chromatographic system**—Proceed as directed in the *Assay*.

**Isosorbide mononitrate related compound A standard solution**—Prepare as directed for *Isosorbide mononitrate related compound A standard preparation* in the *Assay*.

**Isosorbide dinitrate standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in methanol, sonicate and warm if necessary, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.125 mg of isosorbide dinitrate per mL.

**Standard solution**—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed, to a suitable volumetric flask. Dissolve in water, quantitatively add a volume of *Isosorbide mononitrate related compound A standard solution* and a volume of *Isosorbide dinitrate standard stock solution*, and dilute with water to volume to obtain a solution having a known concentration of about 2.0 mg of isosorbide mononitrate per mL, 0.005 mg of isosorbide mononitrate related compound A per mL, and 0.005 mg of isosorbide dinitrate per mL. Filter a portion of the solution, discarding the first few mL of the filtrate.

**Test solution**—Use the *Assay preparation*, prepared as directed in the *Assay*.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of isosorbide mononitrate related compound A and isosorbide dinitrate relative to the amount of isosorbide mononitrate in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$100(CV/W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of isosorbide mononitrate related compound A or isosorbide dinitrate, as appropriate, in the *Standard solution*;  $V$  is the volume, in mL, of the *Test solution*;  $W$  is the amount, in mg, of isosorbide mononitrate in the portion of Diluted Isosorbide Mononitrate used to prepare the *Test solution*, based on the label claim; and  $r_U$  and  $r_S$  are the peak areas of the corresponding components obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% of isosorbide mononitrate related compound A is found; and not more than 0.25% of isosorbide dinitrate is found. Calculate the percentage of each other impurity (other than isosorbide mononitrate related compound A or isosorbide dinitrate) in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak area for each other impurity obtained from the *Test solution*; and  $r_S$  is the sum of the areas of all the peaks: not more than 0.5% of total impurities is found, including isosorbide mononitrate related compound A and isosorbide dinitrate; and not more than 0.5% of total impu-

rities is found, the results for *Test 1* and *Test 2* being considered.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and methanol (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Standard preparation**—Transfer an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS to a suitable volumetric flask, dissolve in water, add a volume of methanol equivalent to 4% of the flask volume, and dilute with water to volume to obtain a solution having a known concentration of about 2.0 mg of isosorbide mononitrate per mL.

**Isosorbide mononitrate related compound A standard preparation**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1.0 mg of isosorbide mononitrate related compound A per mL. Quantitatively dilute a portion of this solution with water to obtain a solution having a known concentration of about 0.05 mg per mL.

**Resolution solution**—Transfer 10.0 mL of *Isosorbide mononitrate related compound A standard preparation*, 1.0 mL of the *Standard preparation*, and 4.0 mL of methanol to a 100-mL volumetric flask, and dilute with water to volume. Filter a portion of the solution, discarding the first few mL of the filtrate.

**Assay preparation**—Transfer an accurately weighed amount of Diluted Isosorbide Mononitrate, equivalent to 100 mg of isosorbide mononitrate, to a 50-mL volumetric flask, dissolve in about 25 mL of water, add 2 mL of methanol, dilute with water to volume, and mix. Filter a portion of the solution, discarding the first few mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 12.5-cm column that contains packing L1. The flow rate is about 1.5 mL per minute, increasing to 3.0 mL per minute at about 8.5 minutes to ensure that the isosorbide mononitrate peak has completely eluted. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for isosorbide mononitrate related compound A, 1.0 for isosorbide mononitrate, and 4.1 for isosorbide dinitrate; and the resolution, *R*, between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the isosorbide mononitrate peaks. Calculate the quantity, in mg, of isosorbide mononitrate (C<sub>6</sub>H<sub>9</sub>NO<sub>6</sub>) in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$CV(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of isosorbide mononitrate in the *Standard preparation*; *V* is the volume, in mL, of the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Mononitrate Tablets

» Isosorbide Mononitrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isosorbide mononitrate (C<sub>6</sub>H<sub>9</sub>NO<sub>6</sub>).

**Packaging and storage**—Preserve in tight containers. Store at a temperature between 20° and 30°.

#### USP Reference standards <11>—

USP Isosorbide RS

[NOTE—The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.]

USP Diluted Isosorbide Dinitrate RS

USP Diluted Isosorbide Mononitrate RS

USP Diluted Isosorbide Mononitrate Related Compound A RS  
1,4:3,5-Dianhydro-D-glucitol 2-nitrate.

C<sub>6</sub>H<sub>9</sub>NO<sub>6</sub> 191.14

#### Identification—

**A: Thin-Layer Chromatographic Identification Test** <201>—

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 120 mg of isosorbide mononitrate, to a suitable container, add 50.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Quantitatively dilute the supernatant (10 in 50) with absolute alcohol.

**Standard solution**: a solution of USP Diluted Isosorbide Mononitrate RS in absolute alcohol containing 0.5 mg of isosorbide mononitrate per mL.

**Application volume**: 20 µL.

**Developing solvent system**: a mixture of chloroform and methanol (95:5).

**Spray reagent**—Dissolve 1 g of soluble starch in 100 mL of boiling water. Cool, add 0.5 g of potassium iodide, and mix to dissolve.

**Procedure**—Examine the plate under short-wavelength UV light, marking any observed spots. Visualize nitrates on the plate by spraying with *Spray reagent* and illuminating with short-wavelength UV light for about 10 minutes. Isosorbide mononitrate and other nitrates appear as a violet spot on a white to light violet background.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution <711>—

**Medium**: water; 900 mL, deaerated.

**Apparatus 2**: 50 rpm.

**Time**: 15 minutes.

Determine the amount of C<sub>6</sub>H<sub>9</sub>NO<sub>6</sub> dissolved by employing the following method.

**Mobile phase**—Proceed as directed in the *Assay*.

**Standard solution**—Transfer 20 mg, accurately weighed, of USP Diluted Isosorbide Mononitrate RS to a 200-mL volumetric flask, dilute with *Medium* to volume, and mix well. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with *Medium* to volume, and mix well.

**Test solution**—Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm, discarding the first few mL.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for

**Procedure:** the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Proceed as directed in the *Assay*. Calculate the percentage of  $C_6H_9NO_6$  dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak responses for the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_6H_9NO_6$  is dissolved in 15 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity*.

#### Related compounds—

##### TEST 1—

**Adsorbent, Standard solution 1, Standard solution 2, Standard solution 3, Application volume, and Developing solvent system**—Prepare as directed in *Related compounds, Test 1* under *Diluted Isosorbide Mononitrate*.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of isosorbide mononitrate, to a suitable container, add 20.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Use the supernatant.

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After developing, dry the plate with warm air for about 10 minutes, dip the plate in a solution prepared by dissolving 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL of water (prepared fresh for each plate), and heat at 105° for 5 minutes. Any spot in the chromatogram obtained from the *Test solution* and corresponding to the  $R_f$  value of the spots obtained from the *Standard solutions* is not more intense than the spot in the chromatogram obtained from *Standard solution 3*: not more than 1.0% of any individual impurity is found. If the spot in the chromatogram obtained from the *Test solution* is nearly as intense as the spot obtained from *Standard solution 3*, further dilute the *Test solution* (1:1) with absolute alcohol, repeat the test, and compare the intensity of the isosorbide spot in the diluted *Test solution* with the intensity of the spots obtained from the *Standard solutions*, correcting the percentage level for the additional dilution of the *Test solution*.

##### TEST 2—

**Mobile phase and Resolution solution**—Proceed as directed in the *Assay*.

**Isosorbide mononitrate related compound A standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate related compound A per mL.

**Isosorbide dinitrate standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg of isosorbide dinitrate per mL.

**Standard solution**—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed, to a suitable volumetric flask. Dissolve in water, quantitatively add a volume of *Isosorbide mononitrate related compound A standard stock solution* and a volume of *Isosorbide dinitrate standard*

*stock solution*, and dilute with water to volume to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate per mL, 0.0005 mg of isosorbide mononitrate related compound A per mL, and 0.0005 mg of isosorbide dinitrate per mL. Filter a portion of the solution, discarding the first few mL of the filtrate.

**Test solution**—Use the *Assay preparation*, prepared as directed in the *Assay*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10% for the isosorbide mononitrate related compound A and isosorbide dinitrate peaks.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Compare the peak areas of the corresponding impurity obtained from the *Test solution* and the *Standard solution*, respectively. The average peak area of the impurity in the *Test solution* is less than or equal to the average peak area of the corresponding peak in the *Standard solution*: not more than 0.5% of isosorbide mononitrate related compound A is found; and not more than 0.5% of isosorbide dinitrate is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Resolution solution**—Prepare a solution of USP Diluted Isosorbide Mononitrate RS and USP Diluted Isosorbide Mononitrate Related Compound A RS having a concentration of 0.0005 mg of each of isosorbide mononitrate and isosorbide mononitrate related compound A per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate per mL. Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of isosorbide mononitrate, to a 200-mL volumetric flask, add 100 mL of water, and sonicate for about 10 minutes. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-



tity, in mg, of isosorbide mononitrate ( $C_6H_9NO_6$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of isosorbide mononitrate in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isosorbide Mononitrate Extended-Release Tablets

» Isosorbide Mononitrate Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isosorbide mononitrate ( $C_6H_9NO_6$ ).

**Packaging and storage**—Preserve in tight containers. Store at a temperature between 20° and 30°.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

### USP Reference standards (11)—

USP Isosorbide RS

[NOTE—The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.]

USP Diluted Isosorbide Dinitrate RS

USP Diluted Isosorbide Mononitrate RS

USP Diluted Isosorbide Mononitrate Related Compound A RS

1,4:3,5-Dianhydro-D-glucitol 2-nitrate.

$C_6H_9NO_6$  191.14

### Identification—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—Proceed as directed for *Identification test A* under *Isosorbide Mononitrate Tablets*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

TEST 1—

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm. The Tablets are placed in a metal helix, prepared by winding 10 inches of a 0.8-mm stainless steel wire around a 9/32-inch shaft and pulling the coils to form a helix 1 inch long.

*Times:* 1, 2, 4, 8, and 12 hours.

Determine the amount of  $C_6H_9NO_6$  dissolved by employing the following method.

*Mobile phase*—Prepare a filtered and degassed mixture of water and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about  $LC/1000$  where  $LC$  is the Tablet label claim in mg.

*Test solution*—Use portions of the solution under test passed through a 0.45- $\mu$ m nylon filter, discarding the first 4 to 6 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph

the *Standard solution*, and record the chromatogram as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Determine the amount, in mg, of isosorbide mononitrate dissolved at each interval by the formula:

$$\frac{r_U \times C_S \times V}{r_S}$$

in which  $r_U$  and  $r_S$  are the peak responses for the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Standard solution*; and  $V$  is the volume, in mL, of *Medium* in the vessel at each time point.

Calculate the amount, in mg, of isosorbide mononitrate removed by sampling at the previous time points by the formula:

$$\sum AD \times \frac{V_S}{V}$$

in which  $AD$  is the amount, in mg, of isosorbide mononitrate dissolved at each time point;  $V_S$  is the volume, in mL, of the sample taken; and  $V$  is the volume, in mL, of *Medium* in the vessel at each time point.

Calculate the percentage of isosorbide mononitrate dissolved at each time point by the formula:

$$\frac{(AD + AR) \times 100}{LC}$$

in which  $AD$  is the amount, in mg, of isosorbide mononitrate dissolved at a given time point;  $AR$  is the amount, in mg, of isosorbide mononitrate removed at the previous time point; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

*Tolerances*—The percentages of the labeled amount of  $C_6H_9NO_6$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 15% and 35%
2	between 28% and 48%
4	between 43% and 68%
8	between 65% and 90%
12	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

*Medium:* Simulated gastric fluid (without enzymes); 500 mL.

*Apparatus 2:* 50 rpm.

*Times:* 1, 2, 6, and 12 hours.

Determine the amount of  $C_6H_9NO_6$  dissolved by employing the following method.

*Mobile phase*—Prepare a filtered and degassed mixture of water and methanol (3:2). Make adjustments if necessary (see *System suitability* under *Chromatography* (621)).

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about 1.2 mg of isosorbide mononitrate per mL.

*Working standard solution*—Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 60  $\mu$ g per mL for Tablets labeled to contain 30 mg and a final

concentration of 120 µg per mL for Tablets labeled to contain 60 mg.

**Test solution**—Use portions of the solution under test passed through a suitable 0.45-µm filter.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Working standard solution*, and record the chromatogram as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the appropriate *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the concentration ( $C_i$ ), in mg per mL, of isosorbide mononitrate removed at each time point  $i$  by the formula:

$$C_i = \frac{r_{U(i)}}{r_S} \times C_S$$

in which  $r_{U(i)}$  is the peak response for the *Test solution* at time point  $i$ ;  $r_S$  is the peak response for the *Working standard solution*; and  $C_S$  is the concentration, in mg per mL, of the *Working standard solution*. Calculate the total amount, in percentage, of drug release at each time point  $i$  by the formula:

$$\{C_i \times [V_0 - ((i - 1)V_i)] + (\sum_{j=1}^{i-1} C_j V_j)\} \times \frac{100}{LC}$$

in which  $C_i$  is the concentration, in mg per mL, of drug removed at time point  $i$ ;  $V_0$  is the initial volume, in mL, of *Medium*;  $V_i$  is the volume, in mL, of sample removed at each sampling time;  $C_j$  is the concentration, in mg per mL, of drug released at time  $j$ ; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—The percentages of the labeled amount of  $C_6H_9NO_6$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 25% and 45%
2	between 35% and 60%
6	between 72% and 90%
12	not less than 80%

**TEST 3**—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** Simulated gastric fluid (without enzymes); 500 mL.

**Apparatus 2:** 50 rpm.

**Times:** 1, 2, 6, and 12 hours.

Determine the amount of  $C_6H_9NO_6$  dissolved by employing the following method.

**Buffer solution**—Transfer 15.4 g of ammonium acetate and 11.5 mL of acetic acid to a 1-L volumetric flask containing about 500 mL of water. Adjust with acetic acid to a pH of 4.7. Dilute with water to volume.

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about 0.12 mg of isosorbide mononitrate per mL.

**Working standard solution**—For Tablets labeled to contain 60 mg, use the *Standard stock solution* with no further dilution. For Tablets labeled to contain 30 mg, transfer 25.0 mL

of the *Standard stock solution* to a 50-mL volumetric flask. Dilute with *Medium* to volume.

**Test solution**—Use portions of the solution under test passed through a suitable 0.45-µm filter.

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and *Buffer solution* (6:3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Working standard solution*, and record the chromatogram as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100 µL) of the appropriate *Working standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the concentration ( $C_i$ ), in mg per mL, of isosorbide mononitrate removed at each time point  $i$  by the formula:

$$C_i = \frac{r_{U(i)}}{r_S} \times C_S$$

in which  $r_{U(i)}$  is the peak response for the *Test solution* at time point  $i$ ;  $r_S$  is the peak response for the *Working standard solution*; and  $C_S$  is the concentration, in mg per mL, of the *Working standard solution*. Calculate the total amount, in percentage, of drug release at each time point  $i$  by the formula:

$$\{C_i \times [V_0 - ((i - 1)V_i)] + (\sum_{j=1}^{i-1} C_j V_j)\} \times \frac{100}{LC}$$

in which  $C_i$  is the concentration, in mg per mL, of drug removed at time point  $i$ ;  $V_0$  is the initial volume, in mL, of *Medium*;  $V_i$  is the volume, in mL, of sample removed at each sampling time;  $C_j$  is the concentration, in mg per mL, of drug released at time  $j$ ; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—The percentages of the labeled amount of isosorbide mononitrate dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	between 20% and 40%
2	between 30% and 50%
6	between 70% and 90%
12	not less than 85%

**Uniformity of dosage units** <905>: meet the requirements for *Content Uniformity*. Proceed as directed in the *Assay*, except to use 1 Tablet instead of the portion of powdered Tablets used in the *Assay preparation*.

#### Related compounds—

**TEST 1—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Standard solution 1**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.0125 mg of isosorbide per mL.

**Standard solution 2**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.025 mg of isosorbide per mL.

**Standard solution 3**—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if nec-

essary, with acetonitrile to obtain a solution having a known concentration of about 0.05 mg of isosorbide per mL.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of isosorbide mononitrate, to a suitable flask containing 20.0 mL of acetonitrile. Sonicate for 10 minutes and then centrifuge. Use the supernatant.

**Application volume:** 20  $\mu$ L.

**Developing solvent system:** a mixture of toluene, ethyl acetate, and isopropyl alcohol (53:32:15).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After developing, dry the plate with warm air for about 10 minutes, dip the plate in a solution prepared by dissolving 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL of water (prepared fresh for each plate), and heat at 105° for 5 minutes. Any spot in the chromatogram obtained from the *Test solution* and corresponding to the  $R_f$  value of the spots obtained from the *Standard solutions* is not more intense than the spot in the chromatogram obtained from *Standard solution* 3: not more than 1% of any individual impurity is found. [NOTE—The  $R_f$  values of isosorbide and isosorbide mononitrate are about 0.2 and 0.6, respectively.] If the spot in the chromatogram obtained from the *Test solution* is nearly as intense as the spot obtained from *Standard solution* 3, further dilute the *Test solution* with acetonitrile (1:1), repeat the test, and compare the intensity of the isosorbide spot in the diluted *Test solution* with the intensity of the spots obtained from the *Standard solutions*, correcting the percentage level for the additional dilution of the *Test solution*.

#### TEST 2—

**Mobile phase**—Prepare a filtered and degassed mixture of water and methanol (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Isosorbide mononitrate related compound A standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.3 mg of isosorbide mononitrate related compound A per mL.

**Isosorbide dinitrate standard stock solution**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.15 mg of isosorbide dinitrate per mL.

**Standard stock solution**—Transfer 2.0 mL of *Isosorbide mononitrate related compound A standard stock solution* and 4.0 mL of *Isosorbide dinitrate standard stock solution* to a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Resolution solution**—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, equivalent to about 24 mg of isosorbide mononitrate, to a 100-mL volumetric flask, add 10.0 mL of *Standard stock solution*, add 20 mL of methanol, and dilute with water to volume.

**Standard solution**—Transfer 10.0 mL of *Standard stock solution* and 20 mL of methanol to a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 60 mg of isosorbide mononitrate, to a 50-mL volumetric flask, add 40 mL of methanol, and sonicate for about 30 minutes with cooling. Warm to ambient temperature, dilute with methanol to volume, and mix. Centrifuge at about 3000 rpm for 10 minutes. Quantitatively dilute the supernatant with water (10 in 50). Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 1.0. [NOTE—The relative retention times are about 0.9 for isosorbide mononitrate related compound A, 1.0 for isosorbide mononitrate, and 5.6 for isosorbide dinitrate.] Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 10% for the isosorbide mononitrate related compound A and isosorbide dinitrate peaks.

**Procedure**—Separately inject equal volumes (about 100  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of isosorbide mononitrate related compound A and isosorbide dinitrate in the portion of Tablets taken by the formula:

$$25(C/W)(r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of the appropriate Standard, USP Diluted Isosorbide Mononitrate Related Compound A RS or USP Diluted Isosorbide Dinitrate RS, in the *Standard solution*;  $W$  is the weight, in mg, of isosorbide mononitrate in the sample used to prepare the *Test solution*; and  $r_u$  and  $r_s$  are the peak areas of the corresponding component obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% of isosorbide mononitrate related compound A is found, and not more than 0.25% of isosorbide dinitrate is found. Calculate the percentage of each other impurity (other than isosorbide mononitrate related compound A or isosorbide dinitrate) in the portion of Tablets taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area for each other impurity obtained from the *Test solution*; and  $r_s$  is the sum of the areas of all the peaks: not more than 0.25% of total other impurities is found, and not more than 0.5% of total impurities is found, including isosorbide mononitrate related compound A and isosorbide dinitrate.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and methanol (8:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Isosorbide mononitrate related compound A standard preparation**—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.15 mg of isosorbide mononitrate related compound A per mL.

**Resolution solution**—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed and equivalent to about 30 mg of isosorbide mononitrate, to a 250-mL volumetric flask. Dissolve in water, add 10.0 mL of *Isosorbide mononitrate related compound A standard preparation*, add 50 mL of methanol, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a concentration of about 0.12 mg of isosorbide mononitrate per mL and about 0.006 mg of isosorbide mononitrate related compound A per mL.

**Standard preparation**—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed, to a suitable volumetric flask. Dissolve in water, add a portion of methanol equivalent to about 20% of the flask volume, and dilute with water to volume to obtain a solution having a known

concentration of about 0.12 mg of isosorbide mononitrate per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 60 mg of isosorbide mononitrate, to a 100-mL volumetric flask, add 50 mL of methanol, and sonicate for about 30 minutes with cooling. Warm to ambient temperature, dilute with methanol to volume, and mix. Centrifuge at about 3000 rpm for 10 minutes. Quantitatively dilute the supernatant with water (10 in 50). Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate.

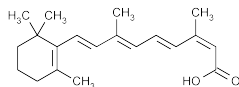
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm  $\times$  12.5-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isosorbide mononitrate ( $C_6H_9NO_6$ ) in the portion of Tablets taken by the formula:

$$500C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of isosorbide mononitrate in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isotretinoin



$C_{20}H_{28}O_2$  300.44

Retinoic acid, 13-*cis*-.

3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-*cis*-4-*trans*-6-*trans*-8-*trans*-nonatetraenoic acid [4759-48-2].

» Isotretinoin contains not less than 98.0 percent and not more than 102.0 percent of  $C_{20}H_{28}O_2$ , calculated on the dried basis.

**Caution**—Isotretinoin is teratogenic. Avoid inhalation and skin contact.

**Packaging and storage**—Preserve in tight containers, under an atmosphere of an inert gas, protected from light.

**USP Reference standards** <11>—

USP Isotretinoin RS

USP Tretinoin RS

**NOTE**—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.

### Identification—

**A: Infrared Absorption** <197M>.

**B: Ultraviolet Absorption** <197U>—

**Solution:** 4  $\mu$ g per mL.

**Medium:** acidified isopropyl alcohol (prepared by diluting 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL). Absorptivities at 354 nm do not differ by more than 3.0%.

**Loss on drying** <731>—Dry it in vacuum at room temperature for 16 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method II** <231>: 0.002%.

### Limit of tretinoin—

**Mobile phase**—Prepare a suitable filtered and degassed mixture of isooctane, isopropyl alcohol, and glacial acetic acid (99.65:0.25:0.1).

**Standard stock solution**—Dissolve an accurately weighed quantity of USP Tretinoin RS in a minimum quantity of methylene chloride, add an amount of isooctane to obtain a solution having a known concentration of about 250  $\mu$ g per mL, and mix.

**Standard solution**—Pipet 1 mL of *Standard stock solution* into a 100-mL volumetric flask, add isooctane to volume, and mix.

**Test solution**—Transfer about 25 mg of Isotretinoin, accurately weighed, to a 100-mL volumetric flask, dissolve in a minimum quantity of methylene chloride, add isooctane to volume, and mix.

**System suitability stock solution**—Dissolve a quantity of USP Isotretinoin RS in a minimum amount of methylene chloride, add an amount of isooctane to obtain an isotretinoin concentration of about 250  $\mu$ g per mL, and mix.

**System suitability solution**—Pipet 1 mL of *Standard stock solution* into a 100-mL volumetric flask, add *System suitability stock solution* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 352-nm detector and a 4.0-mm  $\times$  25-cm column containing 5- $\mu$ m packing L3. The flow rate is about 1 mL per minute. Chromatograph about 20  $\mu$ L of the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively; the resolution,  $R$ , between isotretinoin and tretinoin is not less than 2.0; and the relative standard deviation determined from the tretinoin peak response in replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of tretinoin by the formula:

$$10(C / W)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Tretinoin RS in the *Standard solution*;  $W$  is the weight, in mg, of Isotretinoin taken; and  $r_U$  and  $r_S$  are the tretinoin peak responses obtained from the *Test solution* and the *Standard solution*, respectively. The content of tretinoin is not more than 1.0%.

**Assay**—Dissolve about 240 mg of Isotretinoin, accurately weighed, in 50 mL of dimethylformamide, add 3 drops of a solution of thymol blue in dimethylformamide (1 in 100), and titrate with 0.1 N sodium methoxide VS to a greenish endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 30.04 mg of  $C_{20}H_{28}O_2$ .

## Isotretinoin Capsules

» Isotretinoin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isotretinoin ( $C_{20}H_{28}O_2$ ).

**Caution**—Isotretinoin is teratogenic. Avoid inhalation and skin contact.

**Packaging and storage**—Preserve in tight containers, protected from light. Store at controlled room temperature, in a dry place.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

### USP Reference standards (11)—

USP Isotretinoin RS

USP Tretinoin RS

**NOTE**—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—[**Caution**—Carry out all the tests under subdued light and use low-actinic glassware.]

TEST 1—

*Medium*—

STAGE 1: simulated gastric fluid with pepsin, prepared freshly and purged with nitrogen.

STAGE 2: 0.13 N sodium hydroxide, prepared by transferring 5 g of sodium hydroxide to a 1-L volumetric flask and dissolving in and diluting with water to volume. Prepare fresh, and purge with nitrogen.

**Apparatus** (see *Disintegration* (701))—No disks; the apparatus is adjusted so that the bottom of the basket-rack assembly descends to  $1.0 \pm 0.1$  cm from the inside bottom surface of the vessel on the downward stroke; the 10-mesh stainless steel cloth in the basket-rack assembly is replaced with a 40-mesh stainless steel cloth; a 10-mesh stainless-steel cloth is fitted to the top of the basket-rack assembly.

**Time:** 60 minutes.

**Standard solution**—Transfer about 10 mg of USP Isotretinoin RS, accurately weighed, to a 200-mL low-actinic volumetric flask; add 25.0 mL of *Stage 1 Medium* and about 150 mL of *Stage 2 Medium*; sonicate until completely dissolved (about 20 minutes); and dilute with *Stage 2 Medium* to volume. Pass 20 mL of this solution through a suitable filter, discarding the first 5 mL. Dilute 5.0 mL of the filtrate with *Stage 2 Medium* to 50 mL.

**Sample solution**—Perform a dissolution test on each of 6 Capsules: place 1 Capsule in one of the tubes in each of six basket-rack assemblies. Place each basket in a 1-L beaker containing 100 mL of *Stage 1 Medium* in a bath having a temperature of  $37.0 \pm 0.5^\circ$ . Allow to stand for 30 minutes. Carefully add 800 mL of *Stage 2 Medium* to each beaker. With the disintegration apparatus operating, connect each basket-rack assembly to the drive rod in a timed sequence. After 60 minutes, withdraw 20 mL of *Medium* (*Stage 1* and *Stage 2*), immediately pass the solution through a suitable 0.45- $\mu$ m filter, discard the first 5 mL, and collect the solution in argon-charged, low-actinic glassware. Dilute, if necessary, using low-actinic glassware, with *Stage 2 Medium*, to obtain a theoretical concentration of about 0.0055 mg per mL of isotretinoin, assuming complete dissolution, based on the label claim.

**Capsule shell correction**—Empty the contents of 3 Capsules. Wash the Capsule shells in several 20-mL aliquots of chloroform. Allow the Capsule shells to air dry. Place the Capsule shells in a 1-L flask containing 100 mL of *Stage*

*1 Medium* and 800 mL of *Stage 2 Medium*. Allow the flask to stand for about 1 hour in a bath having a temperature of  $37.0 \pm 0.5^\circ$ , stirring occasionally. Filter, and dilute as described for *Sample solution*.

**Procedure**—Determine the amount of  $C_{20}H_{28}O_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 343 nm, in portions of the *Sample solution* in comparison with the *Standard solution*, correcting for the Capsule shell absorbance, and using *Medium* (*Stage 1* and *Stage 2*) as the blank. Calculate the percentage of  $C_{20}H_{28}O_2$  dissolved by the formula:

$$\frac{(A_U - A_{CS}) \times C_S \times D_U \times 100}{A_S \times LC}$$

in which  $A_U$ ,  $A_{CS}$ , and  $A_S$  are the absorbances obtained from the *Sample solution*, the *Capsule shell correction*, and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Standard solution*;  $D_U$  is the dilution factor of the *Sample solution*; 100 is the conversion factor to percentage; and  $LC$  is the Capsule label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{20}H_{28}O_2$  is dissolved in 60 minutes.

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** 0.05 M phosphate buffer, pH 7.8, containing 0.5% w/v solid *N,N*-dimethyldodecylamine *N*-oxide; 900 mL.

**Apparatus 1:** 20-mesh basket; 100 rpm.

**Time:** 90 minutes.

**Buffer solution**—Transfer 3.4 g of monobasic potassium phosphate to a 1-L volumetric flask, dissolve in and dilute with water to volume, and adjust with phosphoric acid to a pH of  $2.10 \pm 0.05$ .

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (81:19). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 44 mg, accurately weighed, of USP Isotretinoin RS to a 100-mL low-actinic volumetric flask. Add 15 mL of 1-propanol, and sonicate for about 15 minutes. Add 50 mL of *Medium*, and sonicate for 10 minutes. Fill with *Medium* to volume. Transfer 5.0 mL to a 100-mL low-actinic volumetric flask, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about  $L/1000$  mg/mL, where  $L$  is the Capsule label claim, in mg.

**Test solution**—Pass a portion of the solution under test through a suitable 0.45- $\mu$ m filter.

**Chromatographic system**—The liquid chromatograph is equipped with a 358-nm detector and a 4.6-mm  $\times$  5-cm column containing 5- $\mu$ m packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of isotretinoin dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which  $r_U$  and  $r_S$  are the peak responses for the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concen-

tration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the percentage conversion factor; and *L* is the Capsule label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{20}H_{28}O_2$  is dissolved in 90 minutes.

**TEST 3**—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium:** borate buffer, pH 8.0, containing 0.5% cetrimide and 50 mg per L of pancreatin (Prepared by dissolving 12.37 g of boric acid and 14.91 g of potassium chloride in water and diluting with water to 1 L. To 250 mL of this solution, add 19.5 mL of 0.2 M sodium hydroxide solution, and dilute with water to 1 L. Adjust with 0.2 M sodium hydroxide to a pH of  $8.00 \pm 0.05$ , if necessary. Add 5 g of cetrimide, and mix until dissolved. Just before starting the test, dissolve a quantity of pancreatin to obtain a final concentration of 50 mg per L.); 900 mL.

**Apparatus 2:** 75 rpm, with sinkers.

**Time:** 90 minutes.

**Standard solution**—Transfer about 45 mg, accurately weighed, of USP Isotretinoin RS to a 100-mL volumetric flask. Add 60 mL of 0.1 N sodium hydroxide, and sonicate until dissolved. Dilute with 0.1 N sodium hydroxide to volume. Dilute this solution with *Medium* to obtain a final concentration of about  $L/1000$  mg/mL, where *L* is the Capsule label claim, in mg.

**Test solution**—Pass a portion of the solution under test through a suitable 0.45- $\mu$ m filter.

**Mobile phase**—Prepare a filtered and degassed mixture of 0.5% acetic acid in methanol and 0.5% acetic acid in water (71:29). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Chromatographic system**—The liquid chromatograph is equipped with a 353-nm detector and a 4.6-mm  $\times$  25-cm column containing 10- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1800 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of isotretinoin dissolved by the formula:

$$\frac{r_t \times C_s \times 900 \times 100}{r_s \times L}$$

in  $r_t$  and  $r_s$  are the peak responses for the *Test solution* and the *Standard solution*, respectively;  $C_s$  is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the percentage conversion factor; and *L* is the Capsule label claim, in mg.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{20}H_{28}O_2$  is dissolved in 90 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Chromatographic purity—

**Methylene chloride reagent**—Transfer 50 g of sodium bicarbonate to 1000 mL of methylene chloride, shake, and allow to stand overnight. At the time of use, filter suitable portions of this solution, and add 10 mg of butylated hydroxytoluene per mL.

**Mobile phase**—Prepare a filtered and degassed mixture of hexanes, ethyl acetate, and glacial acetic acid (970:30:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve accurately weighed quantities of USP Isotretinoin RS and USP Tretinoin RS in *Methylene chloride reagent* to obtain a solution having known concentrations of about 1 mg of each Reference Standard per mL. Transfer 1.0 mL of this solution, and dilute quantitatively with hexanes to 100.0 mL to obtain a solution having known concentrations of about 0.01 mg of each Reference Standard per mL.

**Standard solution**—Dissolve an accurately weighed quantity of USP Tretinoin RS in *Methylene chloride reagent* to obtain a solution having a known concentration of about 0.5 mg per mL. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with hexanes to obtain a solution having a known concentration of about 1  $\mu$ g per mL.

**Test solution**—Weigh a number of Capsules, equivalent to about 200 mg of isotretinoin. With a sharp blade, carefully open the Capsules, without loss of material, and transfer the contents by pipetting 5 mL of *Methylene chloride reagent* over each Capsule and rinsing with hexanes. Collect the washings in a 500-mL volumetric flask, dilute with hexanes to volume, and mix. Transfer 50.0 mL of this solution to a 200-mL volumetric flask, dilute with hexanes to volume, and mix to obtain a solution having a concentration of about 0.1 mg of isotretinoin per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm  $\times$  25-cm column containing packing L3. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution* [NOTE—The injection volume is about 20  $\mu$ L.], and record the peak responses as directed for *Procedure*: the relative retention times for isotretinoin and tretinoin are about 0.75 and 1.00, respectively; the resolution, *R*, between isotretinoin and tretinoin is not less than 3.0; the tailing factor for the isotretinoin peak is not greater than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than two times the retention time of isotretinoin. Record the chromatograms, and measure the peak responses: the peak response for any impurity is not more than that of the tretinoin response obtained from the *Standard solution* (1.0%); and the sum of all the peak responses, excluding that of isotretinoin, obtained from the *Test solution*, is not more than 1.5 times the tretinoin response obtained from the *Standard solution* (1.5%).

**Assay**—[NOTE—Protect the *System suitability solution*, the *Standard preparation*, the *Assay stock preparation*, and the *Assay preparation* from direct light.]

**Diluent**—Heat 0.1 N sodium hydroxide to about 60° to 70°. Cool it to room temperature and purge with helium or nitrogen. Store it in a plastic container.

**Solvent A**—Prepare a solution of 0.5% acetic acid in methanol.

**Solvent B**—Prepare a solution of 0.5% acetic acid in water.

**Mobile phase**—Prepare a mixture of *Solvent A* and *Solvent B* (71:29). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Dissolve suitable quantities of USP Isotretinoin RS and USP Tretinoin RS in *Diluent* to obtain a solution having known concentrations of about 0.04 mg per mL of isotretinoin and 0.02 mg per mL of tretinoin.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Isotretinoin RS in *Diluent*, and dilute with *Diluent*, stepwise if necessary, to obtain a solution having a known concentration of about 0.04 mg per mL.

**Assay stock preparation**—Transfer not fewer than 10 Capsules to a suitable volumetric flask. Add *Diluent* to the volumetric flask to fill about 50% of the volume, sonicate for

1 hour with occasional shaking to disperse all the contents, and make up the volume with *Diluent* to obtain a solution having a known concentration of about 0.4 mg per mL of isotretinoin.

**Assay preparation**—Transfer 5 mL of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass the solution through a suitable 0.45- $\mu$ m or finer porosity membrane filter.

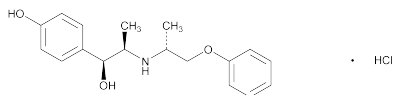
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 353-nm detector and a 4.6-mm  $\times$  25-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the relative retention times for isotretinoin and tretinoin are about 1.0 and 1.3, respectively; the resolution, *R*, between isotretinoin and tretinoin is not less than 2.0; the tailing factor for the isotretinoin peak is not greater than 2.0; and the column efficiency determined from the isotretinoin peak is not less than 2000. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of isotretinoin ( $C_{20}H_{28}O_2$ ) in each of the Capsules taken by the formula:

$$10(CV/N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of isotretinoin in the *Standard preparation*; *V* is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation*; *N* is the number of Capsules taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the isotretinoin peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isoxsuprine Hydrochloride



$C_{18}H_{23}NO_3 \cdot HCl$  337.84

Benzenemethanol, 4-hydroxy- $\alpha$ -[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]-, hydrochloride, stereoisomer.

*p*-Hydroxy- $\alpha$ -[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]benzyl alcohol hydrochloride.  
( $\pm$ )-( $\alpha R^*$ )-*p*-Hydroxy- $\alpha$ -[(1*S*\*)-1-[(1*S*\*)-1-methyl-2-phenoxyethyl]amino]ethyl]benzyl alcohol hydrochloride [579-56-6; 34331-89-0].

» Isoxsuprine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of  $C_{18}H_{23}NO_3 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** <11>—

USP Isoxsuprine Hydrochloride RS

### Identification—

**A: Infrared Absorption** <197K>.

**B: Ultraviolet Absorption** <197U>—

*Solution:* 50  $\mu$ g per mL.

*Medium:* water.

**C:** To 1 mL of a solution (1 in 100), obtained by heating as necessary, add 3 mL of a 1 in 15 solution of sodium nitrite in 2 N sulfuric acid. Add ammonium hydroxide dropwise: a yellow precipitate is formed and it dissolves upon the addition of sodium hydroxide solution (1 in 5).

**D:** To 1 mL of a solution (1 in 100) add 1 mL of phosphomolybdic acid solution (1 in 100): a pale yellow to white precipitate is formed.

**pH** <791>: between 4.5 and 6.0, in a solution (1 in 100).

**Loss on drying** <731>—Dry it at 105° for 1 hour: it loses not more than 0.5% of its weight.

**Residue on ignition** <281>: not more than 0.2%.

**Heavy metals, Method II** <231>: 0.002%.

**Related compounds**—To 10 mg, accurately weighed in a suitable vial, add 1 mL of *N*-trimethylsilylimidazole, and heat at 65° for 10 minutes. Add 5 mL of isooctane, wash with one 3-mL portion of water, and allow the layers to separate. Inject a 2- $\mu$ L portion of the isooctane solution into a gas chromatograph equipped with a 0.3-cm  $\times$  2.0-m glass column packed with packing S1A containing 3% liquid phase G2 and a flame-ionization detector. The column temperature is maintained at 215°, and the injection port and detector are maintained at 250°. The carrier gas is nitrogen, flowing at the rate of 25 mL per minute. Adjust the instrument to provide full-scale response for the major component. Inject a second 2- $\mu$ L portion of the isooctane solution with the attenuator adjusted to an 8-fold increase in sensitivity, and record the chromatogram from 0.5 to 1.5 relative to the retention time of the major peak. Measure the area of all minor peaks, and correct for differences in sensitivity settings. Calculate the percentage of related compounds present taken by the formula:

$$100A / B$$

in which *A* is the sum of the corrected area peaks for all minor peaks, and *B* is the sum of the corrected area peaks for the major and minor peaks. Not more than 2.0% is found.

**Assay**—Transfer about 50 mg of Isoxsuprine Hydrochloride, accurately weighed, to a 1000-mL volumetric flask, add water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Isoxsuprine Hydrochloride RS in the same medium having a known concentration of about 50  $\mu$ g per mL in 1-cm cells at the wavelengths of maximum absorbance at about 269 and 300 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{18}H_{23}NO_3 \cdot HCl$  in the Isoxsuprine Hydrochloride taken by the formula:

$$C(A_{U269} - A_{U300}) / (A_{S269} - A_{S300})$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Isoxsuprine Hydrochloride RS in the Standard solution; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the assay solution (*U*) and the Standard solution (*S*), respectively.

## Isoxsuprine Hydrochloride Injection

» Isoxsuprine Hydrochloride Injection is a sterile solution of Isoxsuprine Hydrochloride in Water

for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{18}H_{23}NO_3 \cdot HCl$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Endotoxin RS

USP Isoxsuprine Hydrochloride RS

**Identification**—To a 60-mL separator transfer 10 mL of pH 9.0 buffer (prepared by mixing equal volumes of 0.1 M monobasic potassium phosphate and 0.1 N sodium hydroxide and, using a pH meter, adjusting to a pH of 9.0 by adding, as necessary, more of either solution) add 1 mL of Injection, and mix. Add 2 mL of chloroform, shake vigorously for 1 minute, filter the chloroform extract through a pledget of cotton, and mix the filtrate with 500 mg of potassium bromide. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the isoxsuprine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoxsuprine Hydrochloride RS that has been treated in the same manner.

**Bacterial endotoxins** (85)—It contains not more than 35.70 USP Endotoxin Units per mg of isoxsuprine hydrochloride.

**pH** (791): between 4.9 and 6.0.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

*pH 4.0 Citrate buffer*—Mix equal volumes of 0.5 M citric acid and 0.5 M sodium citrate, and adjust, by the addition of either solution as necessary, the pH of the solution to  $4.0 \pm 0.2$ .

*Mixed solvent*—Shake 40 mL of ether, 160 mL of iso-octane, and 10 mL of water in a separator, remove and discard the water phase, and pass the solvent phase through a large pledget of cotton to remove excess water.

*Standard preparation*—Transfer about 40 mg of USP Isoxsuprine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 2 N sulfuric acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 2 N sulfuric acid to volume, and mix. The concentration of USP Isoxsuprine Hydrochloride RS in the *Standard preparation* is about 80  $\mu$ g per mL.

*Chromatographic column*—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* (621)), packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 2 g of *Solid Support* and 1 mL of *pH 4.0 Citrate buffer*, and the upper segment is a mixture prepared as directed under *Assay preparation*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 4 mg of isoxsuprine hydrochloride, to a 100-mL beaker, add 1 mL of dimethyl sulfoxide, and allow to stand for about 10 minutes, with occasional swirling. Add 1 mL of *pH 4.0 Citrate buffer* and 3 g of *Solid Support*, mix as directed under *Chromatographic column*, and transfer to the column. Pass 75 mL of *Mixed solvent* through the column, and discard the eluate. Elute the column with a solution prepared by mixing 0.2 mL of bis(2-ethylhexyl)phosphoric acid with 75 mL of *Mixed solvent*, and collect the eluate in a 125-mL separator. Extract the eluate with two 20-mL portions of 2 N sulfuric acid. Transfer the extracts to a 50-mL volumetric flask, dilute with 2 N sulfuric acid to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using a column

blank, prepared with *Mixed solvent*, to set the instrument. Calculate the quantity, in mg, of  $C_{18}H_{23}NO_3 \cdot HCl$  in the portion of Injection taken by the formula:

$$0.05C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Isoxsuprine Hydrochloride RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Isoxsuprine Hydrochloride Tablets

» Isoxsuprine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{23}NO_3 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Isoxsuprine Hydrochloride RS

**Identification**—Transfer a portion of finely powdered Tablets, equivalent to about 10 mg of isoxsuprine hydrochloride, to a 60-mL beaker, add about 20 mL of water, mix, and filter. Transfer the clear filtrate to a 60-mL separator, add 10 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and shake vigorously to mix. Extract with 2 mL of chloroform, filter the extract through a pledget of cotton, and mix the filtrate with 500 mg of potassium bromide. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the isoxsuprine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoxsuprine Hydrochloride RS that has been treated in the same manner.

**Dissolution** (711)—

*Medium*: water; 900 mL.

*Apparatus 1*: 100 rpm.

*Time*: 45 minutes.

*Procedure*—Determine the amount of  $C_{18}H_{23}NO_3 \cdot HCl$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 269 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isoxsuprine Hydrochloride RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{18}H_{23}NO_3 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Buffer solution*—Transfer about 1.32 g of anhydrous dibasic ammonium phosphate to a 1-liter volumetric flask, add about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 7.5, dilute with water to volume, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Isoxsuprine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of isoxsuprine hy-



drochloride, to a 50-mL volumetric flask, and add about 25 mL of *Mobile phase*. Shake by mechanical means for 30 minutes, sonicate for ten minutes to dissolve, dilute with *Mobile phase* to volume, mix, and filter.

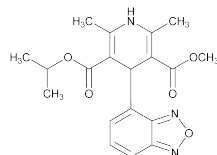
**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 274-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>18</sub>H<sub>23</sub>NO<sub>3</sub> · HCl in the portion of Tablets taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Isosuprine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isradipine



C<sub>19</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub> 371.39

3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-, methyl 1-methylethyl ester, (±)-. Isopropyl methyl (±)-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate [75695-93-1].

» Isradipine contains not less than 98.0 percent and not more than 102.0 percent of C<sub>19</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards <11>—

USP Isradipine RS

USP Isradipine Related Compound A RS

Isopropyl methyl 4-(4-benzofurazanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate.

C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub> 369.38

**Identification, Infrared Absorption** <197K>.

**Melting range** <741>: between 166° and 170°.

**Loss on drying** <731>—Dry it at 105° for 4 hours: it loses not more than 0.2% of its weight.

**Residue on ignition** <281>: not more than 0.1%.

**Heavy metals, Method II** <231>: 0.002%.

**Chromatographic purity**—[NOTE—Use low-actinic glassware throughout this procedure, and otherwise protect the test specimen, the Reference Standard, and all solutions containing them from unnecessary exposure to light.]

*Mobile phase*—Prepare as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Isradipine RS in *Mobile phase*, with the aid of

sonication if necessary, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a known concentration of about 6 µg per mL. [NOTE—If necessary, use 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standard prior to diluting with *Mobile phase*.]

*Resolution solution*—Use the *Standard preparation* prepared as directed in the *Assay*.

*Test solution*—Transfer about 50 mg of Isradipine, accurately weighed, to a 25-mL volumetric flask, and add 5.0 mL of methanol to dissolve, using sonication if necessary. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph replicate injections of the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution,  $R$ , between isradipine and isradipine related compound A is not less than 1.5, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than three times the retention time of isradipine. Record the chromatograms, and measure the responses for all of the peaks: the sum of the peak responses, other than that of isradipine, in the chromatogram of the *Test solution* is not more than four times the isradipine response obtained from the *Standard solution* (1.2%), the response of the largest peak, other than that of isradipine, in the chromatogram of the *Test solution* is not more than 1.6 times greater than the isradipine response obtained from the *Standard solution* (0.5%), and no other peak response, other than that of isradipine, is greater than the isradipine response obtained from the *Standard solution* (0.3%).

**Assay**—[NOTE—Use low-actinic glassware throughout this procedure, and otherwise protect the test specimen, the Reference Standard, and all solutions containing them, from unnecessary exposure to light.]

*Mobile phase*—Prepare a filtered and degassed mixture of water, methanol, and tetrahydrofuran (50:40:10). Make adjustments, if necessary (see *System Suitability* under *Chromatography* <621>).

*Standard preparation*—Dissolve, with the aid of sonication, if necessary, accurately weighed quantities of USP Isradipine RS and USP Isradipine Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise, if necessary, with *Mobile phase* to obtain a solution having known concentrations of 0.2 mg and 10 µg of USP Isradipine RS and USP Isradipine Related Compound A RS, respectively, per mL. [NOTE—If necessary, 1 mL of methanol per 20 mL of *Mobile phase* may be added to dissolve the Reference Standards.]

*Assay preparation*—Transfer about 20 mg of Isradipine, accurately weighed, to a 100-mL volumetric flask. Add sufficient methanol to dissolve, using sonication, if necessary. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 326-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between isradipine and isradipine related compound A is not less than 1.5, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of  $C_{19}H_{21}N_3O_5$  in the portion of Isradipine taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Isradipine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the isradipine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Isradipine Capsules

» Isradipine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isradipine ( $C_{19}H_{21}N_3O_5$ ).

**Packaging and storage**—Store in a tight container at controlled room temperature. Protect from light.

### USP Reference standards (11)—

USP Isradipine RS

USP Isradipine Related Compound A RS

Isopropyl methyl 4-(4-benzofurazanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate.

$C_{19}H_{19}N_3O_5$  369.38

### Identification—

**A:** *Ultraviolet Absorption* (197U)—

*Medium:* methanol.

*Solution*—Transfer the contents of 1 Capsule into a suitable volumetric flask, dissolve the contents in the *Medium* by mechanical shaking for 15 minutes, and dilute with *Medium* to obtain a solution containing 25 µg of isradipine per mL.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium:* 0.1% aqueous solution of lauryl dimethyl amine oxide (prepared by transferring 500 mL of deaerated water into the dissolution vessel, adding 1.65 mL of 30% lauryl dimethyl amine oxide, and mixing); 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{19}H_{21}N_3O_5$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 328 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Isradipine RS in the same *Medium*.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{19}H_{21}N_3O_5$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

NOTE—Isradipine is light sensitive. Throughout the following procedures, protect test or assay specimens, the Reference Standards, and solutions containing them from unnecessary exposure to light. Use low-actinic glassware, unless otherwise directed.

### Chromatographic purity—

*Mobile phase, Resolution solution, and Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Isradipine*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Isradipine RS in *Mobile phase*, with the aid of sonication if necessary, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

[NOTE—If necessary, use 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standard prior to diluting with *Mobile phase*.]

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks: the sum of all peak responses, other than that of isradipine, from the *Test solution* is not more than four times the isradipine response obtained from the *Standard solution* (2.0%); and no single peak response is greater than that of the isradipine peak response obtained from the *Standard solution* (0.5%).

### Assay—

*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Isradipine*.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix the combined contents. Transfer an accurately weighed quantity, equivalent to about 25 mg of isradipine, to a 100-mL volumetric flask. Add 5.0 mL of methanol and 5.0 mL of *Mobile phase*, and sonicate at room temperature for 15 minutes. Shake for 15 minutes in a mechanical shaker. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 5 mL of the filtrate.

*Procedure*—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isradipine ( $C_{19}H_{21}N_3O_5$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Isradipine RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the isradipine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Add the following:

## ▲Isradipine Oral Suspension

### DEFINITION

Isradipine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of isradipine ( $C_{19}H_{21}N_3O_5$ ).

Prepare Isradipine Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Isradipine capsules <sup>a</sup> or powder <sup>b</sup>	100 mg
Glycerin, USP	3 mL
Syrup, NF, a sufficient quantity to make	100 mL

<sup>a</sup> DynaCirc 5-mg capsules, Sandoz Pharmaceuticals, East Hanover, NJ.

<sup>b</sup> Isradipine powder, Sandoz, East Hanover, NJ.

Calculate the required quantity of each ingredient for the total amount to be prepared. If using capsules, empty the required number in a suitable mortar, or use *Isradipine* powder. Add sufficient *Glycerin* to wet the powder, and triturate to a fine paste. Add the *Syrup* in small portions. Add increasing volumes of the *Syrup* to make an isradipine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Syrup* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol, tetrahydrofuran, and water (42:20:38). Filter, and degas.

**Diluent:** Prepare a solution of methanol and 95% ethanol (50:50).

**Standard stock solution:** 1.0 mg/mL of USP Isradipine RS in *Diluent*

**Standard solution:** Prepare 0.1 mg/mL of isradipine from *Standard stock solution* and *Diluent*, and pass through a filter of 0.22-μm pore size.

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of isradipine from Oral Suspension and *Diluent*, and pass through a filter of 0.22-μm pore size.

**Chromatographic system**

(See *Chromatography* ⟨621⟩, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for isradipine is about 6.1 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isradipine (C<sub>19</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of isradipine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of isradipine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• pH** ⟨791⟩: 5.5–6.5

**ADDITIONAL REQUIREMENTS**

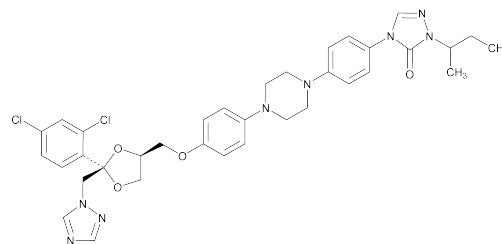
**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature.

**• LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

**• BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded, when stored at controlled cold temperature

**• USP REFERENCE STANDARDS** ⟨11⟩

USP Isradipine RS<sub>▲USP36</sub>

**Itraconazole**

C<sub>35</sub>H<sub>38</sub>Cl<sub>2</sub>N<sub>8</sub>O<sub>4</sub> 705.63

3*H*-1,2,4-Triazol-3-one, 4-[4-[4-[2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-; (±)-1-*sec*-Butyl-4-[*p*-[4-[*p*-[[2*R*\*,4*S*\*)-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ<sup>2</sup>-1,2,4-triazolin-5-one [84625-61-6].

**DEFINITION**

Itraconazole contains NLT 98.5% and NMT 101.5% of C<sub>35</sub>H<sub>38</sub>Cl<sub>2</sub>N<sub>8</sub>O<sub>4</sub>, calculated on the dried basis.

**IDENTIFICATION**

**• A. INFRARED ABSORPTION** ⟨197K⟩

**ASSAY****• PROCEDURE**

**Diluent:** Methyl ethyl ketone and glacial acetic acid (7:1)

**Sample solution:** 0.3 g of Itraconazole in 70 mL of *Diluent*

**Analysis:** Titrate with 0.1 M perchloric acid, determining the endpoint potentiometrically at the second inflection point. Each mL of 0.1 M perchloric acid is equivalent to 35.3 mg of itraconazole (C<sub>35</sub>H<sub>38</sub>Cl<sub>2</sub>N<sub>8</sub>O<sub>4</sub>).

**Acceptance criteria:** 98.5%–101.5% on the dried basis

**IMPURITIES**

**• RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%, determined on 1.0 g

**• ORGANIC IMPURITIES**

**Solution A:** 0.08 M tetrabutylammonium hydrogen sulfate

**Solution B:** Acetonitrile

**Diluent:** Dilute 4.0 mL of hydrochloric acid with methanol to 1 L.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
22	50	50
27	50	50

**System suitability solution:** 10 mg/mL of USP

Itraconazole System Suitability Mixture RS in *Diluent*

**Standard solution:** 1.0 μg/mL of USP Itraconazole RS in *Diluent*

**Sample solution:** 10 mg/mL of Itraconazole in *Diluent*

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 10 μL

System suitability

Sample: System suitability solution

Suitability requirements

System suitability solution: NLT 1.5 peak-to-valley ratio  
Calculate the peak-to-valley ratio, *p/v*, using the following:

$$p/v = H_p/H_v$$

*H<sub>p</sub>* = height above the baseline of the peak due to the *n*-butyl isomer impurity  
*H<sub>v</sub>* = height above the baseline of the lowest point of the curve separating this peak from the itraconazole peak

Analysis

Samples: Diluent, Standard solution, and Sample solution  
Calculate the percentage of each impurity in the portion of Itraconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of each impurity from the Sample solution  
*r<sub>S</sub>* = peak response of itraconazole from the Standard solution  
*C<sub>S</sub>* = concentration of the Standard solution (mg/mL)  
*C<sub>U</sub>* = concentration of the Sample solution (mg/mL)  
Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%) <sup>a</sup>
4-Methoxy derivative <sup>b</sup>	0.20	0.5
4-Triazolyl isomer <sup>c</sup>	0.74	0.5
Propyl analog <sup>d</sup>	0.86	0.5
Isopropyl analog <sup>e</sup>	0.86	0.5
Epimer <sup>f</sup>	0.93	0.5
Itraconazole	1.0	—
<i>n</i> -Butyl isomers <sup>g</sup>	1.05	0.5
Didioxolanyl analog <sup>h</sup>	1.3	0.5

<sup>a</sup> Disregard any peak observed in the Diluent and any peak less than 0.05%.

<sup>b</sup> 2-sec-Butyl-4-[4-[4-(4-methoxyphenyl)piperazin-1-yl]phenyl]-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>c</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-4-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-sec-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>d</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-propyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>e</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-isopropyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>f</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-sec-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>g</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>h</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methyl]-2*H*-1,2,4-triazol-3(4*H*)-one.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%) <sup>a</sup>
Any unspecified impurity	—	0.10
Total impurities	—	1.25

<sup>a</sup> Disregard any peak observed in the Diluent and any peak less than 0.05%.

<sup>b</sup> 2-sec-Butyl-4-[4-[4-(4-methoxyphenyl)piperazin-1-yl]phenyl]-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>c</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(4*H*-1,2,4-Triazol-4-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-sec-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>d</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-propyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>e</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-isopropyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>f</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-sec-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>g</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.

<sup>h</sup> 4-[4-[4-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(2*RS*,4*SR*)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methyl]-2*H*-1,2,4-triazol-3(4*H*)-one.

SPECIFIC TESTS

• OPTICAL ROTATION, Angular Rotation <781A>

Sample: 100 mg/mL in methylene chloride

Acceptance criteria: −0.10° to +0.10° at 20°

• LOSS ON DRYING <731>

Sample: Dry 1 g at 105° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight, light-resistant containers, and store at room temperature.

• USP REFERENCE STANDARDS <11>

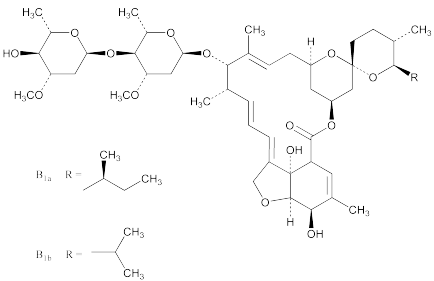
USP Itraconazole RS

USP Itraconazole System Suitability Mixture RS

USP Itraconazole System Suitability Mixture RS contains itraconazole and 6 other minor components

(4-triazolyl isomer, propyl analog, isopropyl analog, epimer, *n*-butyl isomer, and didioxolanyl analog).

Ivermectin



C<sub>48</sub>H<sub>74</sub>O<sub>14</sub> (Component H<sub>2</sub>B<sub>1a</sub>) 875.09

C<sub>47</sub>H<sub>72</sub>O<sub>14</sub> (Component H<sub>2</sub>B<sub>1b</sub>) 861.07

Component H<sub>2</sub>B<sub>1a</sub>:

Avermectin A<sub>1a</sub>, 5-O-demethyl-22,23-dihydro-

(2*aE*,4*E*,8*E*)-(5′,5′,6′,6′,7′,5′,11*R*,13*R*,15*S*,17*aR*,20*R*,20*aR*,20*bS*)-6′-(*S*)-sec-Butyl-3′,4′,5′,6′,7′,10′,11′,14′,15′,17*a*,20′,20*a*,20*b*-tetradecahydro-20′,20*b*-dihydroxy[11′,15′-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-

$\alpha$ -L-arabino-hexopyranosyl)-3-O-methyl- $\alpha$ -L-arabino-hexopyranoside [70161-11-4].

Component H<sub>2</sub>B<sub>1b</sub>:

Avermectin A<sub>1a</sub>, 5-O-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)-.

(2aE,4E,8E)-(5'S,6S,6'R,7S,11R,13R,15S,17aR,20R,20aR,20bS)-3',4',5',6',7,10,11-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'[2H]pyran]-7-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl- $\alpha$ -L-arabino-hexopyranosyl)-3-O-methyl- $\alpha$ -L-arabino-hexopyranoside [70209-81-3].

» Ivermectin is a mixture of avermectin A<sub>1a</sub>, 5-O-demethyl-22,23-dihydro-(component H<sub>2</sub>B<sub>1a</sub>), and avermectin A<sub>1a</sub>, 5-O-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)-(component H<sub>2</sub>B<sub>1b</sub>). It contains not less than 95.0 percent and not more than 102.0 percent for the sum of component H<sub>2</sub>B<sub>1a</sub> plus component H<sub>2</sub>B<sub>1b</sub>, calculated on the anhydrous and alcohol- and formamide-free basis; and the ratio (calculated by area percentage) of component H<sub>2</sub>B<sub>1a</sub>/(H<sub>2</sub>B<sub>1a</sub> + H<sub>2</sub>B<sub>1b</sub>) is not less than 90.0 percent. It may contain small amounts of suitable antioxidant and chelating agents.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 8°. Where the use of an antioxidant is allowed, store at 25°, excursions permitted between 15° and 30°.

**Labeling**—If it is intended for veterinary use only, it is so labeled. Label it to state the name(s) and amount(s) of any added substance(s). Label it also to state that it is for manufacturing, processing, or repackaging.

**USP Reference standards** (11)—

USP Ivermectin RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention times of the component H<sub>2</sub>B<sub>1a</sub> peak and the component H<sub>2</sub>B<sub>1b</sub> peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781S): between -17° and -20° measured at 20°, calculated on the water-, alcohol-, and formamide-free basis.

*Test solution*: 25 mg per mL, in methanol.

**Water**, *Method I* (921): not more than 1.0%.

**Residue on ignition** (281): not more than 0.1%.

**Heavy metals**, *Method II* (231): 0.002%.

**Limit of alcohol and formamide**—

*Internal standard solution*—Dilute 0.5 mL of isopropyl alcohol with water to 100 mL, and mix.

*Standard solution 1*—Transfer 2.0 mL of dehydrated alcohol to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution 2*—Transfer 1.0 mL of formamide to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution 3*—Transfer 5.0 mL of *Standard solution 1* and 5.0 mL of *Standard solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having concentrations of formamide and alcohol of 0.001 and 0.002 mL per mL, respectively. Transfer 2.0 mL of this solution to a 15-mL centrifuge tube, add 2.0 mL of *m*-xylene, insert the stopper, mix, and centrifuge. Remove the upper *m*-xylene layer, and extract it with 2.0 mL of water. Discard the upper layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution*, and

mix. Each mL of this solution contains about 0.0008 mL of alcohol and 0.0004 mL of formamide.

*Standard solution 4*—Transfer 10.0 mL of *Standard solution 1* and 10.0 mL of *Standard solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having concentrations of alcohol and formamide of 0.004 and 0.002 mL per mL, respectively. Transfer 2.0 mL of this solution to a 15-mL centrifuge tube, add 2.0 mL of *m*-xylene, insert the stopper, mix, and centrifuge. Remove the upper *m*-xylene layer, and extract it with 2.0 mL of water. Discard the upper layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution*, and mix. Each mL of this solution contains about 0.0016 mL of alcohol and 0.0008 mL of formamide.

*Test solution*—Transfer 120 mg of Ivermectin, accurately weighed, to a 15-mL centrifuge tube, and dissolve in 2.0 mL of *m*-xylene, heating in a water bath at 45 ± 5°, if necessary. Add 2.0 mL of water, mix, and centrifuge. Transfer the *m*-xylene layer to a 15-mL centrifuge tube, and extract with 2.0 mL of water. Discard the upper *m*-xylene layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution*, and mix.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m fused-silica analytical column coated with a 3-μm G43 stationary phase. The carrier gas is helium, with a 10:1 split ratio and a linear velocity of about 35 cm per second. The chromatograph is programmed as follows. The column temperature is maintained at about 40° for 5 minutes after injection, then increased at a rate of 20° per minute to 180° and maintained at 180° for 2 minutes. The injection port temperature is maintained at about 220°, and the detector temperature is maintained at about 280°.

*Procedure*—Separately inject equal volumes (about 2 μL) of *Standard solution 3*, *Standard solution 4*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for alcohol, formamide, and isopropyl alcohol. Plot the ratios of the peak responses for alcohol and isopropyl alcohol and for formamide and isopropyl alcohol versus concentrations, in mL per mL, of alcohol and formamide, respectively, obtained from *Standard solution 3* and *Standard solution 4*. From the graphs so obtained, and using the ratios of the peak responses for alcohol and isopropyl alcohol and for formamide and isopropyl alcohol obtained from the chromatogram of the *Test solution*, determine the concentrations, *C*, of alcohol and formamide in the *Test solution*. [NOTE—In the event that the peak responses of the *Test solution* are significantly outside the ranges of peak responses obtained from *Standard solution 3* and *Standard solution 4*, prepare additional *Standard solutions*, and chromatograph them to obtain peak responses bracketing those obtained with the *Test solution*.] Calculate the percentages of alcohol and formamide in the portion of Ivermectin taken by the formula:

$$500,000CD/W$$

in which *C* is the concentration of alcohol or formamide, as appropriate, in mL per mL, in the *Test solution*; *D* is the density of alcohol (0.79) or formamide (1.13); and *W* is the weight, in mg, of Ivermectin taken: not more than 5.0% of alcohol and 3.0% of formamide are found.

**Related compounds**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Proceed as directed for *Standard preparation* in the *Assay*.

*Test solution*—Use the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram of the *Test solution* for a period of time equivalent to twice the retention time of the main peak in the chromatogram obtained from the

*Standard solution*, and measure the peak areas. Calculate the percentage of each impurity by the formula:

$$100r_i / (r_s - r_b)$$

in which  $r_i$  is the peak area for each individual impurity in the *Test solution* chromatogram;  $r_s$  is the sum of all peaks in the *Test solution* chromatogram; and  $r_b$  is the total area of all peaks in a blank chromatogram: not more than 2.5% is found for the sum of all peaks with a relative retention time of about 1.3 to 1.4 (corresponding to  $H_4B_{1a}$  isomers and  $\Delta^{2,3}H_2B_{1a}$ ); not more than 1% is found for the peak with a relative retention time of about 0.7 (corresponding to 8a-oxo- $H_2B_{1a}$ ); not more than 0.7% is found for the peak with a relative retention time of about 0.5 (corresponding to avermectin  $B_{1a}$ ); not more than 0.5% is found for any other individual impurity peak; not more than 1% is found for the sum of all unidentified peaks; and not more than 4% is found for the sum of all the peaks, apart from the two main peaks ( $H_2B_{1a}$  and  $H_2B_{1b}$ ). Disregard any peak that is calculated to be less than 0.05%.

#### Assay—

*Mobile phase*—Prepare a mixture of acetonitrile, methanol, and water (53:27.5:19.5), and degas. Make adjustments if necessary (see *System Suitability under Chromatography* <621>). Increasing the proportion of water increases the elution times and allows better separation of impurities.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL.

*Assay preparation*—Transfer about 40 mg of Ivermectin, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Sonicate, if necessary.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.75 for component  $H_2B_{1b}$  and 1.0 for component  $H_2B_{1a}$ ; the resolution,  $R$ , between component  $H_2B_{1b}$  and component  $H_2B_{1a}$  is not less than 3.0; the column efficiency determined from the component  $H_2B_{1a}$  peak is not less than 2000 theoretical plates; the tailing factor for component  $H_2B_{1a}$  peak is not more than 2.5; and the relative standard deviation for six replicate injections is not more than 1.0% determined from the component  $H_2B_{1a}$  peak.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for component  $H_2B_{1a}$  and component  $H_2B_{1b}$ . Calculate the quantity, in mg, of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) and component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ) in the portion of Ivermectin taken by the formula:

$$DC(r_U / r_S)$$

in which  $D$  is the dilution factor, in mL, used to prepare the *Assay preparation*;  $C$  is the concentration, in mg per mL, of component  $H_2B_{1a}$  or component  $H_2B_{1b}$  in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak areas for component  $H_2B_{1a}$  or component  $H_2B_{1b}$  obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ivermectin Injection

» Ivermectin Injection is a sterile solution of Ivermectin in a suitable vehicle. It contains not

less than 95.0 percent and not more than 105.0 percent of the labeled amount of ivermectin, calculated as the sum of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) plus component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ). The ratio of the contents,  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$ , is not less than 90.0 percent.

**Packaging and storage**—Preserve in single-dose or in multi-dose containers, preferably of Type I glass or plastic. Store at a temperature of not more than 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards <11>—

USP Endotoxin RS

USP Ivermectin RS

#### Identification—

**A: Thin-Layer Chromatographic Identification Test** <201>—

*Test solution*—Dissolve a volume of the Injection in methanol, dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.5 mg per mL of ivermectin, and filter.

*Injection volume*: 2  $\mu$ L.

*Developing solvent system*: unsaturated chamber, consisting of a freshly prepared and equilibrated mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).

*Procedure*—Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light: the retardation factor,  $R_f$ , of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**B:** The retention times of the two principal component peaks of ivermectin in the chromatogram of the *Assay preparation* corresponds to that of the two principal component peaks of ivermectin in the chromatogram of the *Standard preparation*, obtained as directed in the Assay.

**Bacterial endotoxins** <85>—It contains not more than 0.016 USP Endotoxin Unit per  $\mu$ g of ivermectin.

**Sterility** <71>—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

#### Chromatographic purity—

*Mobile phase and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.004 mg per mL solution.

*Test solution*—Dissolve a volume of the Injection in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.

*Procedure*—Inject equal volumes (about 20  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(C_S / C_T)(r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of ivermectin in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of ivermectin in the *Test solution*;  $r_i$  is the peak response for each individual peak obtained from the *Test solution*; and  $r_S$  is the ivermectin peak response obtained from the *Standard solution*. Not more than 2.7% of the peak with a relative retention time of about 1.3 to 1.5 to that of the principal peak is found; not more than 1.0% of any other impurity is found; and not more than 6.0% of total impurities is found. Disregard any peak below 0.05%.

**Other requirements**—It meets the requirements under *Injections* (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (106:55:39). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.4 mg per mL solution.

**Assay preparation**—Dilute a volume of Injection in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with 245-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the first peak (component  $H_2B_{1b}$ ) and the second peak (component  $H_2B_{1a}$ ) is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%, determined from the component  $H_2B_{1a}$  peak.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the component  $H_2B_{1a}$  plus component  $H_2B_{1b}$ . Calculate the percentage of label claim of ivermectin ( $H_2B_{1a} + H_2B_{1b}$ ) in the portion of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of ivermectin in the *Assay preparation*;  $r_U$  is the total peak response of  $H_2B_{1a}$  plus  $H_2B_{1b}$  peaks obtained from the *Assay preparation*; and  $r_S$  is the total peak response of  $H_2B_{1a}$  plus  $H_2B_{1b}$  peaks obtained from the *Standard preparation*. Calculate the ratio of the contents, in percent, of  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$  in the portion of Injection taken by the formula:

$$100(r_1 / r_U)$$

in which  $r_1$  is the peak response of  $H_2B_{1a}$  obtained from the *Assay preparation*; and  $r_U$  is as defined above.

## Ivermectin Paste

### DEFINITION

Ivermectin Paste contains NLT 90.0% and NMT 110.0% of the labeled amount of Ivermectin, calculated as the sum of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) and component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ). The ratio of the contents,  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$ , is NLT 90.0%.

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Sample solution:** 0.5 mg/mL of ivermectin dispersed in methanol from a quantity of Paste. Sonicate if necessary until completely dispersed.

**Application volume:** 2 μL

**Developing solvent system:** Methylene chloride, methanol, and ammonium hydroxide (90:9:1)

**Analysis:** Develop the chromatogram in an unsaturated chamber. Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light.

**Acceptance criteria:** Meets the requirements

- **B.** The retention times of the two principal component peaks of ivermectin from the *Sample solution* correspond to those of the two principal component peaks of ivermectin from the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, and water (106:55:39)

**Standard solution:** 0.4 mg/mL of USP Ivermectin RS in methanol

**Sample solution:** Disperse a quantity of Paste in methanol, using sonication if necessary, to obtain a solution containing 0.4 mg/mL of ivermectin.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 245 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between the first peak (component  $H_2B_{1b}$ ) and the second peak (component  $H_2B_{1a}$ )

**Relative standard deviation:** NMT 2.0%, determined from the component  $H_2B_{1a}$  peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ivermectin, component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) and component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ), in the portion of Paste taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = sum of the peak responses for component  $H_2B_{1a}$  and component  $H_2B_{1b}$  from the *Sample solution*

$r_S$  = sum of the peak responses for component  $H_2B_{1a}$  and component  $H_2B_{1b}$  from the *Standard solution*

$C_S$  = concentration of USP Ivermectin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ivermectin in the *Sample solution* (mg/mL)

Calculate the ratio of the contents, in percentage, of the components,  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$ , in the portion of Paste taken:

$$\text{Result} = (r_U / r_T) \times 100$$

$r_U$  = peak response of  $H_2B_{1a}$  from the *Sample solution*

$r_T$  = sum of the peak responses for component  $H_2B_{1a}$  and component  $H_2B_{1b}$  from the *Sample solution*

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of ivermectin, calculated as the sum of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) and component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ). The ratio of the contents,  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$ , is NLT 90.0%.

### IMPURITIES

#### • ORGANIC IMPURITIES

**Mobile phase, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard solution:** 0.004 mg/mL of USP Ivermectin RS in methanol

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Paste taken, disregarding any peak below 0.05%:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for each impurity from the *Sample solution*  
 $r_S$  = peak response of the principal peak from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of ivermectin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 3.0% of any peak with a relative retention time of 1.3–1.5, relative to that of the principal peak

**Any other impurity:** NMT 1.0%

**Total impurities:** NMT 6.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at a temperature not higher than 30°.
- **LABELING:** Label it to indicate that it is for oral veterinary use only.
- **USP REFERENCE STANDARDS** <11>  
USP Ivermectin RS

**Ivermectin Tablets**

» Ivermectin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Ivermectin components  $\text{H}_2\text{B}_{1a}$  ( $\text{C}_{48}\text{H}_{74}\text{O}_{14}$ ) plus  $\text{H}_2\text{B}_{1b}$  ( $\text{C}_{47}\text{H}_{72}\text{O}_{14}$ ). They may contain a suitable antioxidant.

**Packaging and storage**—Preserve in well-closed containers, and store at a temperature below 30°.

**USP Reference standards** <11>—

USP Ivermectin RS

USP 3-*tert*-Butyl-4-hydroxyanisole RS

**Identification**—The retention times of the  $\text{H}_2\text{B}_{1a}$  and  $\text{H}_2\text{B}_{1b}$  peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** <711>—

**Medium:** 0.01 M phosphate buffer, pH 7, with 0.5% of sodium dodecyl sulfate (prepared by dissolving 50 g of sodium dodecyl sulfate in approximately 9 L of water, adding 100 mL of 1 M monobasic sodium phosphate monohydrate, adjusting with sodium hydroxide to a pH of 7, and diluting with water to 10 L); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amount of  $\text{C}_{48}\text{H}_{74}\text{O}_{14}$  (component  $\text{H}_2\text{B}_{1a}$ ) plus  $\text{C}_{47}\text{H}_{72}\text{O}_{14}$  (component  $\text{H}_2\text{B}_{1b}$ ) dissolved by employing the following method.

**Mobile phase**—Prepare a degassed solution of acetonitrile, methanol, and water (53:35:12).

**Standard stock solution**—Prepare a 0.13 mg per mL solution of USP Ivermectin RS in *Medium*.

**Standard solution**—Using the accompanying table, dilute the *Standard stock solution* with *Medium* to volume, and mix.

Tablet Strength (mg per Tablet)	Required Dilution Ratio	Volume of Standard stock solution (mL)	Volumetric Flask Size (mL)
3.0	1 in 40	5.0	200
6.0	1 in 20	5.0	100

**Test solution**—Pass a portion of the solution under test through a suitable filter, and use the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm  $\times$  10-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.81 for  $\text{H}_2\text{B}_{1b}$  and 1.0 for  $\text{H}_2\text{B}_{1a}$ ; the resolution,  $R$ , between the  $\text{H}_2\text{B}_{1a}$  and  $\text{H}_2\text{B}_{1b}$  peaks is not less than 1.5; the capacity factor,  $k'$ , for the  $\text{H}_2\text{B}_{1a}$  peak is not less than 4; the column efficiency determined from both the  $\text{H}_2\text{B}_{1a}$  and  $\text{H}_2\text{B}_{1b}$  peaks is not less than 1500 theoretical plates; the tailing factor for the  $\text{H}_2\text{B}_{1a}$  peak is not more than 2; and the relative standard deviation for replicate injections for the  $\text{H}_2\text{B}_{1a}$  peak is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100  $\mu\text{L}$ ) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the combined quantities, in percentage, of  $\text{H}_2\text{B}_{1a}$  plus  $\text{H}_2\text{B}_{1b}$  dissolved based on the peak responses obtained from the *Test solution* and the *Standard solution* by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[A_S(D_S)L]$$

in which  $A_U$  is the total peak area of  $\text{H}_2\text{B}_{1a}$  plus  $\text{H}_2\text{B}_{1b}$  obtained from the *Test solution*;  $W_S$  is the weight, in mg, of the USP Ivermectin RS taken to prepare the *Standard stock solution*;  $P$  is the purity of the USP Ivermectin RS (percent [w/w]  $\text{H}_2\text{B}_{1a}$  plus percent [w/w]  $\text{H}_2\text{B}_{1b}$ ), expressed as a decimal;  $D_U$  is the *Test solution* dilution factor;  $A_S$  is the total peak area of  $\text{H}_2\text{B}_{1a}$  plus  $\text{H}_2\text{B}_{1b}$  obtained from the *Standard solution*;  $D_S$  is the *Standard solution* dilution factor; and  $L$  is the label claim of ivermectin, in mg per Tablet.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $\text{C}_{48}\text{H}_{74}\text{O}_{14}$  ( $\text{H}_2\text{B}_{1a}$ ) plus  $\text{C}_{47}\text{H}_{72}\text{O}_{14}$  ( $\text{H}_2\text{B}_{1b}$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** <905>: meet the requirements for *Content uniformity*.

PROCEDURE FOR CONTENT UNIFORMITY—

**Mobile phase**—Prepare as directed in the *Assay*.

**Standard solution A**—Use the *Standard preparation* from the *Assay*.

**Standard solution B**—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution containing 0.125 mg per mL.

**Stock sensitivity solution (1%)**—Use the *Stock sensitivity solution* (1%) from the *Assay*.

**Sensitivity solution (0.2%)**—Use the *Sensitivity solution* (0.2%) from the *Assay*.

**Test solution**—Transfer 1 Tablet into each of ten 25-mL volumetric flasks. Add 5.0 mL of water, and sonicate for 10 minutes. Add approximately 15 mL of methanol, sonicate for 5 minutes, and mix. Allow the solution to cool to room temperature. Dilute with methanol to volume, and mix. Pass a portion of each solution through a 1.0- to 1.2- $\mu\text{m}$  chemically resistant filter prior to analysis.

**Chromatographic system** (see *Chromatography* <621>)—Proceed as directed in the *Assay*.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of *Standard solution A* (for the 6 mg per Tablet dose) or *Standard solution B* (for the 3 mg per Tablet dose), the *Sensi-*



tivity solution (0.2%), and the Test solution into the chromatograph, record the chromatograms, and measure the responses of the ivermectin peaks. Calculate the quantity as a percentage of the label claim of ivermectin per Tablet taken by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[(A_S)(D_S)L]$$

in which  $A_U$  is the peak area of  $H_2B_{1a}$  plus the peak area of  $H_2B_{1b}$  obtained from the Test solution;  $W_S$  is the weight, in mg, of the USP Ivermectin RS taken to prepare Standard solution A or Standard solution B;  $P$  is the purity of USP Ivermectin RS (percent [w/w]  $H_2B_{1a}$  plus percent [w/w]  $H_2B_{1b}$ ), expressed as a decimal;  $D_U$  is the Test solution dilution factor;  $A_S$  is the peak area of  $H_2B_{1a}$  plus the peak area of  $H_2B_{1b}$  obtained from Standard solution A or Standard solution B;  $D_S$  is the Standard solution A or Standard solution B dilution factor; and  $L$  is the label claim of ivermectin, in mg per Tablet.

#### Limit of 8a-oxo- $H_2B_{1a}$ —

**Mobile phase**—Proceed as directed in the Assay.

**BHA Working Standard solution**—Dissolve an accurately weighed quantity of USP 3-*tert*-Butyl-4-hydroxyanisole RS in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.96  $\mu$ g per mL.

**Test solution**—Use the Assay preparation.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The column temperature is maintained at 30°. The flow rate is about 1.2 mL per minute. Chromatograph the BHA Working Standard solution and the Test solution, and record the peak responses as directed for Procedure: the relative retention times at 280 nm are about 0.24 for BHA, 0.77 for 8a-oxo- $H_2B_{1a}$ , and 1.0 for  $H_2B_{1a}$ .

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the BHA Working Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of 8a-oxo- $H_2B_{1a}$  as a percentage of the label claim of ivermectin in the portion of Tablets taken by the formula:

$$[100(A_D)(W_S)(P)(D_U)(C_F)]/[(A_S)(D_S)(N)(L)(F)]$$

in which  $A_D$  is the peak area of 8a-oxo- $H_2B_{1a}$  obtained from the Test solution;  $W_S$  is the weight of USP 3-*tert*-Butyl-4-hydroxyanisole RS, in mg, taken to prepare the BHA Working Standard solution;  $P$  is the purity of USP 3-*tert*-Butyl-4-hydroxyanisole RS, expressed as a decimal;  $D_U$  is the Test solution dilution factor;  $C_F$  is the correction factor (equal to 0.98) used to convert mg of 8a-oxo- $H_2B_{1a}$  to mg of ivermectin;  $A_S$  is the peak area of BHA obtained from the BHA Working Standard solution;  $D_S$  is the BHA Working Standard solution dilution factor;  $N$  is the number of Tablets taken to prepare the Test solution;  $L$  is the label claim of ivermectin, in mg per Tablet; and  $F$  is the relative response factor (equal to 1.0): not more than 2.0% of 8a-oxo- $H_2B_{1a}$  is found. The correction factor,  $C_F$ , (equal to 0.98) is calculated by the following formula:

$$[0.90 (\text{molecular weight of } H_2B_{1a}) + 0.10 (\text{molecular weight of } H_2B_{1b})]/(\text{molecular weight of 8a-oxo-}H_2B_{1a}) = 873.10/889.10 = 0.98]$$

#### Assay—

**Mobile phase**—Prepare a mixture of acetonitrile, methanol, and water (53:35:12). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution containing 0.25 mg per mL.

**Stock sensitivity solution (1%)**—Quantitatively prepare a 1 in 100 dilution of the Standard solution in methanol.

**Sensitivity solution (0.2%)**—Quantitatively prepare a 1 in 5 dilution of the Stock sensitivity solution (1%) in methanol.

**Assay preparation**—Transfer the appropriate number of Tablets into a 250-mL volumetric flask according to the accompanying table:

Tablet Strength (mg per Tablet)	Number of Tablets
3.0	20
6.0	10

Add approximately 25 mL of water, and sonicate for 10 minutes. Add methanol to fill the flask three-quarters full, sonicate for 5 minutes or until the Tablets are completely disintegrated, and shake until mixed well. Allow the solution to cool to room temperature. Dilute with methanol to volume, add a magnetic stirrer, and mix until no lumps are present in the solution. Pass a portion of this solution through a 1.0- to 1.2- $\mu$ m chemically resistant filter prior to injection.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 30°. Chromatograph the Sensitivity solution (0.2%) and the Standard preparation at 245-nm detection, and record the peak responses as directed for Procedure: the signal-to-noise ratio for the ivermectin peak obtained from the Sensitivity solution (0.2%) is not less than 10; obtained from the Standard preparation, the relative retention times are about 0.82 and 1.0 for components  $H_2B_{1b}$  and  $H_2B_{1a}$ , respectively; the capacity factor,  $k'$ , for the component  $H_2B_{1b}$  is not less than 3; the column efficiency for component  $H_2B_{1a}$  is not less than 1500 theoretical plates; the tailing factor for component  $H_2B_{1a}$  is not more than 2; and the relative standard deviation for the area response for total ivermectin ( $H_2B_{1a}$  plus  $H_2B_{1b}$ ) for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for component  $H_2B_{1a}$  plus component  $H_2B_{1b}$ . Calculate the percentage of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) plus component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ) as a percentage of the label claim of ivermectin per Tablet taken by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[(A_S)(D_S)(N)(L)]$$

in which  $A_U$  is the total peak response of  $H_2B_{1a}$  plus  $H_2B_{1b}$  obtained from the Assay preparation;  $W_S$  is the weight of the USP Ivermectin RS, in mg, taken to prepare the Standard preparation;  $P$  is the purity of the USP Ivermectin RS (percent [w/w]  $H_2B_{1a}$  plus percent [w/w]  $H_2B_{1b}$ ), expressed as a decimal;  $D_U$  is the sample dilution factor;  $A_S$  is the total peak area of  $H_2B_{1a}$  plus  $H_2B_{1b}$  obtained from the Standard preparation;  $D_S$  is the Standard dilution factor;  $N$  is the number of Tablets taken to prepare the Assay preparation; and  $L$  is the label claim of ivermectin, in mg per Tablet.

### Ivermectin Topical Solution

» Ivermectin Topical Solution is a topical solution in a suitable vehicle. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of Ivermectin, calculated as the sum of component  $H_2B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) plus component  $H_2B_{1b}$  ( $C_{47}H_{72}O_{14}$ ). The ratio of the

contents,  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$ , is not less than 90.0 percent.

**Packaging and storage**—Preserve in well-closed containers. Store at a temperature not more than 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Ivermectin RS

**Identification**—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution*—Dissolve a volume of Topical Solution in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.5 mg per mL of ivermectin, and filter.

*Injection volume:* 2  $\mu$ L.

*Developing solvent system:* unsaturated chamber, freshly prepared and equilibrated mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).

*Procedure*—Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light: the retardation factor,  $R_f$ , of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

**B:** The retention times of the two principal component peaks of ivermectin in the chromatogram of the *Assay preparation* corresponds to that of the two principal component peaks of ivermectin in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**Chromatographic purity**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.004 mg per mL solution.

*Test solution*—Dissolve a quantity of Topical Solution in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.

*Procedure*—Inject equal volumes (about 20  $\mu$ L) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Topical Solution taken by the formula:

$$100(C_S / C_T) (r_i / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of ivermectin in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of ivermectin in the *Test solution*;  $r_i$  is the peak response for each impurity from the *Test solution*; and  $r_S$  is the ivermectin peak response obtained from *Standard solution*. Not more than 2.7% of the peak with a relative retention time of about 1.3 to 1.5 to that of the principal peak is found; not more than 1.0% of any other impurity is found; and not more than 6.0% of total impurities is found. Disregard any peak below 0.05%.

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (106:55:39). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.4 mg per mL.

*Assay preparation*—Dilute a volume of Topical Solution, quantitatively, and stepwise if necessary, with methanol to

obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the first peak (component  $H_2B_{1b}$ ) and the second peak (component  $H_2B_{1a}$ ) is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%, determined from the  $H_2B_{1a}$  peak.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the component  $H_2B_{1a}$  plus component  $H_2B_{1b}$ . Calculate the percentage of label claim of ivermectin ( $H_2B_{1a} + H_2B_{1b}$ ) in the portion of Topical Solution taken by the formula:

$$100(C_S / C_U) (r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of ivermectin in the *Assay preparation*;  $r_U$  is the total peak response of  $H_2B_{1a}$  plus  $H_2B_{1b}$  peaks obtained from the *Assay preparation*; and  $r_S$  is the total peak response of  $H_2B_{1a}$  plus  $H_2B_{1b}$  peaks obtained from the *Standard preparation*. Calculate the ratio of the contents, in percent, of  $H_2B_{1a} / (H_2B_{1a} + H_2B_{1b})$  in the portion of Topical Solution taken by the formula:

$$100(r_1 / r_U)$$

in which  $r_1$  is the peak response of  $H_2B_{1a}$  obtained from the *Assay preparation*; and  $r_U$  is as described above.

## Ivermectin and Clorsulon Injection

» Ivermectin and Clorsulon Injection is a sterile solution of Ivermectin and Clorsulon in a suitable vehicle. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of ivermectin [component  $B_{1a}$  ( $C_{48}H_{74}O_{14}$ ) plus component  $B_{1b}$  ( $C_{47}H_{72}O_{14}$ )] and not less than 95.0 percent and not more than 105.0 percent of the labeled amount of clorsulon ( $C_8H_8Cl_3N_3O_4S_2$ ).

**Packaging and storage**—Preserve in single-dose or multi-dose containers, preferably of Type I glass or plastic. Store at a temperature not higher than 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Clorsulon RS

USP Endotoxin RS

USP Ivermectin RS

**Identification**—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

*Test solution:* about 0.5 mg of ivermectin and 5 mg of clorsulon per mL in methanol.

*Application volume:* 2  $\mu$ L.

*Developing solvent system:* a mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).

**B:** The retention times of the two major peaks in the chromatogram of the *Assay preparation* correspond to those

in the chromatogram of the *Standard preparation*, as obtained in the *Assay for ivermectin*. The retention time of the major clorsulon peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for clorsulon*.

[NOTE—The two major ivermectin components are not separated by this method.]

**Bacterial endotoxins** (85)—It contains not more than 2.3 USP Endotoxin Units per mg of combined ivermectin and clorsulon.

**Sterility** (71): meets the requirements.

**Other requirements**—It meets the requirements under *Injections* (1).

#### Assay for ivermectin—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (530:350:70). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Increasing the proportion of water increases the resolution.

**Standard preparation**—Prepare a solution of USP Ivermectin RS in methanol having a known concentration of about 0.3 mg per mL.

**Assay preparation**—Transfer an accurately measured portion of Injection, equivalent to about 30 mg of ivermectin, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure* [NOTE—The relative retention times are about 0.8 for component B<sub>1b</sub> and 1.0 for component B<sub>1a</sub>]; the resolution, *R*, between component B<sub>1b</sub> and component B<sub>1a</sub> is not less than 2.0; the column efficiency determined from the component B<sub>1a</sub> peak is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections determined for the component B<sub>1a</sub> peak is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of ivermectin component B<sub>1a</sub> (C<sub>48</sub>H<sub>74</sub>O<sub>14</sub>) plus ivermectin component B<sub>1b</sub> (C<sub>47</sub>H<sub>72</sub>O<sub>14</sub>) in the portion of Injection taken by the formula:

$$CP(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation*; *P* is the designated percentage of the sum of component B<sub>1a</sub> plus component B<sub>1b</sub> in USP Ivermectin RS; and *r<sub>U</sub>* and *r<sub>S</sub>* are the sums of the peak area responses for ivermectin component B<sub>1a</sub> plus ivermectin component B<sub>1b</sub> obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for clorsulon—

**Mobile phase**—Prepare a filtered and degassed mixture of chloroform, methanol, and water (900:100:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Prepare a solution of USP Clorsulon RS in methanol having a known concentration of about 2.4 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

**Assay preparation**—Transfer an accurately measured portion of Injection, equivalent to about 240 mg of clorsulon, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L3. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure* [NOTE—The relative retention times are about 0.6 for ivermectin (the major components of ivermectin co-elute) and 1.0 for clorsulon]; the column efficiency determined from the clorsulon peak is not less than 4000 theoretical plates; the tailing factor for the clorsulon peak is not more than 2.0; and the relative standard deviation for replicate injections determined for the clorsulon peak is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of clorsulon (C<sub>8</sub>H<sub>8</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Injection taken by the formula:

$$1000C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clorsulon RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the clorsulon peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ivermectin and Pyrantel Pamoate Tablets

» Ivermectin and Pyrantel Pamoate Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ivermectin components H<sub>2</sub>B<sub>1a</sub> (C<sub>48</sub>H<sub>74</sub>O<sub>14</sub>) plus H<sub>2</sub>B<sub>1b</sub> (C<sub>47</sub>H<sub>72</sub>O<sub>14</sub>) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pyrantel pamoate (C<sub>34</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub>S).

**Packaging and storage**—Preserve in tight containers, protected from light, at a temperature not exceeding 30°, and avoid freezing.

**Labeling**—Label Tablets to indicate that they are intended for veterinary use only. Tablets that can be chewed are so labeled.

#### USP Reference standards (11)—

USP Ivermectin RS

USP Pyrantel Pamoate RS

**Identification**—The retention times of the two major ivermectin peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay for ivermectin*. The retention time of the pyrantel peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay for pyrantel pamoate*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g. Tablets meet the requirements of the test for absence of *Escherichia coli*.

**Uniformity of dosage units** (905): The requirements for dosage uniformity are met if the amount of ivermectin and pyrantel pamoate in each of the 10 dosage units as determined from the Content Uniformity method lies within the range of 75.0% to 125.0% of the label claim, and the RSD is less than or equal to 7%.

If not more than 3 units are outside the range of 75.0% to 125.0% of label claim, and no unit is outside the range of 75.0% to 135.0% of label claim, or if the RSD is greater than 7%, or if both conditions prevail, test 20 additional units. The requirements are met if not more than 3 units of the 30 are outside the range of 75.0% to 125.0% of label claim, and no unit is outside the range of 75.0% to 135.0% of label claim and the RSD of the 30 dosage units does not exceed 9%.

**pH** (791): between 4 and 6, in a solution prepared as follows. Grind about 15 g of Tablets in a blender. Transfer 10 g of the coarse powder thus obtained to a blender jar, add 250 mL of water, previously adjusted to a pH of 7.0 with 0.01 N sodium hydroxide or 0.01 N hydrochloric acid, and blend for about 5 minutes. Allow to settle, and filter a portion of the supernatant. Determine the pH of the filtrate.

#### Assay for ivermectin—

**Mobile phase**—Prepare a mixture of acetonitrile, methanol, 0.05 M monobasic sodium phosphate, and water (1130:670:200:5), adjust with phosphoric acid to a pH of 3.0, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of methanol and water (95:5).

**Alumina column**—Add about 30 mL of water to about 500 g of neutral alumina, and shake for about 2 hours on a reciprocating shaker. Add about 4 g of the resulting suspension to a 10-mm × 10-cm chromatographic tube fitted with a stopcock, tapping the sides of the column to facilitate settling of the alumina to a height of between 5 and 6 cm. Prepare a separate column for each solution to be tested.

**Standard stock solution**—Prepare a solution of USP Ivermectin RS in methanol having a known concentration of about 0.28 mg of USP Ivermectin RS per mL. Transfer 4.0 mL of this solution and 5.0 mL of water to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.56 µg of USP Ivermectin RS per mL.

**Standard preparation**—Transfer 15.0 mL of the *Standard stock solution* to a 50-mL centrifuge tube, add 4.0 mL of water, and mix on a vortex mixer. Extract this solution with 10 mL of hexanes by shaking for about 10 minutes. Centrifuge, and discard the hexanes layer. Repeat the extraction with 5 mL of hexanes by shaking for 5 minutes. Centrifuge, and discard the hexanes layer. Add the aqueous layer to the *Alumina column*, and allow to elute. Discard the first 2 mL of eluant, and collect the next 5 mL of eluant in a stoppered tube.

**Assay stock solution**—Grind an accurately weighed Tablet until it is completely broken up and free from lumps and transfer to a screw-capped, wide-mouth, amber bottle of appropriate volume. Add an accurately measured volume of *Diluent* to the bottle to obtain the estimated concentration of ivermectin of about 0.55 µg per mL. Cap the bottle, and mix on a vortex mixer for about 1 minute. Ultrasonicate for 15 minutes then mechanically shake for an additional 30 minutes. Centrifuge a portion of the suspension thus obtained for about 5 minutes.

**Assay preparation**—Transfer 15.0 mL of the *Assay stock solution* to a 50-mL centrifuge tube, add 4.0 mL of water, and mix on a vortex mixer. Proceed as directed under *Standard preparation*, beginning with "Extract this solution." The solution thus obtained is the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at about 30°. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the relative retention times are about

0.8 for component H<sub>2</sub>B<sub>1b</sub> and 1.0 for component H<sub>2</sub>B<sub>1a</sub>; the resolution, *R*, between the component H<sub>2</sub>B<sub>1b</sub> peak and the component H<sub>2</sub>B<sub>1a</sub> peak is not less than 2.5; the column efficiency determined from the component H<sub>2</sub>B<sub>1a</sub> peak is not less than 2000 theoretical plates; the tailing factor at 5.0% of peak height for the H<sub>2</sub>B<sub>1a</sub> peak is not more than 2.2; and the relative standard deviation for replicate injections determined from the sum of the component H<sub>2</sub>B<sub>1b</sub> peaks and the component H<sub>2</sub>B<sub>1a</sub> peak is not more than 2.0%. [NOTE—After the column has been used, if the system suitability requirements are not met, regenerate the column as follows. Wash the column with 50 mL of water, slowly increasing the flow rate to 1 mL per minute. Make at least seven 100-µL injections of a dimethyl sulfoxide and water solution (1:1) at 5-minute intervals using water as a mobile phase. Purge the column with 100 mL of methanol, 200 mL of methylene chloride, and again with 100 mL of methanol, slowly increasing the flow rate to 1 mL per minute after each solvent changeover. Finally, purge the column with *Mobile phase*, increasing the flow rate to that used for the analysis.]

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of label claim of ivermectin [component H<sub>2</sub>B<sub>1a</sub> (C<sub>48</sub>H<sub>74</sub>O<sub>14</sub>) plus component H<sub>2</sub>B<sub>1b</sub> (C<sub>47</sub>H<sub>72</sub>O<sub>14</sub>)] in the Tablet taken by the formula:

$$100(P(C_S/C_U))(r_U/r_S)$$

in which *C<sub>S</sub>* is the concentration, in µg per mL, of USP Ivermectin RS in the *Standard stock solution*; *C<sub>U</sub>* is the nominal concentration, in µg per mL, of ivermectin in the *Assay stock solution*; *P* is the purity of the USP Ivermectin RS [percent (w/w) H<sub>2</sub>B<sub>1a</sub> plus percent (w/w) H<sub>2</sub>B<sub>1b</sub>], expressed as a decimal; and *r<sub>U</sub>* and *r<sub>S</sub>* are the sums of the peak areas for component H<sub>2</sub>B<sub>1a</sub> and component H<sub>2</sub>B<sub>1b</sub> obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pyrantel pamoate**—[NOTE—Use amber glassware in preparing solutions of pyrantel pamoate, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay for pyrantel pamoate* without prolonged interruption.]

**Extraction solvent**—Prepare a mixture of tetrahydrofuran and trifluoroacetic acid (94:6).

**Mobile phase**—Prepare a degassed mixture of acetonitrile, water, acetic acid, and triethylamine (940:25: 25:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Increasing the amount of acetonitrile in the *Mobile phase* increases retention times.]

**Standard preparation**—Prepare a solution of USP Pyrantel Pamoate RS in *Extraction solvent* having a known concentration of about 1.7 mg per mL. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix. This solution contains about 0.27 mg of USP Pyrantel Pamoate RS per mL. [NOTE—Stable for 72 hours if stored at room temperature in a dark area.]

**Assay preparation**—Grind an accurately weighed Tablet until it is completely broken up and free from lumps and transfer to a 300-mL stock bottle, add 50.0 mL of *Extraction solvent*, mix, sonicate for about 15 minutes, and shake by mechanical means for about 1 hour. Allow to settle, and decant the supernatant into a second 300-mL bottle. Add a second 50-mL portion of *Extraction solvent* to the stock bottle, and shake by mechanical means for about 1 hour. Transfer the contents of the second 300-mL bottle to the stock bottle. Rinse the second bottle with 50.0 mL of *Extraction solvent*, and transfer this rinsing to the stock bottle. Sonicate the stock bottle for about 10 minutes, and centrifuge a portion of the liquid. Transfer an accurately meas-

ured volume of the clear supernatant, equivalent to about 6.5 mg of pyrantel pamoate, to a 25-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 313-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency determined from the pyrantel peak is not less than 8000 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the per-

centage of label claim of pyrantel pamoate ( $C_{34}H_{30}N_2O_6S$ ) in the Tablet taken by the formula:

$$100 (C_s/C_u)(r_u / r_s)$$

in which  $C_s$  is the concentration, in mg per mL, of USP Pyrantel Pamoate RS in the *Standard preparation*;  $C_u$  is the nominal concentration of pyrantel pamoate in the *Assay preparation*; and  $r_u$  and  $r_s$  are the pyrantel peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. [NOTE—Where the test for *Uniformity of dosage units* has been performed using the *Assay for pyrantel pamoate* procedure as a test for *Content uniformity*, use the average of these determinations as the *Assay for pyrantel pamoate* value.]



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